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57th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Date/Place:	**Geneva, Switzerland, August 16 – 20, 2009**
Chairman:	Kurt Hostettmann

Dear Colleagues,

The 57th Congress of the Society of Medicinal Plant and Natural Product research will be held this year in Geneva, Switzerland. The congress venue is going to be at the CICG (Centre International des Conférences Genève) which is very well equipped to host such an important scientific event. As chairman of the Organizing Committee and also currently Director of the Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, I am highly pleased that the proposed programme attracted numerous scientists of 71 different countries who submitted more than 900 abstracts for poster or oral presentation.
The main topics of the Congress are:

- Lead finding from Nature
- Conservation and biodiversity issues
- Plants and aging of the population
- Natural products and neglected diseases
- Anti-cancer agents
- HIV and viral diseases
- Quality control and safety assessments of phytomedicines
- Prevention of metabolic diseases by medicinal plants and nutraceuticals
- Cosmetics, flavours and aromas

The programme of the Congress is offering invited lectures to be delivered by distinguished scientists, short oral communications which will be in parallel sessions and numerous posters. The opening lecture will be given by HRH Princess Chulabhorn Mahidol, President of the Chulabhorn Research Institute in Bangkok. The present issue of *Planta Medica* devoted to our Congress could be realized thanks to the help of Dr. Kuhlmann from Thieme Verlag and also because of the efficient work realized by staff members of my Laboratory, namely Karin Megzari, Martine Cabo, Prof. Jean-Luc Wolfender, Dr. Karine Ndjoko, Dr. Philippe Christen and Dr. Frederic Martin. My thanks go also to the agency selected for organizing this Congress, namely *Kuoni Destination Management* and their collaborators, Laetitia Roch, Franck Grosset and Steeve Girod. Checking more than 900 abstracts represents an enormous work which was achieved by the members of the scientific Committee. I express my gratitude to all of them for helping us to publish abstracts of good quality in this volume of *Planta Medica*.

I hope that everybody will enjoy their stay in Geneva.

Prof. Kurt Hostettmann
Chairman, Organizing Committee of the 57th International Congress & Annual Meeting of the Society for Medicinal Plant and Natural Product Research

Lectures

L1 Recent Investigation of Diverse Cytotoxic Natural Products from Thai Bioresources

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It is considered that because of the structural and biological diversity of their constituents, terrestrial plants offer a unique and renewable resource for the discovery of potential new drugs and biological entities. The potential of large areas of tropical rainforests remains virtually untapped. With much of the biological diversity found in tropical and subtropical regions, the investigation of these resources is of paramount importance. It has been estimated that about half of the plants in the world are found in the tropics. However, only a small percentage of the world's flowering plants have as yet been analysed for their possible medicinal uses. Apart from the abundance of plants, Thailand is also endowed with a variety of endophytic fungi which are potential sources for the production of a diverse array of bioactive metabolites, endophytic fungi associated with Thai medicinal plants are of our special interest and in the past few years we have investigated some of such endophytic fungi. In addition to our current interests in the natural products from plants and endophytic fungi, we are also interested in the marine natural products isolated from marine organisms found along Thai coastal lines from the Gulf of Thailand to the Andaman Sea. In this presentation, our recent investigation on the chemistry as well as biological activities, especially cytotoxic activity of some natural products derived from the above mentioned Thai bioresources will be presented. **References:** [1] Youngsa-ad, W. et al. (2007) Planta Med. 73:1491 – 1494. [2] Prachyawarakorn, V. et al. (2008) Planta Med. 74:69 – 72. [3] Chomcheon, P. et al. (2009) Phytochemistry 70:121 – 127.

L2 New chemistry from South East Asian medicinal plants

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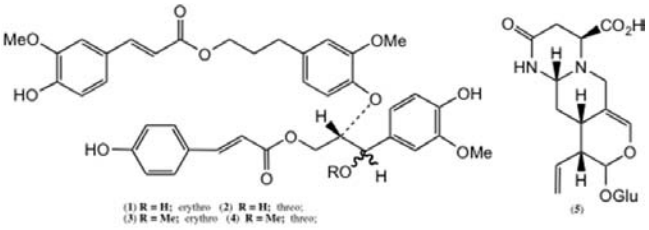
[1]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane Q 4072, Australia; [2]Department of Chemistry, Faculty of Mathematics and Natural Science, Tanjungpura University, West Kalimantan, Indonesia; [3]Faculty of Pharmacy, Airlangga University, East Java, Indonesia; [4]School of Pharmacy, The University of Queensland, Brisbane Q 4072, Australia

Indonesia comprises only 1.3% of the earth's land surface, but has 11% of the world's higher plants, with over 1000 different plant extracts used in traditional medicines. [1] In a collaborative program on the chemistry of Indonesian medicinal plant species, we have examined the natural products chemistry of plants of the genus *Durio* and *Fagraea*. Both triterpene and lignan metabolites were isolated from *Durio* along with well known compounds such as 3-hydroxymellein that are characteristic of fungi. The relative and absolute configuration of a set of new neolignan metabolites (1) – (4) were explored by NMR and by CD studies. The genus *Fagraea* was generally characterized by iridoid glycosides, however the unusual new terpene alkaloid fagraeoside (5) was isolated from *Fagraea racemosa*.



Acknowledgements: AusAID **References:** [1] Anonymous (2000) *World Resources 2000 – 2001, people and ecosystems: the fraying web of life,* World Resources Institute, Washington DC; 246 – 248

L3 Novel $GABA_a$ ligands, inspired by nature

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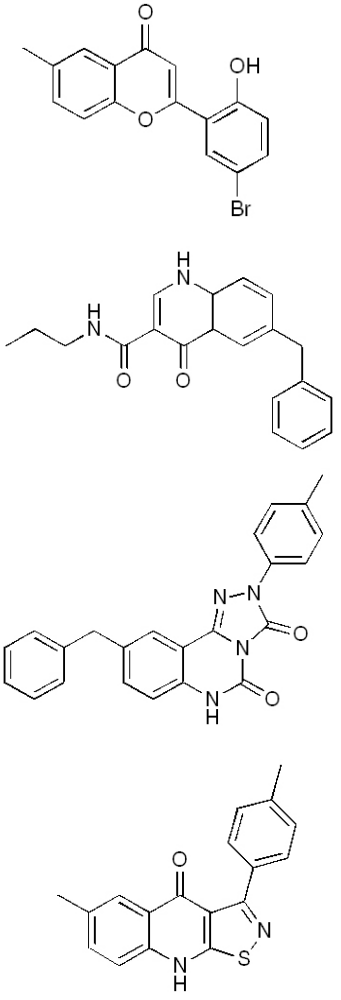
The major inhibitory neurotransmitter γ-amino butyric acid (GABA) exerts its inhibitory effect by binding to three different classes of receptors: $GABA_A$-, $GABA_B$- and $GABA_C$ receptors. $GABA_A$- and $GABA_C$ are both ligand gated chloride ion channels, while $GABA_B$ are G-protein coupled receptors. $GABA_A$ receptors are important therapeutic targets for anxiety disorders, cognitive disorders, epilepsies, mood disorders, schizophrenia and sleep disorders. Due to the commercial interest, many compound classes, such as benzodiazepines, steroids, and barbiturates, are known to allosterically modify the effect of GABA by binding to distinct sites of the $GABA_A$ receptors. The pharmacological properties of the benzodiazepines (anxiolytic, anti-convulsant, muscle relaxant and sedative/hypnotic) make them the most frequently used $GABA_A$ receptor modulating drugs in the clinic. We have developed several new classes of ligands that bind to the benzodiazepine binding site of the $GABA_A$ receptors, and used the knowledge to fine-tune a pharmacophore model that has suggested yet new and very potent ligands. Examples of compounds, all with sub-nM K_i-values, that we have worked with are shown below.



L4 Opportunities and challenges that face those interested projects that link natural product chemistry, plant uses and conservation

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Changes in land use, habitat loss, pollution and climate change are all factors that are contributing to a decrease in biodiversity. Stopping this decline is an important challenge. We are not only losing plant species that have potential to be developed as foods but also those that have medicinal and pesticidal properties. The development of DNA-based

phylogenies has furthered our understanding of plant relationships and has provided robust frameworks onto which, geographical distribution, uses of plants as well as their chemistry can be superimposed. This enables us to identify families and species that justify further research. However, obtaining plants for this research can be a major challenge. The 1992 United Nations Conference on Environment and Development (Rio Summit) resulted in a series of international plants to support conserving plants for sustainable use, including the Convention on Biological Diversity (CBD). The CBD was the first international convention to state that a county has "sovereign rights" over its genetic resources and that if these resources are used by others then any benefits that arise from this use should be shared. This has resulted in countries developing "Access and Benefit Sharing" legislation that covers what a scientist needs to do before working on genetic resources but also what should happen if products are developed from the genetic resource. In some countries the use of traditional knowledge is also covered in this legislation. Many researchers are unsure as to which permits they need. In an age when we need to increase efforts to conserve plants and the traditional knowledge about their uses it is importance to not only protect the sovereign rights of countries but also facilitate mechanism that enable communities maximise the uses of their plant-resources. Including the identification of alternative species they might need if their flora changes through the influences of climate change.

L5 Recent study on the Chemistry of Sumatran Medicinal Plants

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Sumatra is the fourth largest island in the world and known to be very rich with varieties of tropical rainforest plants. Many of these plants have been used traditionally for centuries for many purposes such as medicines, coloring matters, food, spices, insecticides, aromatics, etc. In continuation of our work to study the chemistry of Sumatran Traditional Medicinal Plants [1], recently we investigated the chemical constituents of two species of *Lerchea* (Rubiaceae) as well as liverworts *Bazzania* sp, fungus *Scleroderma* sp and lichen *Stereocoulon* sp. In addition, in collaboration with National Agency of Drug and Food Control of the Republic of Indonesia (BPOM RI) we have also studied the chemistry and standardization of extracts of some widely used Sumatran Medicinal Plants which surprisingly contain high flavonoid contents i.e *Syzygium polyanthum* (Weigh.) Walp. traditionally used as anti-diabetic, *Scurulla ferruginea* Danser (Loranthaceae) as anti-cancer, *Piperomia pelucida* (L.) Kunth. (Piperaceae), Sida *rhombifolia* L. (Malvaceae) as anti-rheumatic, *Gynura procumbens* (Lour.) Merr. (Asteraceae) as anti-pyretic and anti-inflammatory, *Pluchea indica* (L.) Less, (Asteraceae), as anti-pyretic, *Uncaria gambier* (Hunter) Roxb. (Rubiaceae) as anti-diarrhoea and industrial sources of tannins. The isolation, structure elucidation and analysis of chemical contents of these Sumatran medicinal plants will be discussed. **Reference:** [1] Arbain, D. (2008) *Science and Culture* 74:65 – 70.

L6 Natural products for the treatment of infectious neglected diseases

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Neglected, mostly tropical diseases affect large, poorest populations often living in rural areas, urban slums or in conflict zones. Approximately 1 billion people, one sixth of the world's population, suffer from one or more neglected tropical diseases, yet there is almost no effective treatment. Despite the recently boosted research activity, infectious diseases, such as AIDS, tuberculosis, diarrhea, or vector-borne parasitic diseases, such as malaria, trypanosomiasis and leishmaniasis continue to take an enormous toll on human health. The absence of vaccines and the emergence of drug-resistant strains render the eradication and the control of these diseases nearly impossible. Hence, the search for new drugs against these diseases has become a pressing, global demand. Except for malaria, natural resources have remained an untapped resource for the discovery of new drug leads for the treatment of infectious neglected diseases (INDs). Our recent research mostly on endemic Turkish plants and marine organisms have led to the identification of many natural products of various classes, e.g. iridoids, alkaloids, flavonoids, fatty acids, saponins, terpenoids and steroids, which are generally selectively toxic to the parasitic protozoa, i.e. *Plasmodium falciparum*, *Trypanosoma brucei, T. cruzi* and *Leishmania donovani* [1 – 4]. We have also isolated or synthesized a number of natural products (quinones, sesquiterpenes etc) with significant activity against the tubercle bacillus, *M. tuberculosis*, as well the causative agents of food poisoning and diarrhea, *E. coli* and *S. aureus*. The potential cellular target of some of these antibacterial and antimycobacterial natural products has been identified [3,4]. This lecture will give a summary of our results concerning the isolation, characterization and biological activity of mostly secondary metabolites that could serve as potential drug scaffolds against INDs. **References:** [1] Tasdemir, D. et al. (2006) Antimicrob. Agents Chemother. 50:1352 – 1364. [2] Tasdemir, D. et al. (2005) Phytochemistry 66:355 – 362. [3] Tasdemir, D. et al. (2007) Bioorg. Med. Chem. 15:6834 – 6845. [4] Karioti, A. et al. (2008) Phytomedicine 15:1125 – 1129.

L7 Natural Products for the treatment of HIV/AIDS

Klimkait T[1,2], Hamy F[1,2], Vidal V[1,2], Gericke N[1], Sanglier JJ[1], Guitard P[1], Giger R[1], Molac B[1], Matter A[1]
[1]Esperanza Medicines Foundation (EMF), Basel, Switzerland; [2]InPheno AG, Basel, Switzerland

Access to anti-viral drug (ARV) therapy remains a serious issue for poor people in developing countries. Despite the influx of major funds in Sub-Saharan Africa over the last few years (10 billion USD in 2008) only about one third of all patients get regular access to ARV therapy. For people living with HIV/AIDS that do not fulfill the criteria of fullblown AIDS, there are very few options. Traditional medicines have long filled this gap albeit under conditions that are not optimal. EMF would like to contribute in three ways to better health care, i) in supplementing ARV therapy in fullblown AIDS patients with medicines that can contribute to the well being, quality of life and life span, ii) supporting AIDS patients that are unable to access ARV therapy with alternative medicines, iii) in providing such medicines to people with HIV/AIDS that are not yet fully symptomatic. The goal of EMF is to discover, develop and ultimately bring to market a diverse portfolio of three types of products: i) Food supplements that fulfill safety criteria and have a favorable nutritional effect in people with HIV/AIDS, ii) Complementary medicines that are safe and demonstrably, in limited Phase I/phase II clinical trials have a positive and measurable effect on the quality of life of people with HIV/AIDS, and iii) novel anti-AIDS therapies registered for treatment of HIV/AIDS. EMF is a charity-funded, not-for-profit organization that has since its inception in 2004 studied a large variety of more than 12'000 Natural Products for use in above settings. Important partners in this endeavor were the Natural Products Unit at Novartis AG, and are InPheno AG in Basel for the systematic testing of antiviral activity in a variety of cellular systems employing several HIV-1 substrains that are representative of the African epidemiology, CSIR in Pretoria, SA (Dr. Vinesh Maharaj) and, most recently, the Molecular Biology Institute in Yaoundé, Cameroun (Dr. Céline Nkenfou Nguefeu) and the Institute of Pharmaceutical Biology, Pharmazentrum, University Basel (Prof. M. Hamburger & Dr. O. Potterat). A number of antivirally attractive product candidates have been discovered that are now in the process of being tested for their pharmacological suitability and developability. Some concrete examples of such products will be presented.

L8 Anti-cancer Agents from Plants, Current and Future Prospects

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Plants remain an important source of new anti-cancer drugs. The U.S. National Cancer Institute maintains an active program to discover and develop new drugs from plant sources. Plant materials are collected world-wide and processed to yield extracts which are screened in-house to identify promising leads. These extracts are also made available to scientists world-wide for independent study. The NCI's development efforts include full pre-clinical studies and clinical trials with the goal of FDA approval of new therapies. This talk will present a summary of past successes, current efforts and possible directions for new research on plant natural products.

L9 Chemical constituents from Chinese liverworts and their biological significances

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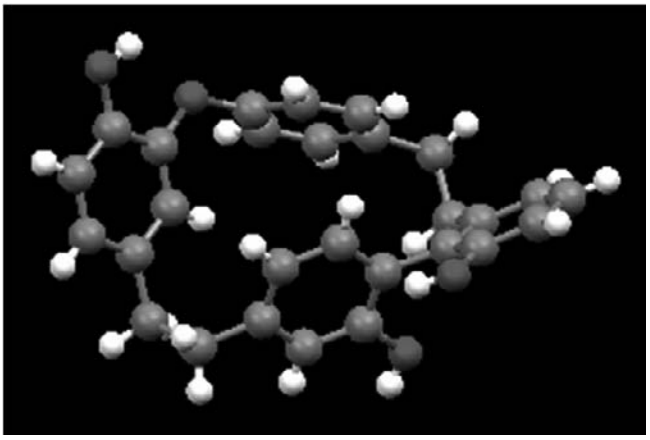
The dimorphic yeast *Candida albicans* is the most prevalent human fungal pathogen due to its high frequency of clinical isolation and the much amount of morbidity and mortality it causes. The natural high tolerance for antifungal drugs and the increase of clinically resistant *C. albicans* strains have lent the urgency for the development of new antifungal drugs. Such compounds should inhibit the morphological transition from yeast to filament which is one property contributing to *C. albicans* virulence. As a part of our ongoing research program on the isolation and identification of potentially antifungal compounds from the Chinese liverworts, over 30 macrocyclic bisbibenzyls have been isolated in our lab, including new bibenzofurane bisbibenzyls, which paves the way for screening the small molecule that block morphogenesis in *C. albicans* and obtaining structure-activity relationship data. Riccardin D, one of the macrocyclic bibibenzlyls was found to inhibit *C. albicans* biofilm formation with a sub-MIC concentration, which is associated with its block of hyphal growth. The underlined mechanism was also investigated.



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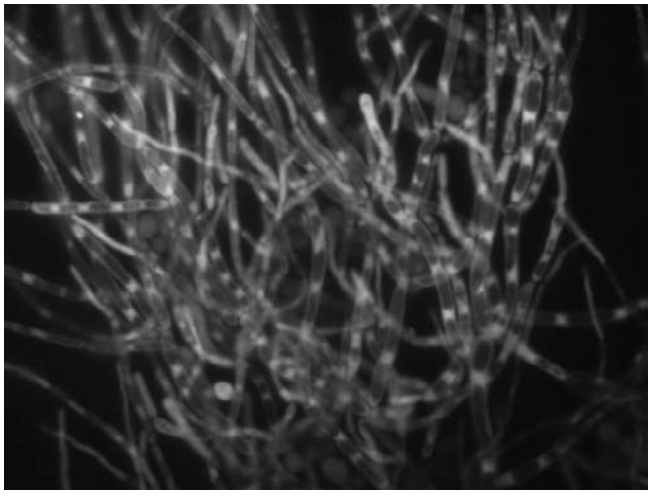

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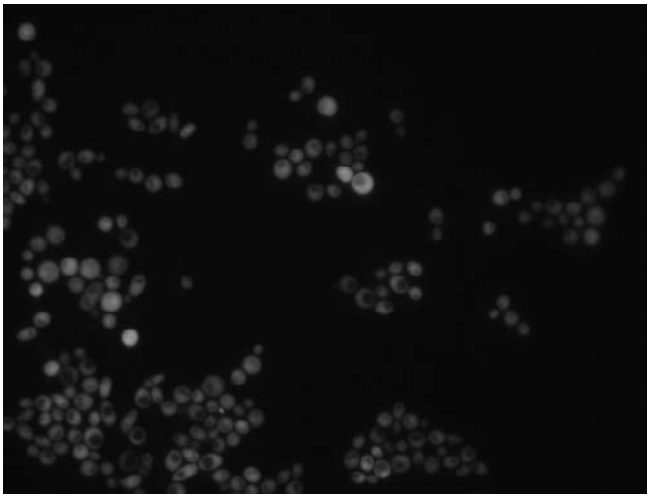

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L10 Phytomedicines Safety and Pharmacovigilance: Some Important Considerations

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Contrary to popular belief that herbal medicines are not innocuous there is sufficient evidence to question this premise. There is an urgent need, therefore, to perform more evidence-based herbal studies and provide conclusive evidence of their quality, efficacy and safety, which require well designed randomized controlled clinical trials to establish their therapeutic risk-benefit profiles [1]. Although the number of reports stating adverse drug reactions (ADRs) of phytomedicines is increasing, most of these cases are poorly documented and accordingly pre- and post-marketing surveillance of herbal medicine presents a unique challenge when compared with conventional medicines. The likelihood of their pharmacodynamic and pharmacokinetic interactions with conventional drugs warrants further investigations by modern *in vitro* and *in silico* predictive tools with special reference to their possible metabolisms by cytochrome P450 s and their potential interactions with P-glycoprotein. *In silico* simulations of absorption, distribution, metabolism and elimination (ADME) as well as toxicity can be used successfully to predict the disposition of certain secondary metabolites. The use of these innovations coupled with hyphenated techniques (e.g LC/MS/MS) may provide a reliable and comprehensive characterization of these herbal preparations and aid in the elucidation of their potential toxicity. Application of post-genomic techniques such as DNA microarrays may discern patterns of genomic changes that can be used as biomarkers to predict specific effects and provide an insight into the pharmacodynamics, pharmacogenomics and toxicogenomics of herbal medicines. The presentation intends to incorporate all the aforementioned modern technologies to better understanding of ADRs associated with herbal medicines in relation to disease state, geriatrics as well as pregnancy and lactation which remain major causes of morbidity and mortality.

Reference: [1] Mills, S. and Bone, K. (2005) The essential guide to herbal safety. Elsevier. Philadelphia USA.

L11 Phytopharmaceuticals for Dementia Therapy

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The rising 'epidemic' of diseases like Alzheimer's has not been met by effective symptomatic treatments or preventative strategies. Two current prescription drugs are acetylcholinesterase (AChE) inhibitors derived from plants: galantamine from *Galanthus and Narcissus* and rivastigmine based on the structure of physostigmine from *Physostigma venenosum* [1]. Clinical evidence relating to cognition for other plants includes extracts from the European sage (*Salvia officinalis*) and lemon balm (*Melissa officinalis*), for complex mixtures of traditional Chinese

medicines, *Ginkgo biloba*, and huperzine A from the moss *Huperzia serrata* [1,2]. Equally important, behavioural and psychological symptoms most often challenge carers and lead to institutionalization. Both *M. officinalis and Lavandula angustifolia* alleviate agitation and there is unexplored potential for other plants to alleviate symptoms, such as *Hypericum perforatum* for depression and *Valeriana officinalis* for anxiety and sleep disorders. These plant species for dementia therapy are examined from the perspectives of traditional medical uses, relevant bioactivities and active phytochemicals, and clinical controlled trial evidence. Recent research in the North England Universities Medicinal Plant Research Centre includes: identification of butyrylcholinesterase inhibitory activity in the *Narcissus* flowers [3], more potent 5HT1A receptor interactions for *M. officinalis* compared to *L. angustifolia* essential oils [4], GABA channel activity in both oils [5,6], significant improvements in memory in normal elderly volunteers tested after acute oral sage compared to placebo [7], a survey of the wide variety of plants and their compounds that inhibit AChE [1], and evidence that cholinesterase inhibitors have more than symptomatic effects by reducing amyloid in the human brain [8]. *Acknowledgements: The Alzheimer Society UK supported essential oil pharmacological studies.* **References:** [1] Houghton, P.J. et al. (2006) Nat. Prod. Rep. 23:181 – 199. [2] Howes, M.-J.R. et al. (2003) Phytother. Res. 17:1 – 18. [3] Tasker, A. et al. (2008) Neurosci. Lett. 442:297 – 299. [4] Elliott, M.S.J. et al. (2007) Int. J. Essent. Oil Ther.1:143 – 152. [5] Huang, L. et al. (2008) J. Pharm. Pharmacol. 60:1515 – 1522. [6] Abuhamdah, S. et al. (2008) J. Pharm. Pharmacol. 60:377 – 384. [7] Scholey, A.B. et al. (2008) Psychopharmacology 198:127 – 139. [8] Ballard, C.G. et al. (2007) Neurology 68:1726 – 1729.

L12 Mimopezil, A Huperzine A Derivative Undergoing Clinical Development For The Treatment Of Alzheimer's Disease

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Mimopezil is a novel cholinesterase inhibitor, non-enzymatically transformed after administration into the active compound Huperzine A. Huperzine A is a quinolizidine alkaloid with potent and selective acetylcholinesterase inhibition properties, originally isolated from a Chinese club moss (*Huperzia serrata* or *Lycopodium serrata*). The plant has been used for centuries in China to treat disorders such as memory loss, schizophrenia and hypertension. Huperzine A's pharmacokinetic profile implies a two to four times a day dosing schedule. In the search for huperzine A derivatives adapted to clinical use, mimopezil was selected among over 100 other compounds for its optimal profile in potency, selectivity, and progressive biotransformation into huperzine A. These characteristics allowed to target a once daily oral dosing schedule and to develop an injectable, monthly sustained-release formulation. Mimopezil is undergoing clinical development for the treatment of Alzheimer's Disease. The safety, tolerability, pharmacokinetics, pharmacodynamics and preliminary efficacy of oral mimopezil in humans have been assessed in 5 phase I studies. Efficacy, safety and tolerance of mimopezil administered orally (1.5 mg and 2 mg 1x/day) versus placebo and donepezil was assessed in a phase IIa study in patients with mild to moderate AD, with positive results on cognitive impairment. However, it is well-known that treatment by the oral route in AD patients is often associated with lack of compliance resulting in variable drug exposure and potentially reduced treatment effect. To overcome this drawback, a sustained-release formulation of mimopezil (implants for s.c. injection) was developed. In a phase I study, single or repeated administration of these implants to healthy volunteers at doses of 3 to 15 mg monthly resulted in a prolonged release of huperzine A for up to four weeks. The safety and efficacy of mimopezil monthly implants versus daily oral administration of donepezil is currently being tested in a phase IIb, randomised, double-blind, double-dummy, active controlled study. A total of 158 patients have been enrolled at 27 sites over 3 continents. Preliminary results of this study will be available and disclosed at the conference.

L13 Screening botanicals for new taste and flavor compounds

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The food industry is looking for magic bullets to enhance taste [1]. This presentation discusses an approach based on a low-throughput screening of natural products, selected on the basis of the botanical literature, from personal curiosity, or from field work. Human perception of food is relayed by specific sensors, such as the taste and trigeminal receptors, located in the mouth [2,3] or by the odor receptors located in the nasal cavity. In addition, enzymes and bacteria process food during mastication, thereby inducing flavor changes. Because sophisticated analytical equipment cannot detect a taste or an odor active compound, the analytical strategy is to taste or smell each fraction to track the taste or odor active molecule. Some examples will be discussed, such as the discovery of new molecules having a trigeminal effect in black cardamom, *Amomum tsao-ko* Crevost et Lemarié (Zingiberaceae), a commonly used spice in Sichuan cookery, which produces a pleasant, refreshing trigeminal effect in the mouth [4]. Olfaction and retro-aromatic effects are an important aspect of food acceptance. The role of micro-organisms in the bio-generation of odorant molecules in the mouth has been studied in dentistry, but not as an added benefit for food perception. The importance of cysteine-*S*-conjugates in vegetables, as flavour precursors in the mouth, will be demonstrated [5]. **References:** [1] Chandrashekar, J. et al. (2006) Nature 444:288 – 294. [2] Wenner, M. (2008). Sci. Am. 299:96 – 99. [3] Shepherd, G.M. (2006) Nature 444: 316 – 320. [4] Starkenmann, C. et al. F. (2007) J. Agric. Food Chem. 55:10902 – 10907. [5] Starkenmann, C. et al. (2008) J. Agric. Food Chem. 56:975 – 980.

L14 Nutraceutical Discovery – from plant extracts to functional food

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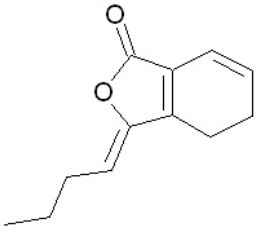
[1]DSM Nutritional Products Ltd., P.O. Box 2676, CH-4002 Basel, Switzerland; [2]Novartis Pharma AG, CH-4056 Basel, Switzerland

Nutrition may have a significant additional impact on health and opens the possibility for the development of defined nutritional products with desired health benefits. Nutraceuticals as food supplements should have a long-term effect in preventing a wide range of medical conditions, but many products lack efficacy and cannot deliver what they promise. DSM Nutritional Products follows a scientific and target-based discovery approach to find and develop new products in this field. We have acquired and created several plant extract libraries from different geographical locations and with a large diversity of plant types. Our approach in the discovery of new products is based on high-throughput screening (HTS) in microtiter plate format. A large number of extracts and natural products is tested in a sequence of assays (e.g. binding, enzyme or *in vitro*). The number of tests per assay is reduced at each level, while the quality and information is increasing. Active compounds are then evaluated for various aspects like toxicity, abundance in food chain plant, production costs, etc. Passing leads are further on tested for efficacy *in vivo* and finally candidates also for human evidence. Projects using screening activity are for example mental performance, metabolic health, and healthy aging/wellness.



Ligustilide from the TCM source (TCM = traditional chinese medicine) *Ligusticum chuanxiong* will be examined as an example.

Acknowledgements: We would like to thank B. Müller, K. Arnosti and B. Colletto for their valuable contributions in the discovery process.

Workshops

WS 1
Workshops for Young Researchers: Validation of Analytical Methods

Chair: Bilia AR[1]
Co-Chair: Rollinger JM[2], Wolfender JL[3]

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Guidelines and validation issues for method validation

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The demonstration of the ability of an analytical method to quantify is of great importance to ensure quality, safety and efficacy of pharmaceuticals. Consequently, before an analytical method can be implemented for routine use, it must first be validated to demonstrate that it is suitable for its intended purpose. The analyst refers to guidelines and regulatory documents, and therefore the validity of the analytical methods is dependent on the guidance, terminology and methodology, proposed in these documents. It is therefore of prime importance to have clear definitions of the different validation criteria used to assess this validity. The harmonization of validation of analytical procedures was developed by numerous members of the scientific community to understand the objectives of a procedure and to propose protocols that will include these criteria and these objectives. A comparative commentary of official documents regulating the validation of analytical methods (ICH, FDA, ISO, etc.) and understanding the basics in statistical requirements will help to consider some validation protocols and examples. This seminar will help you to understand the current definitions in method validation and to show the latest statistical tools which can help producing more reliable results in a faster and consistent manner.

WS 2
Workshops for Young Researchers: Cell Culture

Chair: Hensel A
Co-Chair: Tasdemir D[2], Efferth T[3]

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Common practice of (animal) cell culture: requirements, problems and answers

Deters A
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The frequency of prokaryotic and eukaryotic cell cultures used for biomedical and pharmaceutical research rises with accelerated progress and growing knowledge. Cell cultures permit the investigation of natural products regarding to their bioactivity, the (industrial) production of medicinal used products and is a real alternative to animal experiments. The cells themselves and their use as a test system create unique requirements on the scientists working with bacterial, animal or plant cells. Independent of the used cell type considerable results and reliable information will only be obtained if the tests are carried out with optimal growing cells and using an appropriate experimental set up. The work with cells is extremely complex and offers a lot of sources of errors and problems that need to be solved before the investigations. The lecture will give a survey of common requirements of animal cells in regard to their environment, the correlation between physical incubation parameters and the composition of media and buffers. In connection with these generalities the most frequent problems and their solution will be discussed. Some examples of experimental set ups will illustrate different designs of cell based studies with following fundamental steps: In general an experiment starts with the characterization of physico-chemical properties of the natural product giving a direction to the necessary pre-investigations. Major topics to consider for the achievement of significant results are appropriate controls and the most adequate methods for analysis. A suitable statistical evaluation completes the warranty to achieve reliable information concerning the bioactivity of natural compounds

WS 3
Permanent Committees on Manufacturing and Quality Control of Herbal Remedies and Regulatory Affairs of Herbal Medicinal Products

Chairs: Meier B[1] and Vlietinck AJ[2]

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Are European Pharmacopoeia (Ph. Eur.) monographs on Extracts a useful basis for the development of herbal medicinal products?

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The general monograph on Extracts, which classifies extracts into standardised, quantified and other extracts, and the majority of individual extract monographs detailed in the 6th Edition of the Ph. Eur were elaborated before the Herbal Medicinal Products Committee (HMPC) began publishing Community Monographs on medicinal plants and their extracts. However, the HMPC is now publishing an ever increasing number of Community Monographs, many of which are already the subject of Ph. Eur monographs. As the HMPC Community Monographs are referred to in the quality assessment for the licensing/registration of herbal medicinal products it is important that there are no discrepancies between these monographs and those of the Ph. Eur. However, problems may arise because Community Monographs are based on historical data whereas Ph. Eur monographs are being updated in response to current scientific knowledge and improved analytical methodology. The appointment of observers by both the HMPC and the Ph. Eur should help in understanding the rationale for the decisions taken by each organisation and lead to harmonisation in the key areas of nomenclature, production and assay methods. This understanding and co-operation should be strengthened by proposals to have meetings between the relevant groups from the two organisations. The aim of this workshop is to bring together representatives from the Ph. Eur Phytochemistry Groups of Experts, the HMPC Quality Working Group and the pharmaceutical industry to present their views as to both the usefulness and shortcomings of the Eur. Ph. monographs on extracts and to propose resolutions to some of the current problems. These presentations will be followed by a panel and audience discussion to further explore this important area of herbal medicinal product development.

WS 4
Permanent Committee on Biological and Pharmacological Activity of Natural Products: Phytoestrogens: risks and benefits for human health

Chair: Butterweck V

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Chemistry, exposure and biotransformation of phytoestrogens

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Phytoestrogens are polyphenolic non-steroidal plant compounds with estrogen-like biological activity. Based upon their chemical structure, the most common phytoestrogens can be classified into four main groups, i. e. isoflavonoids, flavonoids, stilbenes and lignans. Isoflavonoids can be subclassified into isoflavones and coumestans, whereas the flavonoids consist of flavones, flavanones and chalcones. Less common phytoestrogens belong to the terpenoids and saponins. For each group, the chemistry, dietary sources, exposure and biotransformation of the most interesting compounds will be discussed. Since most phytoestrogens are structurally quite similar to the estrogen 17β-estradiol, they may exhibit selective estrogen receptor modulation activities. Therefore, the structure-activity relationship of various isoflavonoids, prenylated flavonoids and stilbenes will be discussed in terms of hormonal as well as non-homonal biological effects.

Benefits: Biological effects of phytoestrogens following long term exposure

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Isoflavones (ISO) are bioactive food ingredients of the traditional East Asian diet and currently discussed as alternatives to classical hormone replacement therapies. The initial "health claim" towards menopausal applications of ISO containing products stems from epidemiologic observations as soy isoflavones seem to reduce the prevalence of hormone-dependent cancers, e. g. endometrial, breast or prostate cancer. These claims were further supported by the observation, that neonatal exposure to ISO can prevent DMBA induced mammary cancer in rats. Although there are numerous studies on ISO phytoestrogens, experimental animal data on their long-term effects eventually supporting or disapproving observations in epidemiological studies are scarce. Therefore we and others [1] performed dietary exposure studies in rats with ISO-free diets or diets specifically enrichted in ISO or pure genistein. Differently to others we started the exposure already prior to mating of the parental animals and it was maintained throughout the life of the offspring up to day 97. The dietary exposure to ISO improved bone strength, did not stimulate proliferative activities within the mammary gland, but dramatically affected estrogen responsiveness in the uterus. The first two observations clearly represent beneficial properties of life-long dietary exposure to ISO. How the finding on induction of increased estrogen responsiveness by ISO has to be interpreted within in the frame of risk/benefit debate of ISO is subject of further studies. In summary, chronic dietary exposure with soy ISO may have beneficial impact on mammary gland and bone health and clearly is different to results from acute therapeutic application studies to treat already existing symptoms. A possible link of these findings to modifications of the epigenome will be discussed and is also subject of further studies. In conclusion, these findings are of particular relevance consumers of Oriental diets or those supplementing their diet with ISOs and as a consequence potentially undergo a significant health impact. **Reference:** [1] Mardon, J. et al. (2008) Exp. Biol. Med. 233: 229 – 237.

Risks: Nutritional and toxicological considerations of phytoestrogens

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The term "phytoestrogens (PE)" defines a structurally diverse class of natural compounds that possess the ability to alter various components of the endocrine system (e. g. receptors, metabolizing enzymes, transporters) and potentially induce adverse health effects in exposed individuals and populations. Particularly nuclear receptors which include steroid receptors are known to bind many of such substances mimicking or antagonizing the effects of estrogens or androgens and this may have substantial impacts for female and male fertility. By the example of the isoflavone genistein (Gen) a risk assessment with focus on fertility and hormone dependent cancer is performed. Soy products contain significant amounts of Gen. Those products are used to improve health. i. e. they are consumed as drugs to ameliorate for example psycho-vegative climacteric complaints or even to prevent cancer. Another exposure to Gen results from the intake of processed soy products which often are supplemented with isoflavones. An increasing number of babies are fed with industrially-prepared formula milk or vegetable based puree, however the effects of PEs contained in these products on babies' health remain unclear. Data from animal experiments provide convincing evidence that a prepubertal exposure to PEs may not affect the onset of reproductive function but reduces fertility outcome and accelerates reproductive ageing. In contrast to these adverse effects of PEs prepubertal exposure followed by a life-long intake of PEs at concentrations, naturally present in vegetarian food, reduces the risk of steroid dependent cancer. Taken together, to perform a reliable assessment of the magnitude of any adverse effect of PEs on the health of individuals or populations improved experimental and epidemiological approaches are required to model demographic effects of PEs on fertility and the risk of cancer.

WS 5
Permanent Committee on Breeding and Cultivation of Medicinal Plants: Genetic Resources, Conservation and Breeding

Chair: Franz C

Genetic Resources, Conservation and Breeding

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The conservation of genetic resources of medicinal plants is an intrinsic factor for detection and further exploitation of new natural products for the benefit of humans and animals. All measures to maintain the biodiversity by avoiding over-collection or habitat destruction are therefore urgently needed. Based on the issues of the Convention on Biological Diversity (CBD), a European Conservation of Plant Genetic Resources Working Group in the field of Medicinal and Aromatic Plants (ECPGR-WG-MAP) was established in agreement with the Global Strategy for Plant Conservation (GSPC) in order to stop the drop in biodiversity and to ensure the continued availability of MAP genetic resources. The *in-situ* and *ex-situ* conservation measures of the respective species together with a harmonized documentation of botanical, chemotaxonomical and genetic data will be presented and discussed. In the second part of the Workshop the actual state-of-the-art in the use of genetic resources for breeding programmes will be presented exemplarily for *Artemisia sp., Hypericum perforatum, Salvia officinalis* and *Thymus vulgaris.* Yield, phytochemical characters, tolerance against biotic and abiotic stress and homogeneity of cultivars are the most important issues, and recommending procedures to optimize the quality of medicinal plants will complete the discussion.

Short lectures

SL1 Cystus052, a plant extract against seasonal and pandemic influenza virus

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Influenza still represents a major threat leading to zoonosis. The appearance of highly pathogenic avian influenza viruses of the H1N1 and H5N1 subtypes being able to infect humans reveals the urgent need for new and efficient countermeasures against this disease. Several antiviral compounds have been developed against influenza virus; their long-term efficacy is often limited, because of their toxicity or the emergence of drug-resistant virus mutants. Moreover, neuraminidase inhibitors the most common anti-influenza agents are less effective against new H5N1 isolates. In this regard, we were able to show that a polyphenol rich plant extract from a special variety of *Cistus incanus* named Cystus052 exhibits antiviral activity against influenza viruses *in vitro*, in a mouse model and a randomized, placebo controlled clinical study. The recovery from clinical symptoms was 2.5 days faster in the Cystus052 group compared to patients taken the placebo. The protective effect of Cystus052 appears to be mainly due to binding of the polymeric polyphenol components of the extract to the virus surface, thereby inhibiting binding of the hemagglutinin to cellular receptors. The antiviral potential of Cystus052 against seven H5N1 viruses by IC 50, EC 50, Km, Vmax and Ki values indicated that Cystus052 was much more potent than oseltamivir. In addition, using an *in vitro* infectivity inhibition assay we found that a single treatment of Cystus052 was up to 100-fold more effective against these H5N1 viruses compared to oseltamivir, during the first 24 hours after infection. We conclude that Cystus052 given prior to infection might be an effective antiviral with prophylactic potential against influenza viruses including A/H5N1.

SL2 Potent antiviral effect of a polyphenol-enriched *Rumex acetosa* L. extract against herpes simplex virus type 1 via interaction with viral envelope proteins

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Herpes Simplex virus type 1 (HSV-1) is the prototype member of the herpes virus subfamily Alphaherpesvirinae. It is extremely widespread and causes a broad range of diseases ranging from localized skin infections to serious infections of the central nervous system like encephalitis. After primary infection HSV-1 persists in sensory neurons, establishes latency and can be periodically reactivated. A polyphenol-enriched plant extract from *Rumex acetosa* L. (Polygonaceae), standardized on oligomeric and polymeric procyanidins, was tested for antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro* using plaque reduction assay and MTT assay. The extract inhibited HSV-1 replication in a dose-dependent manner. IC_{50} values, as determined by MTT assay on Vero cells was 0.4 µg/mL. Respective CC_{50} values were calculated with 45.2 µg/mL, indicating a CC_{50}/IC_{50} ratio of 113. The antiviral activity was confirmed by plaque reduction assay. To determine the mechanism of this antiviral effect, the extract was added at different stages during the viral replication cycle. The strongest antiviral activity in MTT-assay was observed when the extract was added before attachment of HSV-1 to Vero cells. With an adsorption assay it was clearly verified that the attachment of virus to the host cells was inhibited. Therefore the effect of the test extract on HSV-1 membrane proteins was investigated. In immunoblot experiments a significant interaction between the viral surface glycoprotein D (gD) and the compounds of the *R. acetosa* extract became apparent. Thus this extract is assessed as strong inhibitor of virus attachment to the host cell due to interaction of the procyanidins with viral envelope proteins.

SL3 Inhibition of P-glycoprotein at the blood brain barrier by phytochemicals derived from traditional Chinese medicine

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The blood brain barrier (BBB) controls the transport of xenobiotic compounds from blood into brain and maintains the brain's integrity towards harmful insults. A major functional constituent of BBB represents the efflux transporter, P-glycoprotein (P-gp) I capillary endothelium. P-gp is highly expressed at the luminal membrane of brain capillary endothelium cells. Hence, P-gp still represents a major obstacle to the effective treatment of common central nervous system diseases. One attractive concept in experimental neurology to overcome failure of drug treatment is to selectively modulate BBB function by P-gp inhibitors to facilitate drug penetration into the brain. To identify novel P-gp inhibitors, we applied the calcein assay in flow cytometry, spectrofluorometry, and confocal microscropy. The assays were done with P-gp-expressing CEM/ADR5000 and P-gp-negative parental CCRF-CEM cells. In parallel, brain capillaries were isolated from pigs and porcine brain capillary endothelial cells were cultured. Protein and mRNA expression profiles were determined by microarry analyses, real-time RT-PCR, and Western blot. We analyzed 70 phytochemicals, twelve of which strongly interacted with P-gp. Intracellular calcein fluorescence increased to > 500% of controls (fluorescence in absence of P-gp inhibitors), suggesting high affinity of these compounds to P-gp. In conclusion, identification of novel P-gp inhibitors from phytochemicals derived from TCM may have high impact on the development of strategies to modulate BBB function for therapy of brain diseases.

SL4 Protein kinase inhibitors from the endophytic fungus *Stemphylium globuliferum*

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The endophytic fungus *Stemphylium globuliferum* was isolated from stem tissues of the Moroccan medicinal plant *Mentha pulegium*. Extracts of the fungus, which was grown on solid rice medium, exhibited considerable cytotoxicity when tested *in vitro* against L5178Y cells. Chemical investigation yielded eight new secondary metabolites, alterporriol F, alterporriol G and its atropisomer H, alterporriol I and its atropisomer J, altersolanol K, altersolanol L and stemphypyrone, beside eight known compounds. The structures were determined on the basis of one- and two-dimensional NMR spectroscopy and mass spectrometry. Among the alterporriol-type anthranoid dimers, the mixture of alterporriols G and H exhibited considerable cytotoxicity against L5178Y cells with an EC_{50} value of 2.7 µg/mL, whereas the other congeners showed only modest activity. The compounds were also tested for protein kinase inhibitory activity in an assay involving 24 different kinases. Compounds methylalaternin, macrosporin, altersolanol A and the mixture of alterporriol G and H were the most potent and also selective inhibitors, displaying EC_{50} values between 0.64 and 1.4 µg/mL toward individual kinases.

SL5 Differential cytotoxic and prooxidnant activity of knipholone and knipholone anthrone

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Knipholone (KP) and knipholone anthrone (KA) are natural 4-phenylanthraquinone structural analogues with established differential biological effects including *in vitro* antioxidant [1] and antimicrobial properties [2]. The present study was designed to investigate the comparative *in vitro* cytotoxic activity and the possible mechanism of action of these two compounds. We demonstrated that KA is by order of magnitude more cytotoxic to mammalian cells than KP. In parallel with the demonstrated cytotoxic effect, KA but not KP induces prooxidative DNA damage in the presence of copper ions. In order to establish the possible involvement of reactive oxygen species in the KA-mediated prooxidative effect, we investigated the protective effect of several metal chelators and reactive oxygen species scavengers. Our data suggest that reactive oxygen species such as hydrogen peroxide are involved and a good correlation between prooxidative action, antioxidant effect and cytotoxicity is established for these two structural analogues. The chemistry, pharmacology and potential medicinal/toxicological potential of these compounds are discussed. *Acknowledgements: This research was supported by the HEFCE capability funding.* **References:** [1] Habtemariam, S. (2007) J. Food Chem. 102:1042 – 1047. [2] Bringmann, G. et al. (2008) Nat. Prod. Rep. 25:696 – 718.

SL6 Rapid and efficient purification of hypericin and pseudohypericin and inhibition of thioredoxin reductase

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Hypericins have many biological and pharmacological activities and in particular reported as potent anticancer molecules [1]. These molecules are widely distributed in plant kingdom but their availability is limited due to the low content in the plants. A rapid and efficient isolation of hypericin and pseudohypericin from *H. perforatum* hydro-alcoholic dried extracts has been developed. Briefly, the method consists of a partition of the extract between organic and aqueous layers, further purification with Sephadex LH-20 column chromatography and a final separation of constituents using Sephadex LH-60 column chromatography. The three-step fractionation resulted in 98% content in total naphthodianthrones. To the best of our knowledge this is the first report on the separation of hypericin from pseudohypericin using size exclusion chromatography [2]. Pure hypericin and pseudohypericin were tested on both cytosolic (TrxR1) and mitochondrial thioredoxin reductases (TrxR2). Hypericin and, particularly, pseudohypericin acted as strong inhibitors of thioredoxin reductases, both in the dark and in ambient light. Further, pseudohypericin, similarly to hypericin, also inhibits glutathione reductase. Pseudohypericin IC_{50} values for TrxR1 and TrxR2 were 4.6 and 7.9 μM, respectively. Hypericin appeared to be more effective on mitochondrial thioredoxin reductase reaching an IC_{50} value of about 50 μM, a value that is noticeably lower than that measured for TrxR1 (198 μM). The inhibition profile was similar to that produced by hypericins on glutathione reductase. As the thioredoxin system is highly overexpressed in cancer cells, its inhibition by hypericins, natural compounds that are known to display appreciable anticancer properties, can offer new clues on their mechanism of action and may open interesting perspectives for future tumor therapy. **References:** [1] Kubin, A. et al. (2005) Curr. Pharm. Design 11:233 – 253. [2] Karioti, A. et al. (2009) J. Sep. Sci. *in press*.

SL7 Molecular chaperons mediated pathways of stress protective and anti-aging effects of adaptogens

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The aim of our studies on plant adaptogens, e.g. ADAPT-232 forte – a fixed combination of standardized extracts of *Eleutherococcus senticocus* (Rupr. et Maxim) Harms root, *Schisandra chinensis* (Turzc) Baill., root, *Rhodiola rosea* L., root, was *to give a rationale* to their antifatigue, stress-protective and life-span enhancing effects on the molecular level. In several studies on animals we demonstrated that these effects are associated with the regulation of key mediators of stress response, such as molecular chaperons (e.g. Hsp70) [1], stress-activated c-Jun N-terminal protein kinase (JNK1) [2], Forkhead box O (FoxO) transcription factor DAF-16 [3], cortisol [2,4] and nitric oxide (NO) [2]. Adaptogens prevent stress-induced NO increase [2], which is known to inhibit the production of cellular energy (ATP) through two mechanisms: inhibition of mitochondrial respiration by inhibition of cytochrome P450 and the inhibition of glycolysis [5]. That results in increased performance and endurance [1]. The key point of the mechanism of action of ADAPT-232 is up-regulation, of a "stress sensor" protein Hsp70 [1], which plays an important role in cell survival and apoptosis. Hsp70 inhibits the NOS II gene expression and interacts with glucocorticoid receptors directly and via the JNK pathway affecting thereof the circulating cortisol and NO. Adaptogen-induced up-regulation of Hsp70 triggers stress-induced JNK-1 and DAF-16-mediated pathways of regulation the resistance to stress, resulting in enhanced mental and physical performance and increased of longevity [6]. **References:** [1] Panossian, A. et al. (2009) Phytomedicine, DOI: 10.1016/j.phymed.2008.12.003. [2] Panossian, A. et al. (2007) Drug Target Insights 1:39 – 54. [3] Wiegant, F.A.C.et al. (2009) Biogerontology 10:27 – 42. [4] Olsson, E.M.G. et al. (2009) Planta Med. 75:105 – 112. [5] Brown, G.C. (2001) Biochim. Biophys. Acta 1504:46 – 57. [6] Panossian, A. and Wikman, G. (2009) Curr. Clin. Pharmacol. *In press*.

SL8 Evaluation of the Antioxidant and Anti-Diabetic effects of Baicalin in Type 2 Diabetic Goto Kakizaki (GK) rats

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Oxidative stress, claimed to be triggered directly by hyperglycemia, is increased in Type 2 diabetes and is the root cause of many diabetic complications. Numerous studies have shown that current treatments with Western drugs do not eradicate the probability of developing complications. In this study, Goto-Kakizaki (GK) type 2 diabetic rat models were used to look into the effect of combining the anti-diabetic drug, metformin, with baicalin, a compound from *Scutellaria baicalensis*, which is recognized for its radical scavenging ability. Three groups of GK rats were given the following treatments orally for 30 days: (1) Metformin 500 mg/kg, (2) Baicalin 120 mg/kg, (3) Metformin 500 mg/kg + Baicalin 120 mg/kg. Vehicle-treated diabetic controls were also used to obtain data for comparison. The rats in both baicalin and combined treatment groups had elevated superoxide dismutase (SOD) and catalase (CAT) antioxidant enzyme activities compared to the diabetic control ($p < 0.05$). In addition, combined treatment caused noteworthy increase in pancreatic insulin levels and declines in plasma triglycerides (TG) and cholesterol (TC) levels as well as glucose concentrations. The study thus demonstrated the potential ability of baicalin to enhance the antidiabetic effect of metformin as well as reduce oxidative stress when used alone or in combination with metformin.

SL9 Preferred, Novel and Neglected Scaffolds: Natural Products in a Drug Discovery Program

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Over the past few years, Sequoia Sciences has identified preferred, neglected, and novel drug-like scaffolds from its extensively purified li-

brary of plant compounds. Some of these scaffolds have more chiral centers and non-aromatic rings than synthetically inspired compounds. Since the majority of known scaffolds have been neglected in drug discovery [1] can neglected and novel plant scaffolds inspire the drugs of 2020? Certain representative scaffolds will be presented and compared to known drugs and published synthetic scaffolds that have undergone lead optimization. Payne [2] stated "Right now, there are no novel mechanism-of-action antibacterials in Phase I, nor are there even good preclinical leads with promising Gram-negative activity." Is this an accurate assessment of antibacterial discovery programs foretelling more doom in the hospitals? If so, can antibacterial therapeutics that improve the effectiveness of existing antibiotics satisfy demand until 2030? Sequoia will present data demonstrating a plant-inspired compound that increases the effectiveness of gram-negative antibiotics. The story starts from a plant and evolves to a semisynthetically produced novel scaffold that inhibits the expansion of gram-negative biofilms and potentiates the activities of antibiotics. **References:** [1] Lipkus, A.H. et al. (2008) J. Org. Chem. 73:4443 – 4451. [2] Payne, D.J. et al. (2007) in Nat. Rev. Drug Discov. 6:29 – 40.

SL10 New antiplasmodial natural products from cyanobacteria: linking their ecological role to their therapeutic potential

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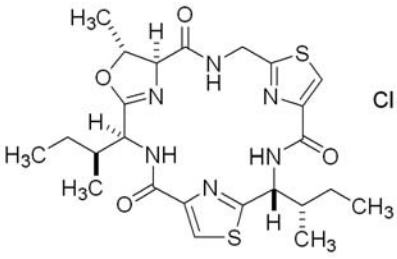
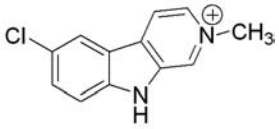
Cyanobacteria (blue-green algae) produce many metabolites that are directed towards competing photoautotrophs. Such algicidal compounds might offer new approaches for the selective inhibition of the malaria parasite, *Plasmodium falciparum*, as this organism contains an organelle (apicoplast) of algal origin [1]. In this communication, we report the identification of two classes of cyanobacterial secondary metabolites with antiplasmodial activity.



Aerucyclamide B



Nostocarboline

Aerucyclamides A-D [2] are heterocyclic peptides that are ribosomally produced [3] by *Microcystis aeruginosa* PCC7806. Nostocarboline is a chlorinated N-methylated carbolinium alkaloid from *Nostoc* 78 – 12A [4]. Both compounds display submicromolar IC_{50} values against *Plasmodium falciparum*, with a pronounced selectivity towards rat myoblasts. Their respective potential ecological roles and therapeutic potentials will be discussed. **References:** [1] a) Burja, A.M. et al. (2001) Tetrahedron 57:9347 – 9377. b) Gademann, K. et al. (2008) Curr. Org. Chem. 12:326 – 341. [2] a) Portmann, C. et al. (2008) J. Nat. Prod. 71:1193 – 1196. Portmann, C. et al. (2008) J. Nat. Prod. 71:1891 – 1896. [3] Ziemert, N. et al. (2008) Appl. Environ. Microbiol. 74:1791 – 1797. [4] a) Becher, P.G. et al. (2005) J. Nat. Prod. 68:1793 – 1795. b) Barbaras, D. et al. (2008) Biorg. Med. Chem. Lett. 18:4413 – 4415. c) Portmann, C. et al. (2009) ChemBioChem 10:889 – 895.

SL11 The characterization, total synthesis and antiprotozoal activities of novel bichalcones from *Rhus pyroides*

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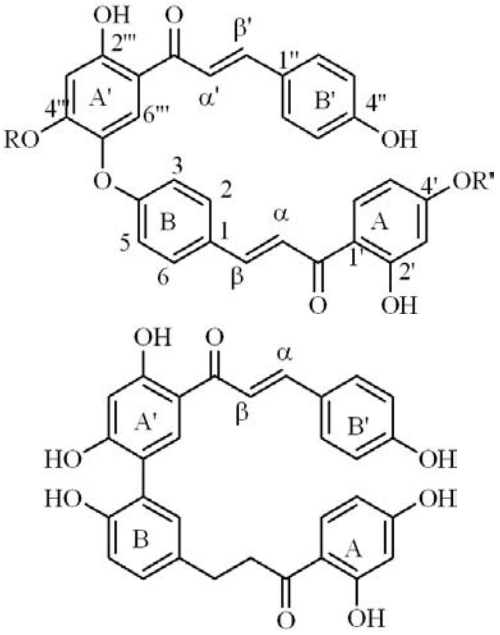
Rhus pyroides is a tree which is resistant to attack by common pests including the corn cricket. Early investigations were directed to determining if anti-feeding metabolites were present in the plant [1]. As part of our effort to look for anti-protozoal substances we obtained from the leaves of the same plant novel *O*-linked chalcone dimer (**1**) and the previously unknown C-C coupled dimer (**2**) [2]. We also reported on the synthesis of the *O*-linked dimers (achieved through application of the Ullmann coupling of appropriately substituted chalcone moieties), and their antiproliferative properties. We have now concluded an investigation that culminated in the total synthesis of the C-C linked dimers from simple and available resorcinol and 4-hydroxybenzaldehyde and have also determined their antiprotozoal and cytotoxic properties. Key steps included the solvent-free syntheses of chalcones, and the first application of the Suzuki-Miyaura coupling reaction in the synthesis of bichalcones. The present work constitutes a general method for the rapid syntheses of a number of rhuschalcone VI related bichalcones. The synthesis and biological properties will be presented.



References: [1] Masesane, I.B. et al. (1999) Phytochemistry 53:1005 – 1008. [2] Mdee, L.K. et al. (2003) J. Nat. Prod. 66:599 – 604.

SL12 Mozambique Centre of Research and Development on Ethnobotany

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In this communication we present the Center of Research and Development in Ethnobotany, created by the Scientific Council on Ethnobotany (COCIE) of the Ministry of the Science and Technology of Mozambique. This center, located at Namaacha and inaugurated in June of 2009, is responsible for the coordination of the research activities, interconnection with other institutions, definition of research priorities and promotion of small companies formation on the basis of native plants. Of its

plan of share already in course they are to enhance: Make a list of the most useful medicinal and feeding plants used in the different regions of the country; Accomplishment of taxonomic studies and study of the *in vitro* propagation methods for preservation and conservation of species at risk; Accomplishment of chemical, pharmacological and toxicological studies aiming at to prove the traditional uses of the plant-target and to guarantee its quality, security and effectiveness; Creation of a nutritional table with data regarding the Mozambique native feed plants; Creation of a garden including medicinal and alimentary plants and promotion of its use and conservation next to the local communities. Expected outcomes includes the basic formation of students concerning exploitation and conservation of the medicinal, culinary and aromatic plants; development of plant products and the consequent transfer of technology to small companies; technician formation specialists (BSc, MSc and PhD degrees) in taxonomy, vegetal biology, ethnopharmacology and biodiversity conservation of Mozambican plants. Research team is constituted by researchers from the above-mentioned institutions.

SL13 The African Herbal Pharmacopeia – Challenge and Potential

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The African continent with an estimated 216 634 000 ha of closed forest area encloses some 40 – 45.000 higher plant species that present enormous industrial potential. Africa contributes 25% of the global pool of plant genetic resources currently being traded. While over 5.000 plants are known to be used medicinally, few have been described and studied. This groß under-utilisation is further challenged with massive loss of biodiversity. The African continent is known to have the highest rate of deforestation in the world (1% loss compared to the global rate of 0.6%). In spite of these challenges, Africa has contributed to the world's leading commercial medicinal plants, albeit on the low side (83 out of the 1100). Among them are the following: Madagascan Periwinkle (*Catharanthus roseus*), Devil's Claw (*Harpagophytum procumbens*), Rauwolfia (*Rauwolfia vomitoria*) amongst others. With so much potential and diversity, why is African 'absent' on the international scene. It is becoming increasingly clear that the potential for the business and agricultural sectors is enormous unless African countries prepare internationally recognised medicinal plant standards. The absence of the latter is a major barrier to regional and international trade. It could also explain why African Herbal Medicine has not been mainstreamed in the countries health system. The preparation and publication of the African Herbal Pharmacopeia by the Association of African Medicinal Plant Standards (AAMPS; http://www.aamps.org) proposes to help address some of these lacunae. Over 50 important medicinal African plants have been described and all the relevant data has been incorporated to promote the cultivation and trade of these important medicinal plants. The present paper will elaborate on the challenges in documenting and studying African medicinal plants and the potential they represent for the continent in terms of trade and business.

SL14 Multi-disciplinary approach of *Tahitian vanilla* biodiversity assessment

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Three vanilla species are cultivated in the world: *V. planifolia*, *V. pompona* and *V. tahitensis*. Tahitian vanilla is characterized by anise notes [1] and oily texture. Its diversification seems to have occurred in French Polynesia since its introduction and despite its vegetative mode of propagation [2]. Vanilla growers distinguish about twenty cultivars according to their morphological traits but in local farms, two cultivars are mainly produced: "Tahiti" and "Hapape". More than two hundred plants have been collected in the Polynesian islands and grown in a preservation shade house. Tahitian vanilla biodiversity is investigated using morphological, genetic, aromatic and lipidic traits. To assess genetic diversity, fingerprints (AFLP) and chromosome counts were realized. The chemical composition of the pods was investigated by HPLC analysis of aromatic compounds and fatty acids. Many morphological traits were also measured. We report here the results of the diversity characterization of five cultivars which were chosen for their large variation in morphological traits. The five cultivars show differences in their fingerprints and/or in their diploidy level. Moreover, they are well discriminated by their aroma and fatty acid compositions. The two most distinct cultivars, according to their genetic pattern, also present the most divergent chemical compositions. Such a combined analysis may provide useful information for breeding programs by allowing i) the selection of the best cultivars according to their aromatic and fatty acid composition, ii) the identification of genes related to the flavor and fatty acid biosynthesis for the selection of the best hybrids. **References:** [1] Da Costa, C. et al (2006) Dev. Food Sci. 43:161 – 164. [2] Duval, M.F. et al. (2006) Les Actes du BRG 6:181 – 196.

SL15 Quality control of herbal medicines in Japan

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Herbal medicines in Japan are mostly used for the Kampo medicine, (the medicine traditionally practiced in Japan, based on ancient Chinese medicine). The Kampo medicine was established by the 18th century in Japan. A Kampo prescription traditionally is administered in many forms, but mainly in the form of decoction of five to ten different crude drugs (herbs). Nowadays, Kampo medicines are mainly distributed in the shape of ready-made granules, powders or tablets, containing a spray-dried decoction of crude drugs. The quality of the crude drugs is controlled by the Japanese Pharmacopoeia (JP) and the Japanese Herbal Medicine Codex (JHMC, non-JP crude drug standard). The 15th edition of JP (JP15) with the supplement 1 contains 153 crude drugs and 54 powdered ones. The JHMC covers additional 39 crude drugs and 2 powdered one. For each crude drug, the origin, physical properties and criteria for identification are rigorously specified by respective testing method. In addition, purity test and chemical assay are required. The quality of the ready-made Kampo products for ethical uses is controlled by the regulations for manufacturing control and quality control of ethical extract products in Kampo medicine formulations (the Kampo extract preparation GMP). The quality of the proprietary ones is also controlled by the corresponding regulations. These regulations are self-imposed ones of Japan Kampo-Medicine Manufactures Associations (JKMA: http://www.nikkankyoorg/frame. html). In the GMP, each crude drug must satisfy the criteria of JP or JHMC. Also, it is required to obtain data of TLC profile to identify individual crude drugs. In addition, quantitative HPLC analyses of the main constituents of at least two crude drugs are needed as indicator ingredients. The requirement of each Kampo extract preparation on TLC profile and HPLC analysis is written in JP or the corresponding letter of the approval. JP15 with the supplement 1 contains 8 monographs of Kampo extracts. It is planned that the monographs of the top 20 Kampo extracts at least appear in the JP16. In Japan, 148 Kampo formulae are approved for ethical use and the total sales of the top 20 extract products account for about 67%, in the whole sales of ethical Kampo products.

SL16 Falcarinol (Panaxynol) is a CB_1 cannabinoid receptor antagonist and induces pro-allergic effects in skin

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In our ongoing search for new cannabinoid receptor (CB) ligands we isolated falcarinol (panaxynol) from the endemic Sardinian plant species *Seseli praecox* Gramisans. This polyyne is also found in different food plants, such as carrots, parsley, celery, and in the medicinal plant *Panax ginseng* C.A. Meyer. We show that falcarinol exhibits non-selective binding affinity to human CB receptors ($hCB_1 = 3.78 \pm 0.23\,\mu M$; $hCB_2 = 2.36 \pm 0.4\,\mu M$) whereas its natural derivative falcarindiol does not bind. Since purified falcarinol was highly unstable under all conditions tested we repeatedly isolated this compound for biological characterization. Major breakdown products were identified and one new polyyne was isolated. Based on experiments measuring intracellular

calcium and cAMP using CB_1/CB_2 transfected cell lines and selective antagonists, falcarinol is a weak partial CB_2 agonist but a more significant CB_1 inverse agonist. In CB receptor expressing human HaCaT keratinocytes falcarinol (5 – 20 μM) but not falcarindiol increased the expression of the pro-allergic chemokines CCL2/MCP-1 and IL-8 and blocked the inhibition of TNF-alpha/tolerogen-stimulated CCL2/MCP-1 and IL-8 expression exerted by the endocannabinoid anandamide. Intriguingly, falcarinol strongly aggravated histamine-induced allergic reactions in skin prick tests performed on humans. Given the known contact allergic potential of topical falcarinol and the known anti-allergic effects mediated by anandamide and Δ9-tetrahydrocannabinol (THC) in the skin, falcarinol-associated dermatitis may be directly related to its blockage of the CB_1 receptor and increased IL-8 and CCL2/MCP-1 expression. Overall, falcarinol may facilitate sensitization to other allergens rather than being an allergen itself.

SL17 Antiadhesive natural products for a new cytoprotective strategy against the early stages of infection by pathogens

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Because of immense resistance problems the effective antibiotic, virucidic and parasitic therapy will emerge as a major goal within the near future. Antiadhesive compounds (entry blockers) reveal a new class of cytoprotective agents, interacting with outer surface structures from pathogens, responsible for the recognition of host cells and the initiation of adhesion. Inhibition of the adhesion will result in a strongly diminished infection rate. Because many bacterial adhesins are interacting with host cells via carbohydrate-protein interaction rhamnogalacturonans from *Glycyrrhiza glabra* and *Abelmoschus esculentus* were shown to inhibit strongly the *in situ* adhesion of *Helicobacter pylori* and *Campylobacter jejuni* against intact intestinal tissue. Structure-activity relations indicated highly acidic polymers with a high degree of glucuronic acid to be most active. *In vivo* infection studies in *C. jejuni* infected broilers showed, that the oral use of such polysaccharides is inactive due to intestinal metabolism of the polysaccharides. Using low-molecular compounds, acidic N-Phenylpropenoyl-amino acid amides were shown to be highly effective against the adhesion of *H. pylori*, leading to a new class of antiadhesives with good intestinal adsorption and better pharmacokinetic potential. Beside these compounds, which are interacting directly with the adhesins, tannin-like polyphenols were shown to change the protein structure of viral adhesins, resulting in an reduced adhesion of herpes simplex virus (HSV-1). The interaction of defined oligomeric procyanidins from *Rumex acetosa* L. with outer surface adhesins of the virus and with the gingipain-adhesins from *P. gingivalis* was shown. Gen expression analysis of *P. gingivalis*, treated with defined oligomeric procyanidins clearly indicated that the polyphenols not only blocked the adhesion, but also the signal transduction for activation of virulence factors. Summarizing, the antiadhesive potential of this new concept is reviewed and critically assessed.

SL18 Nasal formulations of a polyphenols-enriched extract of Herba Cisti

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The aqueous extract ECce20 of *Cistus creticus* ssp. *eriocephalus*, herba (CCE, previously studied as *Cistus incanus*) is rich in polyphenols, including flaven-3-ols and proanthocyanidins [1]. The reported antiviral and anti-inflammatory activity of some extracts [2,3] has led us to formulate and characterise a nasal formulation for local delivery using a *CCE* hot water extract [4], supplied by the extract company Finzelberg. Following determination of the extract's solubility in ethanol/water solvent mixes, optimised formulations containing 1, 10, 50 and 100 mg/ml powdered extract and benzalkonium chloride (0.01% w/v as preservative) in ethanol/water (5:95) were developed. Solutions were delivered as an aerosol from metered dose nasal sprays (Valois VP3). Aerosols were sized by laser diffraction; the weight of each actuation was determined by difference. Solubility and delivery of *Cistus* extract was determined by absorbance at 368 nm using UV Spectrophotometer. The mean size of all aerosols was in the range 40 – 41 μm, with a weight, per actuation, of 101.5 mg. These are functions of the 100 μl metered dose volume. The mean delivered dose per actuation of *CCE*-extract over the first 55 actuations of the product was 0.088 ± 0.062 mg (1 mg/ml), 1.086 ± 0.011 mg (10 mg/ml), 5.494 ± 0.175 mg (50 mg/ml) and 11.296 ± 0.090 mg (100 mg/ml). The developed, preserved formulation has an aerosol droplet size appropriate for nasal delivery, delivers a consistent uniformity of dose (by weight) and delivers between 88 and 113% of the nominal dose of *CCE*-extract per actuation for the four concentrations of extract examined. **References:** [1] Danne, A. et al. (1993) Phytochemistry 34:1129 – 1133. [2] Droebner, K. et al. (2007) Antivir. Res. 76:38 – 47. [3] Taila, S. et al. (2008) J. Pharm. Pharmacol. 60: 62 – 63. [4] Obolsky, D. (2008) Phytochemical and in vitro NF-kappa B inhibitory/Antioxidant Profiling of *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter & Burdet; MSc dissertation, The School of Pharmacy, University of London.

SL19 Research into chemistry and biological activities of *Prangos* Lindl. (Apiaceae) species of Turkey

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Prangos Lindl. (Apiaceae) is represented in the world by 40 species and altogether 43 taxa. 17 taxa belonging to 16 species are recorded in Turkey. Herbal parts of these species called caksir are used as fodder and the roots are used as aphrodisiac like members of some other related genera such as *Ferula* L., *Ferulago* W. Koch and *Peucedanum* L. We have analyzed essential oils of different parts of the following species: *Prangos bornmuelleri* Hub.-Mor. et Reese (New name: *Ekimia bornmuelleri* (Hub.-Mor. et Reese) H. Duman et M.F. Watson, P. *ferulacea* (L.) Lindl., *P. turcica* A. Duran, M. Sagiroglu et H. Duman, *P. pabularia* Lindl., *P. platychlaena* Boiss. et Tchihat., *P. ilanae* Pimenov, Akalin et Kljuykov, *P. uechtritzii* Boiss. et Hausskn., *P. heyniae* H. Duman et M.F. Watson, *P. denticulata* Fisch. et Mey., *P. baseri* A. Duran et M. Ozturk. A new acetylenic derivative was isolated from the fruit oil of P. *platychlaena* subsp. *platychlaena*. Essential oil compositions of all the other oils are presented in a cumulative manner as analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Some of the species were subjected to tests for antibacterial, antifungal, antiprotozoal and insecticidal (anti-mosquito) activities.

SL20 The Kava Anxiety Depression Spectrum Study (KADSS): A randomized, placebo-controlled, cross-over trial using an aqueous extract of *Piper methysticum*

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Rationale: *Piper methysticum* (Kava) has been withdrawn in European, British, and Canadian markets due to concerns over hepatotoxic reactions. The WHO recently recommended research into 'aqueous' extracts of Kava. Objective: To conduct the first documented human clinical trial assessing the anxiolytic and antidepressant efficacy of an aqueous extract of Kava. Design and Participants: The Kava Anxiety Depression Spectrum Study (KADSS) was a 3-week placebo-controlled, double-blind, crossover trial that recruited 60 adult participants with one month or more of elevated generalized anxiety. Five Kava tablets per day were prescribed containing 250 mg of kavalactones per day. **Results:** The aqueous extract of Kava reduced participants' Hamilton Anxiety

Scale (HAMA) score in the first controlled phase by -9.9 (CI: 7.1, 12.7) vs. -0.8 (CI: -2.7, 4.3) for placebo, and in the second controlled phase by -10.3 (CI: 5.8, 14.7) vs. +3.3 (CI: -6.8, 0.2). The pooled effect of Kava vs. placebo across phases was highly significant ($p < 0.0001$), with a substantial effect size ($d = 2.24$, $\eta p^2 = .428$). Pooled analyses also revealed highly significant relative reductions in Beck Anxiety Inventory and Montgomery-Asberg Depression Rating Scale scores. The aqueous extract was found to be safe, with no serious adverse effects, and no clinical hepatotoxicity. **Conclusions:** The aqueous Kava preparation produced significant anxiolytic and antidepressant activity, and raised no safety concerns at the dose and duration studied. Kava appears equally effective in cases where anxiety is accompanied by depression. This should encourage further study and consideration of globally re-introducing aqueous rootstock extracts of Kava for the management of anxiety.

SL21 Identifying $GABA_A$ receptor ligands in black pepper by activity profiling, LC-TOFMS, and offline microprobe NMR

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The pharmacological treatment of insomnia, epilepsy and panic disorders today includes the use of classical benzodiazepines and nonbenzodiazepines. However, therapy is accompanied by well-known side-effects resulting from insufficient target selectivity for different $GABA_A$ receptor subtypes. Highly selective $GABA_A$ receptor ligands are therefore an unmet medical need [1]. Rational lead discovery approaches are not possible due to the lack of a high-resolution structure of this pentameric transmembrane receptor [2]. We combine extract library screening with HPLC based activity profiling of extracts as an effective avenue to new natural product leads [3]. In a program for discovery of new scaffolds for $GABA_A$ receptor agonists, a library of 880 extracts was screened in a functional assay in *Xenopus laevis* oocytes transiently expressing recombinant $GABA_A$ receptors of defined subunit composition ($\alpha_1\beta_2\gamma_{2S}$). An ethyl acetate extract of black pepper (*Piper nigrum* L., Piperaceae) fruits significantly potentiated GABA induced chloride current. A combination of HPLC based activity profiling, LC-PDA-TOFMS and offline microprobe NMR analysis allowed rapid identification of the active compounds with mg-amounts of extract. The major active compound was identified as piperine. A set of 12 structurally related, weakly active or inactive amides was also characterized. Structural features critical for $GABA_A$ agonistic activity of the piperine scaffold could be identified by the combination of structural and pharmacological data of this compound series. **References:** [1] Whiting, P.J. (2006) Curr. Opin. Pharmacol. 6:24 – 29. [2] Barrera, N.P., Edwardson, J.M. (2008) Trends Neurosci. 31:569 – 576. [3] Hamburger, M., Potterat, O. (2006) Curr. Org. Chem. 10:899 – 920.

SL22 Chemical investigations of Carallumas of India

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Caralluma (Asclepiadaceae) is a genus comprising of around 350 species. They occur mainly in India, the Middle East and Africa. They are succulent herbs and are almost leafless. *C. edulis* and *C. tuberculata* are reported to be sold as vegetable in markets in India and Pakistan. They are eaten raw or pickled. Some of them are considered to be famine food for the tribals [1,2]. An extract of *C. fimbriata* is being sold in the west (mainly US) under the trade names "slimalluma", "synex diet", "slimarex "etc. They are being claimed as appetite suppressants and wait loss promoters. Some of the Carallumas are accredited with medicinal properties. *C. stalagmifera* is claimed to be useful in migraine [3], *C. edulis* in leprosy, diseases of blood and diabetes [4] and *C. negevensis* in lung diseases and cancer [5)]. Chemical investigations of Carallumas of India have yielded a number of new steroidal glycosides over the years. Flavonoidal glycosides are the other class of compounds reported from Carallumas. An account of steroidal glycosides isolated till date from different Carallumas will be given. Conclusion: 1) Carallumas are rich source of steroidal glycosides, mostly of the pregnane type. 2) Despite a close morphological resemblance, different species or varieties of *Caralluma* elaborate different metabolites. 3) Steroidal glycosides of Carallumas are structurally related to those of *Hoodia gordonii*. 4) Luteolin-4'-neohesperidoside is a common constituent of all the Carallumas investigated. **References:** [1] Ram Gandhi, (1999) Carallumas of the Indian sub-continent. The Indian society of Cactii and succulents, New Delhi. [2] Ahmad, V.U. et al. (1988)J. Nat. Prod. 51:1092 – 1097. [3] Sreenivasacharyulu, M. (1939) "Yogaratnakaram", Vol. 2, Swatantra Press, Nellore, pp. 678 (Telugu). [4] Chopra, R.N. et al. (1956) Glossary of Indian Medicinal Plants. CSIR, New Delhi, 51. [5] Braca, A. et al. (2002) Tetrahedron 58:5837 – 5842.

SL23 Non-invasive infrared spectroscopic techniques for the characterization of medicinal plants and their constituents

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Near- and mid-infrared spectroscopy (NIRS; 10.000 – 4.000 cm^{-1}; MIRS; 4.000 – 400 cm^{-1}) are non-invasive spectroscopic tools enabling a fast qualitative and quantitative characterization of medicinal plants and their constituents down to the ppm-level. Treatment of spectra recorded with chemometrical and multivariate approaches allows determining chemical (e.g. secondary plant metabolites, leading compounds) and physical parameters (e.g. water, alcohol content) simultaneously by one single measurement lasting only a few seconds. Liquid plant extracts are investigated in the transflection mode at thermostated conditions using light-fibre optics, dried parts of plant (flowers, leaves, roots) also in the reflection mode using a sample desk. For the quantitative analysis of secondary metabolites including 3',4',5'-trimethoxyflavone in *Flos Primulae veris*, hypericin and hyperforin in *St. John's Wort*, etheric oils in *Achillea* species, a reference method based on liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE) is applied. Qualitative cluster analysis not only allows identifying different parts of a plant but also enables to distinguish different species, which is essential also in traditional Chinese medicine (TCM). For the investigation of active ingredients distribution within a plant part imaging spectroscopy with a resolution down to 5 µm combined with hierarchical cluster analysis (HCA) offers an immense potential as a novel screening tool. In the present contribution the main advantages of the novel quality control IRS tool in medicinal plant analysis are pointed out and discussed in detail by several applications.

SL24 Polyphenolic fingerprint of methanolic extracts of *Mentha* sp. cultivated in Slovakia

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Mentha L. species (Lamiaceae) represent a large source of natural antioxidants, mainly of phenolic origin [1]. HPLC-DAD and LC/MS/MS were used for a qualitative examination of polar flavonoids and phenolcarboxylic acids in methanolic extracts of leaves of different *Mentha* sp. Flavonoid-*O*-glycosides eriocitrine (**1**), luteolin-7-*O*-glucuronide (**2**), luteolin-7-*O*-rutinoside (**3**), luteolin-7-*O*-glucoside (**4**), naringenin-7-*O*-rutinoside (**5**), apigenin-7-*O*-rutinoside (**6**), hesperetin-7-*O*-rutinoside (**7**), diosmin (**8**) were identified as major flavonoid constituents. Beside the caffeic acid derivatives lithospermic acid (**9**) and rosmarinic acid (**10**), salvianolic acid B (**11**) was detected which has not been described for *Mentha sp.* previously. This work additionally presents the flavonoid spectrum of *M.x villosa* Huds. (cv. 'Snežná') for the first time. The polyphenolic fingerprints of *Mentha* leaves indicate the metabolic plasticity of the investigated species and beside the essential oil also flavonoids and phenolcarboxylic acids could serve as additional markers for their differentiation.

Mentha L.	1	2	3	4	5	6	7	8	9	10	11
M.x piperita cv. 'Perpeta'	+++	+	++	tr	tr	+	++	++	+	++	+
M. spicata	tr	-	+	tr	tr	tr	+	+	+	+++	tr
M. spicata var. *crispa*	+	tr	+	tr	tr	+	++	+	++	+++	++
M.x villosa	+++	+	+++	+	+	+	++	++	tr	+++	tr
M.x villosa cv. 'Snežná'	+++	+	+++	+	+	+	++	++	+	+++	tr
M. longifolia	+++	tr	++	+	+	++	tr	tr	+	+++	tr
M. longifolia var. *lavandulodora*	+	tr	++	+	tr	+	+++	+++	+	+++	tr

+++, ++, + (level of presence), – (absence), tr (trace)

Acknowledgements: VEGA grant SR 1/4289/07 **Reference:** [1] Fialova, S. et al. (2008) Acta Facult. Pharm. Univ. Comenianae 55: p 96 – 102.

SL25 Authentication of skullcap (*Scutellaria lateriflora* L.) – a commonly adulterated medicinal plant

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INTRODUCTION: The North American herb skullcap (*Scutellaria lateriflora*) is widely used as a mild sedative, but it has been plagued by adulteration and substitution problems for more than a century. **Aims:** To identify reliable HPLC and HPTLC methods for the authentication of *S. lateriflora* raw material. **Methods:** A total of 45 samples were analysed by LC-MS, HPLC and HPTLC, including commercial raw material (genuine and substituted) and authentic herbarium material of *S. lateriflora*, five other *Scutellaria* taxa, and the potentially hepatotoxic substitute *Teucrium chamaedrys*. Four flavonoids (baicalein, baicalin, scutellarin and chrysin) were quantified and several others identified in the samples. The dataset was also subjected to principal components analysis. **Results:** Genuine *S. lateriflora* was shown to possess a characteristic chemical profile and was readily distinguishable from the other taxa examined, both by HPLC and HPTLC. *S. lateriflora* had a high content of baicalin (1790 ± 550 µg/g) and a low content of scutellarin (100 ± 70 µg/g). *S. galericulata* had a somewhat similar profile, but also contained chrysin (39 ± 4 µg/g), whereas *S. baicalensis* (leaf), *S. altissima* and *S. barbata* contained scutellarin as the main flavonoid. *T. chamaedrys* contained teucrioside but none of the flavonoids typical of *Scutellaria*. Four lots of commercial raw material offered as *S. lateriflora* in the global market in 2007 were found to consist of a different species of *Scutellaria*. CONCLUSION: Genuine *S. lateriflora* can readily be identified from likely adulterants by either HPTLC or HPLC, but adulteration still occurs and rigorous authentication of raw materials is essential.

SL26 Effects of plant density on essential oil composition of sweet basil (*Ocimum basilicum* L.) cultivated in Turkey

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This research was carried out at the Field Crops Department of Faculty of Agriculture, University of Ankara in order to investigate essential oil composition of *Ocimum basilicum* with respect to three different plant densities (30 x 20 cm, 40 x 20 cm, 50 x 20 cm). Field experiment was established in 2008 and three cuttings were obtained during vegetation period. Essential oils obtained by hydrodistillation from basil herb and oils were analyzed by GC-MS. Cutting times were performed in 23 July, in 26 August, in 6 October, respectively. Linalool was determined as a main component that changed between 56.70 – 70.31% in the first cutting, 57.91 – 64.08% in the second cutting, 49.56 – 64.77% in the third cutting. Linalool content decreased regularly from first cutting to third cutting, whereas it increased by decreasing the plant density. The other important components were identified as γ-cadinene (7.75%), β-cubebene (3.97%), α-bergamotene (3.14%) in the first cutting; γ-cadinene (7.23%), α-bergamotene (4.93%), eucalyptol (3.22%) in the second cutting; γ-cadinene (9.34%), α-bergamotene (5.88%), α-amorphene (3.89%) in the third cutting. γ-cadinene was found as the second important compound in all cuttings. Average essential oil ratio was recorded as 0.58% (in first cutting), 0.53% (in second cutting) and 0.21% (in third cutting). In all cuttings, the lowest essential oil ratio was obtained in 30 x 20 cm of plant density. The highest essential oil percentage was recorded in first cutting, while the lowest ratio was determined in third cutting.

SL27 Computational evaluation of Isoorientin (*C*-glycosyl flavone) on PPAR-gamma receptors and HMG-CoA reductase using MOE 2008.10

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Peroxisome proliferators-activated receptor-gamma (PPAR-gamma) plays an essential role in lipid and glucose homeostasis. Numerous studies and comprehensive reviews have documented various naturally derived ligands as PPAR-γ a potential source of novel anti-diabetic compounds from plants and herbs [1]. Isoorientin, a C-glycosyl flavone, has been isolated as an antidiabetic and antihyperlipidemic agent from aerial parts of *Gentiana ollivieri* Griseb. [2]. The objective of this study is to find out, the relation between these receptors and ligand. We used docking property and site finder and electrostatic map tools of molecular operating environment (MOE) 2008.10 computer programme from Chemical computing group. Protein structures were taken from Protein Data Bank PDB and operated with Protonate 3D and minimized. Ligands were designed by LigX. Results shown that, E score1: -16.0501 and E refine: -32.3072 for isoorientin-PPAR gamma docking study, E score1: -9.8957 and E refine: -17.2581 for isoorientin-HMG-CoA docking study. Data obtained from experiments demonstrated that isoorientin can be candidate as a good multi-target drug template. *Acknowledgements: Authors to thank Ms. Patricia Middleton from Chemical Computing Group INC. for supply MOE 2008.10 programme.* **References:** [1] Salam, N.K. et al. (2008) Chem. Biol. Drug Des. 71:57 – 70. [2] Sezik, E. et al. (2005) Life Sci. 76:1223 – 1238. [3] Labute, P. et al. (2008) Electrostatic Maps, Chemical Computing Group, Montreal, Canada.

SL28 Phytoequivalence in the global marketplace for botanical products (II): Phytochemical composition and antioxidant capacity of standardized commercial *Andrographis paniculata* extracts

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In our effort to understand the phytoequivalence of botanical extracts used in complementary medicines, we examined batch-to-batch phytochemical variability of standardized commercial *Andrographis paniculata* (Acanthaceae) extracts sourced from India. *A. paniculata* is used in Ayurvedic medicine as a liver stimulant and for the treatment of jaundice and in Chinese medicine to alleviate body heat and to dispel toxins. Andrographolide and related diterpenoids have been isolated from this species. Commercial extracts are standardized to andrographolide. Significant quantitative variation in andrographolide content has been observed among accessions of *A. paniculata* from Thailand and India. Manufacturers modify extraction parameters to achieve consistent composition and to compensate for seasonal variability of the starting material. The downside of this method of standardization is the process-induced quantitative variation in other chemical constituents. Using HPLC/DAD/MS-MS, we characterized 12 different batches of standardized extracts sourced from one manufacturer. Results revealed the presence of 21 compounds with variation in number and quantity among the tested batches. Five major constituents, andrographoside, iso-, neo-, deoxy-, and dehydroandrographolide, were tentatively identified. Andrographoside showed maximum quantitative variation (18 fold). In order to determine how the phytochemical differences between the extracts relate to their pharmacological activity, we determined the antioxidant capacity of the extracts using DPPH free radical scavenging and oxygen radical antioxidant capacity (ORAC) assays. Although neither andrographolide nor its major derivatives exhibited DPPH radical scavenging activity, we observed a ~2.5 fold variation in the EC_{50} of the whole extracts suggesting that other components contributed to batch-to-batch variation. Ongoing work is directed at the elucidation of the pharmacological significance of batch-to-batch variations to develop better criteria for the determination of phytoequivalence of "standardized" extracts.

SL29 Evidence based efficacy of adaptogens in fatigue

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The aim of this systematic review was to assess the level of scientific evidence of efficacy of adaptogens on cognitive functions in fatigue. The results were evaluated by Natural Standards Evidence-Based Validated Grading Rationale (NSR) and by European Medicines Agency Assessment Scale (EMEAS), as recommended. There is strong scientific evidence (grade A) that treatment with Rhodiola SHR-5 extract is able to improve cognitive performance across "attention" tasks. These results were observed in three randomized, placebo controlled, and double-blind clinical trials (257 patients). In addition, good (grade A level) scientific evidence was estimated in chronic fatigue syndrome. Grade B level was also documented for *Schisandra*, which was shown to increase endurance and mental performance in 2377 patients over eight trials. Grade B level can also be accepted for *Eleutherococcus* in 729 patients with mild fatigue and weakness. Grade C level of evidences, mainly due to conflicting results obtained in different studies, is related to *Ginseng*, which can improve cognitive functions. In conclusion, adaptogens can be defined as a pharmacotherapeutic group of herbal preparations increasing tolerance to mental exhaustion and to enhancing attention and mental endurance in cases of decreased performance, such as in fatigue and in sensation of weakness.

SL30 Anti-inflammatory and antioxidative effects of leaf extract from *Acanthopanax trifoliatus*

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Acanthopanax trifoliatus is a Thai plant belonging to the ginseng family or Araliaceae, which has been traditionally used for the treatment of oxidative-stress related diseases such as lung hemorrhages, bruises, ulcers, partial paralysis, and neurosis [1 – 2]. Its leaves are also popularly consumed as tonic vegetables. Our recent work has shown that the decoction extract from the leaves of *A. trifoliatus* significantly exhibited *in vitro* antioxidant activity determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and thiobarbituric acid reactive substances (TBARS) method for lipid peroxidation of rat brain homogenate [3]. From our previous finding, we evaluated its ability to inhibit inflammation using carrageenan-induced rat paw edema model [4]. Two hours after inflammatory induction, *A. trifoliatus* leaf extract showed inhibitory effect in dose-dependent manner. At the dose of 600 mg/kg, the extract exhibited a significant anti-inflammation (41% inhibition, $P< 0.05$), whereas the non-steroidal anti-inflammatory drug, indomethacin (20 mg/kg), showed 35% inhibition ($P< 0.05$). High performance liquid chromatography-mass spectrometry (HPLC-MS) of the leaf extract revealed peaks corresponded to some flavonoids and polyphenolics including rutin, isoquercetin, quercitrin, chlorogenic acid, 3,5-di-*O*-caffeolylquinic acid and 4,5-di-*O*-caffeoylquinic acid which have been reported to exhibit antioxidant and anti-inflammatory activities [5 – 12].
Acknowledgements: The Graduate Program Development under the Collaboration between Thailand Institute of Scientific and Technological Research and Universities.
References: [1] Perry, L.M. and Metzger, J. (1981) Medicinal Plant of East and Southeast of Asia. MIT Press. London. [2] Loi, D.T. (2000) Glossary of Vietnamese Medical Plants. S and T Pub. Ha Noi. [3] Sithisarn, P. and Jarikasem, J. (2009) Med. Prin. Pract. (accepted). [4] Winter, W.D. et al. (1987) J. Pharmacol. Exp. Ther. 244:51 – 57. [5] Yonathan, M. et al. (2006) J. Ethnopharmacol. 108:462 – 470. [6] Jung, H.A. et al. (1999) Arch. Pharm. Res. 22:213 – 218. [7] Kozlov, A.B. et al. (1994) Biochem. Pharmacol. 47:795 – 799. [8] Guardia, T. et al. (2001) Il Farmaco 56:683 – 687. [9] Nakajima, Y. et al. (2007) Life Sci. 80:370 – 377. [10] Peluso, G. (1995) J. Nat. Prod. 58:639 – 646. [11] Wagner, C. et al. (2006) Brain Res. 1107:192 – 198. [12] Camuesco, D. et al. (2004) Br. J. Pharmacol. 143:908 – 918.

SL31 α-Mangostin, a major xanthone from *Garcinia mangostana* and other phenolic constituents, with potent antibacterial and analgesic activities

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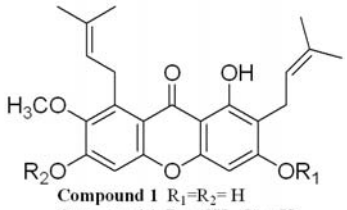
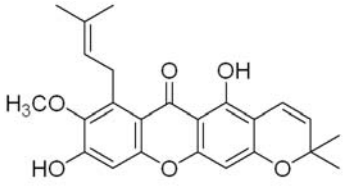
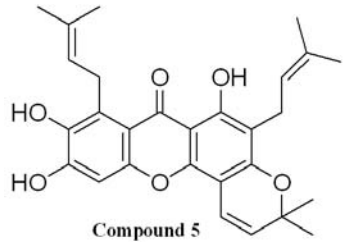
Genus *Garcinia* is rich in xanthones and prenylated phenolic compounds. Fruits of *Garcinia mangostana,* distributed in Southeast Asia have common folk uses like treatment of diarrhoea, anti-inflammatory and ulcers healing. It is a rich source of mangostin-type of xanthone with variety of biological activities. Repeated chromatographic separation and purification of the total alcohol extract of the pericarps of the titled fruits afforded six compounds identified as α-mangostin (**1**) β-mangostin (**2**), 1-hydroxy 3,6,7- trimethoxy 8-(3-methylbut-2-enyl)-xanthone (**3**), mangostanin (**4**), 1,6,7-trihydroxy 6',6'-dimethyl-2 H-pyrano (2',3':3,4)-2,8-di(3-methylbut-2-enyl)xanthone (**5**) and catchin (**6**). Structural elucidation was achieved utilizing different spectroscopic techniques, including 1D and 2D NMR. α-Mangostin showed strong central and peripheral analgesic effects in addition to a potent antibacterial activity particularly against *Bacillus subtilis and Staphylococcus aureus* with MIC 1.6 and 3.2 μg/ml, respectively.



Compound 1 $R_1=R_2=H$
Compound 2 $R_1=CH_3$, $R_2=H$
Compound 3 $R_1=R_2=CH_3$



Compound 4



Compound 5

SL32 POCU1b reverses diabesity induced in rats fed a high-fat diet

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Diabesity, obesity-dependent diabetes, has emerged as a major public health problem that is increasing in frequency. This study investigated the effects of POCU1b, an herbal medicine, in rats previously fed a high-fat diet for 120 days to induce obesity and related diabetes. High-fat fed rats became obese and developed hyperglycemia and hyperinsulinemia, indicating that they were insulin resistant. A high-fat diet with or without 1% POCU1b were then administrated orally for 7 weeks. The treatment of POCU1b significantly decreased body weight compared with vehicle-treated control without change of high-fat diet intake. Plasma glucose and insulin were restored to levels of normal chow-fed rats, and

circulating triglyceride and cholesterol were significantly decreased. POCU1b treatment also reverses the altered circulating adiponectin level. Adipose tissue mass, adipocyte hypertrophy, and deposition of triglyceride in liver were significantly decreased. These changes were accompanied by significant improvement of insulin sensitivity in POCU1b-treated rats. These data indicate that POCU1b provides an effective means of countering obesity and related diabetes induced by consumption of a high-fat diet.

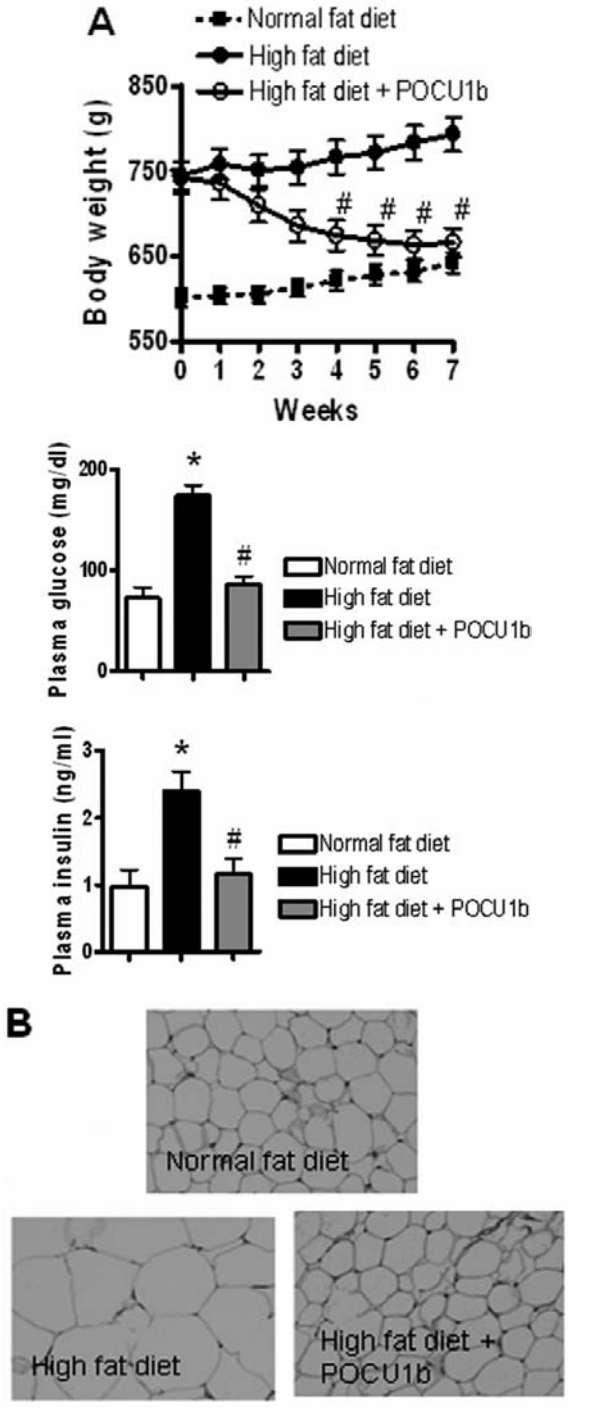

Figure 1. Effects of daily administration of POCU1b on (A) body weight, plasma glucose, plasma insulin, and (B) adpocyte hypertrophy in rats with diet-induced diabesity. Representative images (x 40 magnifications) of epididymal adipocytes. All values are means ± SE. *P < 0.01 vs. normal fat deit, #P < 0.01 vs. high fat diet.

SL33

Cytotoxic phytochemicals

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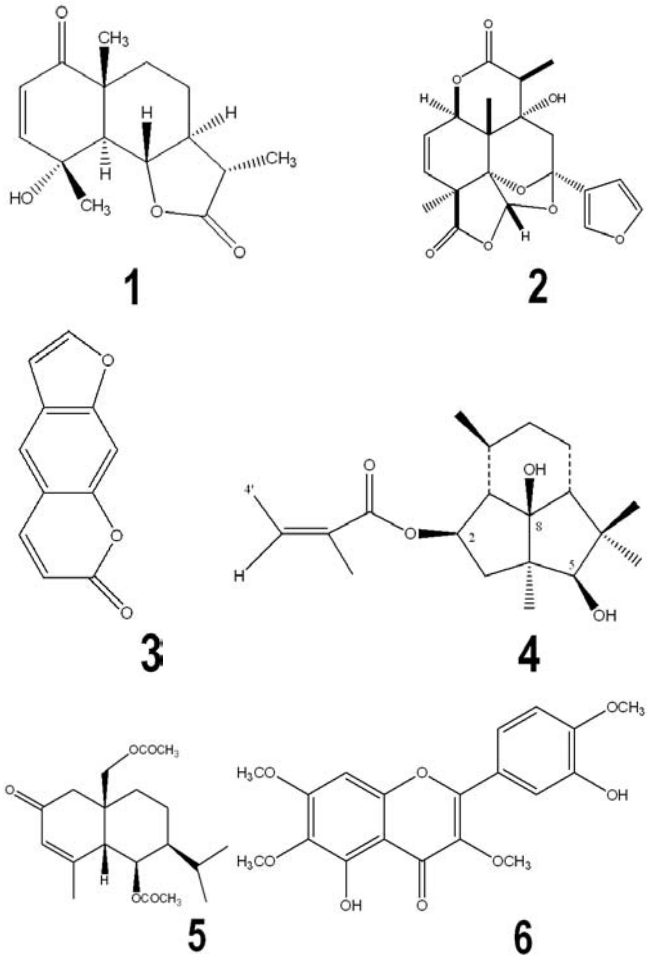
Six pure compounds were isolated and showed potent growth inhibition; vulgarin (1), isolated from *Artemisia judaica*, inhibited the growth of human colorectal cancer cells (27 – 100%, IC_{50} = 14 µg/ml) and human melanoma cells (24 – 100%, IC_{50}= 12 µg/ml) with 7 – 14% growth inhibition of the normal human fibroblasts. Saudinolide (2), isolated from *Cluytia richardiana*, inhibited the growth of colorectal cancer cells (16 – 100%, IC_{50}= 15 µg/ml) and melanoma cells (26 – 100%, IC_{50}= 12 µg/ml) with 14 – 18% growth inhibition of normal fibroblast cells. Psoralen (3), isolated from *Ruta chalepensis*, inhibited the growth of colorectal cancer cells (22 – 100%, IC_{50}= 20 µg/ml) and melanoma cells (41 – 100%, IC_{50}= 12 µg/ml) with 6 – 23% growth inhibition of normal fibroblasts. On the other hand, 2β-angeloyloxy-5β,8β-dihydroxypresilphiperfolane (4), isolated from *Senecio hadiensis*, inhibited the growth of colorectal cancer cells (28 – 100%, IC_{50}= 12 µg/ml) and melanoma cells (26 – 100%, IC_{50}= 12 µg/ml) while exerting 6 – 34% growth inhibitory effect on normal human fibroblasts. Moreover, plectranthone (5) and casticin (6) were both isolated from *Plectranthus cylindraceus*. Compound 5 inhibited the growth of colorectal cancer cells (15 – 100%, IC_{50}= 20 µg/ml) and melanoma cells (39 – 100%, IC_{50}= 12 µg/ml) with 8 – 25% growth inhibition of normal fibroblasts, while compound 6 had very potent growth inhibitory effects on both colorectal cancer cells and melanoma cells (80 – 100%, IC_{90}= 10 and 12 µg/ml, respectively) with 4 – 35% effect on normal human fibroblasts.



SL34 Macelignan suppresses *Porphyromonas gingivalis* supernatant-stimulated urokinase-type plasminogen activator expression via signal transduction in human KB oral cells

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Macelignan, a bioactive compound isolated from *Myristica fragrans* Houtt. or nutmeg, has been reported for its anti-cariogenic and anti-biofilm activities for dental plaque control [1,2]. However, its efficacy to block the expression of urokinase-type plasminogen activator (uPA), a serine protease that is expressed in various inflamed and normal healing cell types in response to cytokines and bacterial products, for periodontal inflammation treatment has not been investigated. This study was aimed to examine whether macelignan suppressed *Porphyromonas gingivalis* supernatant-stimulated uPA expression through regulation of mitogen-activated protein kinase (MAPK) and activating protein (AP)-1 signalings in human KB oral cells by performing casein zymography, reverse transcription-PCR, Western blotting, and reporter gene assays. The main caseinolytic band secreted from the cells was found to be migrated at 54 kDa and represented uPA. Macelignan dose-dependently inhibited the expression of uPA activity, protein, and gene in KB cells in response to *P. gingivalis* supernatant. In accordance with these findings, macelignan effectively decreased phosphorylation of p38 and *c-jun* N terminal kinase (JNK) in *P. gingivalis* supernatant-stimulated KB cells. The levels of c-jun phosphorylation and c-fos expression, which composed of AP-1 transcription factor for uPA gene expression, were also reduced by macelignan in KB cells exposed to *P. gingivalis* supernatant. In linear with these results, macelignan was found to block *P. gingivalis* supernatant-stimulated AP-1 activity in KB cells. These results suggest that macelignan decreased *P. gingivalis* supernatant-stimulated uPA expression by blocking AP-1 activity which may be facilitated by inhibiting phosphorylation of p38 and JNK in KB cells. **References:** [1] Chung, J.Y. et al. (2006) Phytomedicine 13:261 – 266. [2] Yanti et al. (2008) Phytother. Res. 22:308 – 312.

SL35 *Tulbaghia alliacea*: A potential anti-cancer phytotherapy

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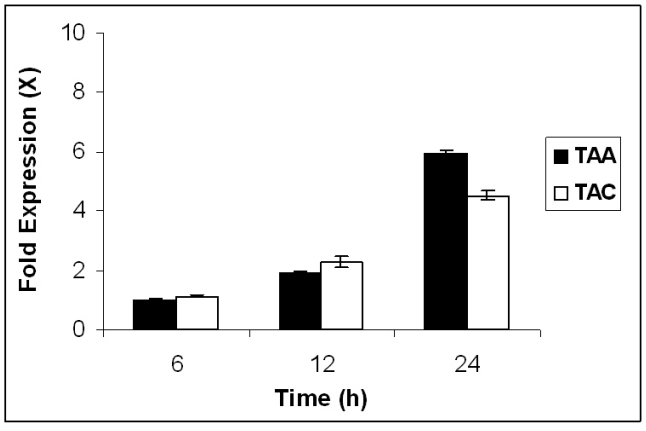
Tulbaghia alliacea is an indigenous garlic plant used in traditional medicine as an anti-cancer remedy. To evaluate this claim, five human cancer cell lines were treated with *Tulbaghia alliacea* aqueous (TAA) and chloroform extract (TAC), for their potential to induce apoptosis (0 – 10 mg/ml over 24 hours) *in vitro*. Using phosphatidyl serine externalisation, Caspase-3 cleavage, mitochondrial depolarisation and DNA fragmentation as markers, this study showed that both extracts induced apoptosis in three of these cell lines (Jurkat, MCF7 and MG63) while the other two cell lines (HeLa and H157) were completely resistant.



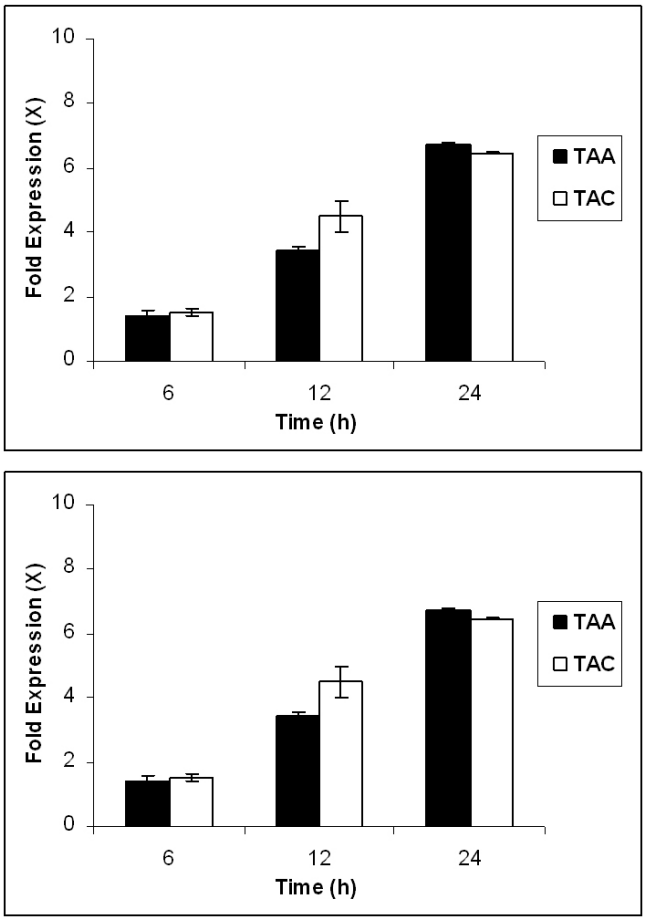


Figure 1: FLTR-Effects of TAA and TAC (6 mg/ml) on Bax, Caspase 3 and Caspase 9

Gene product studies through real time PCR (Fig. 1) revealed that TAA and TAC significantly induced the expression of *Caspase-3*, *Caspase-9 and Bax*, over time ($P < 0.001$). Whilst a previous study showed that *Tulbaghia violacea* extracts induced apoptosis [1], this is the first report on the apoptotic effects of *T. alliacea* in Jurkat, MCF7 and MG63 cancer cells during *in vitro* conditions. **Reference:** [1] Bungu, L. et al. (2006) Afr. J. Biotechnol. 5:1936 – 1943.

SL36 Production of medicinal and aromatic plants for drug industries in Egypt

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The flora of Egypt includes about 2000 species of plants distributed in its different localities that vary in type of soil and prevailing climatic and other environmental conditions that hence encourage the growth of a wide range of plant species. In addition, many medicinal plants have been successfully introduced and acclimatized in Egypt. The medicinal plants are in great demand in folk medicine. The modern pharmaceutical industry also requires a large quantity of medicinal plants for manufacture of drugs. Cultivation of medicinal and aromatic plants in Egypt is taking place mainly for feeding drug industries and for exportation. It had been cultivated in Delta and Nile Valley especially in Upper Egypt (the old soil). Recently, its cultivation moved to the new reclaimed soils in order to save the fertile soils (the old soil) for the production of strategically crops i.e. cotton, rice and wheat. Several species of medicinal and aromatic plants were subjected to cultivation in the new reclaimed soils in order to achieve the technological package for maximum production of the different studied species. Some of these species are used in drug industry and others are used in production of raw materials for cosmetic industry. New techniques in irrigation (dripping or sprinkler), fertilization, mechanization and organic farming systems

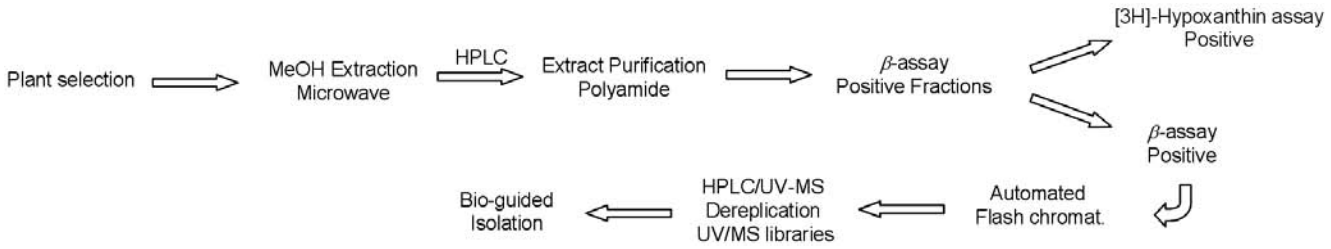

Fig. SL37

were applied in the production of medicinal and aromatic plants in reclaimed soils. Some wild species were subjected for cultivation and production in the new reclaimed lands. The presentation focused on the production of medicinal plants in Egypt either in old soil or in the new reclaimed lands.

SL37 Rapid screening and targeted isolation of antimalarial hits using β-Hematin assay

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Malaria is one of the most devastating infectious diseases killing approximately one million people annually, mostly young children [1]. Natural resources – especially plants with the examples of quinine and artemisinin – have already been demonstrated to be a very successful source of effective and safe antimalarial drugs. The assay on β-hematin is based on the selective detection of the non polymerized hematin after complexation with pyridine [2]. The test was implemented in our laboratory and optimized for the screening of crude plant extracts and pure natural products on 96 well-plates [3]. A total of 70 natural products belonging to various chemical classes and 320 extracts have been tested qualitatively. Inhibitors of the β-hematin synthesis were submitted to the *in vitro* test on *P. falciparum*. Qualitative response obtained correlated more that 80% of cases. 50% of the identified hits presented IC_{50} < 10 mg/ml. A strategy based on the colorimetric test and chemical screening was settled in order to rapidly identify antimalarial hits [3]. **References:** [1] Ioset, J.-R. (2009) Curr. Org. Chem. 12:643 – 666. [2] Egan, T.J. et al. (2005) J. Inorg. Biochem. 99:1532 – 1539. [3] Vargas, S. (2009). PhD Thesis (Submitted).

SL38 Antimicrobial interactions between medicinal plants in African traditional medicine

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In African traditional medicine it is well known that traditional healers often combine various plant species in order to enhance efficacy [1]. Using an anti-infective model, various plants from different geographical areas within southern Africa were examined to validate their use in combination. Two indigenous medicinal plants (*Salvia chamelaeagnea* and *Leonotis leonurus*) were studied to determine their anti-infective properties in combination. Synergistic interactions were observed for the Gram-positive test organisms. *Artemisia afra,* a renowned medicinal plant in South African traditional medicine [2], is commonly used in combination with other species to treat respiratory infections. Some examples from these interactions will be demonstrated i.e. the combination of *A. afra* with *Osmitopsis asteriscoides.* The essential oils and extracts demonstrated varied interactions. Diarrhoeal diseases are one of the highest causes of mortality in southern Africa [3]. With this in mind, an investigation was undertaken to determine which antidiarrhoeal plants are used by the Zulu people residing in the Maputaland area. The two plants most often used in combination are *Psidium guajava* and *Brachylaena huillensis*. The fractional inhibitory concentration index (ΣFIC) for this combination ranged between 0.09 (synergistic) to 2.25 (non-interactive) when tested against pathogens associated with diarrhoea. In Swaziland, the bark of *Ozoroa sphaerocarpa, Breonadia salicina* and *Syzygium cordatum* are traditionally used in a triple combination for the treatment of diarrhoea. When investigated against *Escherichia coli,* higher efficacy was found in the 1:1:1 combination than when tested independently. These examples validate the traditional use of combination therapy. **References:** [1] Hutchings, A. et al. (1996) Zulu Medicinal Plants – an Inventory. University of Natal Press. Pietermaritzburg, South Africa. [2] Thring, T.S.A. et al. (2006) J. Ethnopharmacol. 103:261 – 275. [3] Bradshaw, D. et al. (2003) S. Afr. Med. J. 93: 682 – 688.

SL39 Pharmacognostic standardization and monograph development of Artemisinin from *Artemisia annua* grown in Nigeria: Step towards local production of Artemisinin based combination therapies

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Towards combating the epidemic of malaria in Nigeria, cultivated *Artemisia annua* was subjected to various investigations. Isolation and characterization of artemisinin, along with antimalarial screening were carried out in order to standardize the product and produce a monograph. These studies resulted in the 0.5% artemisinin yield employing a modified method [1], melting point of 155°C, TLC fingerprints and white spindle shaped crystals. Pharmacognostic investigation on *Artemisia annua* yielded the presence of alkaloids, sterols, terpenes, anthraquinone, flavonoids and carbohydrates, with absence of saponins and tannins. Moisture content of 13.1%, total ash value of 12.6%, acid insoluble value of 1.9%, water soluble extractive value of 22.7% and alcohol soluble extractive value of 14.3%. NMR and HPLC analyses were further carried out to determine the identity, purity and quality of the artemisinin obtained and the result indicated a high degree of these parameters. Antiplasmodial activity (*in vitro*) of the isolated artemisinin from *A.* annua against chloroquine resistant *Plasmodium falciparum* (strain K1) parasite lactate dehydrogenase (pLDH) assay [2] yielded a comparable antimalarial activity to the reference drug. Efforts are currently on to determine the artemisinin contents in the different *A. annua* biomasses obtained from different pilot farms in Nigeria as guide to further cultivation expansion. These results along with others being compiled are aimed at local production of ACTs in the country as Nigeria strives to meet the Millennium Development Goals (MDGs) *Acknowledgments: The Artemisinin Development Company Abuja Nigeria, & Prof. Andi Brisibe.* 1. Klayman D.L. et al. (1984) J. Nat. Prod. 47:715 – 717. 2. Makler, M.T. et al. (1993). Am. J.Trop. Med.Hyg. 48:739 – 741.

SL40 Integrated approaches for finding leads from Ayurveda – Way forward

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Resurged interest in Traditional, Alternative & Complementary Medicines (TACM) has opened new areas of exploration. India, with oldest civilization history harbours many TACM. Ayurveda dates back to 5000 B.C., is developed based on the daily life relationship between human and nature. They are a part of health care industry among world countries in order to explore new chemical entities [1]. Ayurveda reports more than 2000 plants, which may be explored through modern scientific approaches. Safety and efficacy of these products are always a cause of concern. Quality control, validated manufacturing processes and post marketing surveillance are the key points to ensure their safety and efficacy. Marker analysis based on chemo profiling and characteristic fingerprints for individual plants could help to develop uniform standardization tool for their promotion [1,2]. Their regulations vary among countries causing difficulty in maintaining uniform standards. Integrated approaches may help in developing therapeutic lead with understanding on their mechanism of action and interactions for synergy [3]. Thus development of TACM from Ayurveda will help to cherish them. In this regard several approaches involved in developing Ayurveda will be discussed including, features in development of Indian TACM including Ayurveda, evaluation of their quality, safety and efficacy and harmonization of regulation and promotion with international co-ordination. **References:** [1] Mukherjee, P.K. (2002) Quality Control on Herbal Drugs. Business Horizons Ltd. New Delhi. [2] Mukherjee, P.K. and Verpoorte, R. (2003) GMP in Herbal Drugs. Eastern Publishers. New Delhi. [3] Mukherjee, P.K. and Wahile, A. (2006) J. Ethnopharmacol. 103:25 – 35.

SL41 *In vitro* and *in vivo* immunomodulatory activity evaluation of *Bacopa monniera*, Scrophulariaceae

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Bacopa monniera, also referred to as *Bacopa monnieri*, *Herpestis monniera*, water hyssop, and "Brahmi," (family Scrophulariaceae) has been used in the Ayurvedic system of medicine for centuries [1]. Aerial parts of *Bacopa monniera* were extracted with distilled water and then successively with methanol. Methanol extract was evaluated for immunomodulatory activity using *in vitro* and *in vivo* methodologies. Effect of Bacopa extract (832 – 6.5 μg/ml) on secretion of mediators like nitric oxide (NO) and superoxide on isolated murine peritoneal macrophages were evaluated by using Griess reagent and nitro blue tetrazolium (NBT) dye reduction assay respectively. The extract showed significant ($P< 0.05$) stimulation of release of NO at 416 μg/ml (SI, stimulation index = 1.67) and 208 μg/ml (SI = 2.14) ($P< 0.05$). NBT reduction were significant at 52 μg/ml (SI = 1.34) as compared to control (SI = 1). The extract was also evaluated for *in vivo* phagocytic activity by carbon clearance assay in mice and it showed significant increase in the phagocytic index (K) at 100 (K = 0.104) and 200 mg/kg (K = 0.116) as compared to control (K = 0.062). The extract significantly ($P< 0.05$) enhanced total leucocytes count at 50 mg/kg (10.82×10^3cells/cmm) and for 100 mg/kg (11.93×10^3cells/cmm) of cyclophosphamide induced myelosuppression (6.02×10^3cells/cmm) in mice (n = 6). Data was analyzed by one way ANOVA followed by Dunnet's multiple comparisons test. The results suggest that *Bacopa* extract influences secretion of mediators *in vitro* and phagocytosis *in vivo*. Reference 1: Anon. (2004) Monograph-*Bacopa monniera*; Alternative Medicine Review; Thorne Research, Inc. Volume 9, Number 1; pp 79 – 85.

SL42 Efficacy of Brazilian propolis extract and gel for the management of denture stomatitis

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Denture stomatitis presents as a chronic diseases in denture-bearing patients, especially under maxillary prosthesis. Despite the existence of a great number of antifungal agents, treatment failure is observed frequently. Propolis, a natural product, possesses well-documented antifungal and anti-inflammatory activities. The purpose of this study was to evaluate the clinical efficacy of a news Brazilian propolis extract and gel formulation in patients diagnosed with denture stomatitis. Forty-five complete-denture wearers with denture stomatitis were enrolled in this pilot study. At baseline, clinical evaluation was performed by a single clinician and instructions for denture hygiene were provided. Fifteen patients received Daktarin® (Miconazole gel), 15 received propolis extract (BPE) and 15 received propolis gel. All patients were recommended to apply the product four times a day during one week. Clinical evaluation was repeated by the same clinician after treatment. All patients treated with Brazilian propolis extract, Brazilian propolis gel and Daktarin® had complete clinical remission of palatal edema and erythema. This new Brazilian propolis gel formulation had efficacy comparable to Daktarin® and could be an alternative topical choice for the treatment of denture stomatitis. *Acknowledgements: FAPEMIG – Fundação de Apoio a Pesquisa do Estado de Minas Gerais; CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico; CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Pharm. Dra. Sheila Rago Lemos Abreu (Pharmanéctar- Belo Horizonte- Brazil).*

SL43 Herbs for the treatment of diabetes and hypertension: remedies from traditional and ethnic formulations

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Patients with diabetes have a much higher rate of hypertension than would be expected in the general population. In general, only 25 percent of patients with hypertension have adequate control of their blood pressure. Fortunately, reductions in blood pressure can decrease the risk of this complication. Malaysia has rich and biologically diverse natural resources with over 15,000 flowering plants and 2,000 medicinal plants. An inventory of selected ethnic communities in East and West Malaysia was undertaken in an effort to identify potentially antidiabetic and antihypertensive plant species used within the traditional pharmacopoeia of the communities. Respondents were randomly selected and interviews were conducted with community elders and persons knowledgeable in traditional medicine. Specific questionnaires were used and whenever possible plants documented in the survey were processed for voucher specimens. The data revealed that there are variations and differences in the method of preparation and utilization of plants as remedies for diabetes and hypertension. While there has been a notable decrease in the practice of using plant-based remedies by the ethnic communities; interest in developing product based on such knowledge is on the upsurge around the world. Twenty species common to the ethnic communities studied are selected for discussion in this presentation. *Acknowledgements: University of Malaya, University Malaysia Pahang.* **References:** [1] Epstein, M. and Sowers, J.R. (1992) Hypertension 19:403 – 418. [2] Bakris, G. et al. (2000) Postgrad Med. 107:53 – 56, 61 – 64. [3] Goh, S.H. et al. (1995) Malaysian Medicinal Plants for the Treatment of Cardiovascular Diseases. Pelanduk Publications, Kuala Lumpur.

SL44 Efflux modulators from *Momordica balsamina* L. in multidrug resistant bacterial strains

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Bacterial infections are becoming more challenging to treat as a result of the emergence of multidrug resistant (MDR) bacteria. The genetic and physiological basis for the MDR phenotype of clinical isolates has been associated with porin deficiencies and over-expression of efflux pumps which, when present in the same organism, decrease the permeability of the bacteria to two or more unrelated antibiotics. The problem of resistant bacteria (Gram-positive and Gram-negative) highlights the urgent need for new drugs. A solution is the development of efflux pump inhibitors that will restore the activity of the antibiotic to which the bacteria became resistant [1]. In this study we evaluated the efflux modulating effect of cucurbitane-type triterpenes isolated from the methanol extract of aerial parts of *Momordica balsamina* L. in some Gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and Gram-negative (*E. coli* and *Salmonella enteritidis*) bacterial strains. The previously developed semi-automated real-time fluorometric method was used to monitor the accumulation and extrusion of the fluorochrome ethidium bromide, in the presence and absence of tested compounds [2]. The tested compounds have shown to increase the accumulation of ethidium bromide in the Gram-positive bacteria tested, with higher accumulation than observed in positive controls, such as thioridazine. No increase in EB accumulation was observed in *S. enteridis* and *E. coli* strains tested. These findings show that these compounds have potential to be used as efflux pump inhibitors of Gram- positive bacteria, and thus to restore the activity of antibiotics. This is especially important due to the increase of multidrug resistance in Gram-positive pathogenic bacteria like *S. aureus*, *S. pneumoniae* and *Enterococcus spp*. *Acknowledgements: The authors wish to thank the Science and Technology Foundation, (FCT, grant SFRH/BD/22321/2005).* **References:** [1] Pietras, Z. et al. (2008) Curr. Drug Targets 9:719 – 728. [2] Viveiros, M. et al. (2008) Int. J. Antimicrob. Ag. 31:458 – 462.

SL45 Functional genomics of immuno-modulatory activities of medicinal plant extracts/ phytocompounds in human dendritic cells/ monocytes

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Echinacea spp. extracts and the derived phytocompounds have been used as botanical drugs or nutraceuticals for immuno-modulatory functions [1]. Dendritic cells (DCs) play an important role in both innate and adaptive immunities. Recently, we investigated differential gene expression profiles in human immature DCs (iDCs) in response to treatment with a butanol fraction extracted from *Echinacea purpurea*, denoted [BF/S+L/Ep]. DNA microarray results showed significant up regulation of specific genes for cytokines (IL-8, IL-1β, and IL-18) and chemokines (CXCL 2, CCL 5, and CCL 2) within 4 h after treatment. Bioinformatics analysis revealed a key-signaling network involving immune-modulatory molecules leading to the activation of a downstream adenylate cyclase 8. Proteomic analysis showed increased expression of antioxidant and cytoskeletal proteins after this treatment [1,2]. Human monocytes (THP-1) were also tested under LPS stimulation with [BF/S+L/Ep], along with three anti-inflammatory phytocompounds (emodium, shikonin and cytopiloyne). Initially (within 0.5 h), shikonin [3] and emodin significantly inhibited the expression of approximately 50 genes, most notably cytokines TNF-α, IL-1 and IL-4, chemokines CCL4 and CCL8, and inflammatory modulators NFATC3 and PTGS2. Cytopiloyne and BF/S+L/Ep did not inhibit early expression of these 50 genes, but inhibited the late-stage expression (~12 hours) for many of them, particularly IL-4, NFATC3 and PTGS2, and the cell migration and chemokine molecules CDH1 and ITGA2. The ERK 1/2 activation pathway was identified as the putative target of BF/S+L/Ep and cytopiloyne. These studies provide useful information for future development of phytocompounds/extracts as defined health supplements or herbal medicines. *Acknowledgements: Agriculture Biotechnology Research Center, Academia Sinica, Taipei, Taiwan. National Science Council, Taiwan.* **References:** [1] Wang, C.Y. et al. (2008) BMC Genomics. 9:479. [2] Wang, C.Y. et al. (2006) Genomics 88:801-808. [3] Staniforth, V. et al. (2004) J. Biol. Chem. 279:5877 – 5885.

SL46 *Stevia rebaudiana*, a novel source of phytoceuticals with anticancer potential

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Functional foods or nutraceuticals are assuming a middle ground between food and drugs due to a growing body of evidence that supports their role in maintaining health and contributing to the treatment of diseases. Since Hippocrates advised to "Let food be thy medicine and medicine be thy food," we have defined medicines and foods based on what is known about each substance in terms of efficacy, safety and the significance of its perceived contribution to health. Oxidants, by-products of normal metabolism cause extensive damage to DNA, protein and lipid leading to aging and degenerative diseases such as cancer, cardiovascular disease, cataracts and others. At least two major human problems, aging and cancer, involve ROS mediated DNA damage [1 – 3]. With this as background, here, we evaluated the neutraceutical properties of *Stevia rebaudiana* Bertoni with a view to develop this amazing herb as "Edible source of anticancer agent'. *Stevia* is gaining significance in different parts of the world and is expected to develop into a major source of high-potency sweetener, for the growing natural food market. Exploiting the use of this non-caloric sweetener will open up new avenues in the food and herbal medicine as an alternative source of natural sweetener [4]. Results showed the preventive effect of the crude extract and the ethyl acetate fraction of *S. rebaudiana* on Fenton reaction-induced damage of pBluescript II SK (-) supercoiled DNA maintained in *E. coli* XL-1 strain in comparison to quercetin and stevioside, the principal sweetening agent in *Stevia.* Densitometric analysis confirmed the experimental data. **References:** [1] Kumar, A. and Chattopadhyay, S. (2007) Food Chem. 100:1377 – 1384. [2] Cerutti, P.A. 1994 Lancet 344:862 – 863. [3] Ghanta, S. et al. (2007) J. Agri. Food Chem. 55:10962 – 10967. [4] Kinghorn, A.D. and Soejarto, D.D. 1985 In: Economic and Medical Plant Research, Vol. 1, Ed. By Wagner, H. Hikino, H. and Armsworth, N.R. Academic Press, London.

SL47 *In vitro* and *in vivo* inhibitory effects of (*S*)-armepavine against hepatic fibrosis in rats

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Activation of hepatic stellate cells (HSCs) plays a crucial role in liver fibrogenesis. (*S*)-armepavine (Arm, $C_{19}H_{23}O_3N$), an active compound from *Nelumbo nucifera*, has been shown to exert immunosuppressive effects. In this study we investigated whether Arm could exert anti-hepato-fibrogenic effects *in vitro* and *in vivo*. A cell line of rat HSCs (HSC-T6) was stimulated with tumor necrosis factor-α (TNF-α) or lipopolysaccharide (LPS) to evaluate the inhibitory effects of Arm. *In vivo* therapeutic studies were conducted in both bile duct-ligated (BDL) and thioacetamide (TAA)-intoxicated rats. BDL or TAA rats were given Arm (3 or 10 mg/kg) by gavage twice daily for 3 or 4 weeks, respectively, starting from the onset of BDL or TAA. Liver sections were taken for fibrosis scoring, immuno-fluorescence staining and quantitative real-time mRNA measurements. One-way analysis of variance was used for comparison of parameters. *In vitro,* Arm (1 – 10 μM) concentration-dependently attenuated TNF-α- and LPS-stimulated α-SMA protein expression and collagen deposition by HSC-T6 cells without adverse cytotoxicity. Arm also suppressed TNF-α-induced NFκB and AP-1 activation and MAPK (p38, ERK1/2, and JNK) phosphorylations. *In vivo,* Arm treatment significantly reduced (a) plasma AST and ALT levels, (b) hepatic α-SMA expression and collagen contents, (c) α-SMA- and NFκB-immuno-positive cells, (d) mRNA expression levels of *IL-6, TGF- β1, TIMP-1, col 1α2, iNOS,* and *ICAM-1* genes, and (e) fibrosis scores of BDL and TAA rats as compared with vehicle treatment. Our study results showed that Arm exerted both *in vitro* and *in vivo* antifibrotic effects in rats, possibly through anti-NF-κB activation pathways.

SL48 Metabolome and bioactivities: artificial neural networks for the prediction of the *in vitro* antioxidant and antimicrobial activities of essential oils

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Our aim is to develop tools for predicting the bioactivity of complex natural products of defined chemical composition or metabolome. For long time aromatic plants have been at the centre of intensive research for their antioxidant, antibiotic, and antiinflammatory activities, among others. Their bioactivity relies on a well characterised subset of their metabolome, namely essential oils. We here report on the use of artificial intelligence to predict the antioxidant and antibiotic activities of essential oils as a first step towards linking metabolome and bioactivities. Multilayer, feed forward artificial neural networks were developed and run using the Fast Artificial Neural Network (FANN) software. The chemical composition of 81 essential oils and their antioxidant (DPPH and linoleic acid models) or their antimicrobial (inhibition disc diameter for *Candida albicans, Clostridium perfringens, Escherichia coli, and Staphylococcus aureus* in disc-diffusion tests) activities were extracted from the scientific literature and used as input/output values, respectively. The artificial neural networks could predict the antioxidant capacities of essential oils of known chemical composition in both DPPH and linoleic acid assays with an average error of only 3.16% and 1.46%, respectively. The antimicrobial activity could also be predicted but within the intrinsic limitations of the disc-diffusion test. These results confirm that artificial neural networks can be used as reliable, fast and cheap tools for predicting bioactivities of natural products with well defined metabolomes. Limiting factors for their performance are the inherent errors of the in vitro assays and the complexity of the network.

SL49 Comfrey root extract ointment in the treatment of acute upper or lower back pain: results of a double-blind, randomised, placebo-controlled, multicenter trial

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The objective was to show the superiority of Comfrey root extract ointment (Kytta-Salbe® f; Merck Selbstmedikation GmbH) to placebo ointment in patients with acute upper or low back pain. The double-blind, multi-centre, randomised clinical trial with parallel group design was conducted over a period of 5 days ± 1 day. The 120 patients were treated with verum or placebo ointment 3 times a day, 4 g ointment per application. The trial included four visits. The primary efficacy variable was the area-under-the-curve (AUC) of the Visual Analogue Scale (VAS) on active standardised movement values at visits 1 to 4. The pain intensity on VAS was assessed at performance of standardised, muscle group specific tests. The secondary objectives of the trial were back pain at rest using assessment by patient on VAS, pressure algometry (pain-time curve; AUC over 5 days), global assessment of efficacy by the patient and the investigator, consumption of analgesic medication, and functional impairment measured with the Oswestry Disability Index. There was a significant treatment difference between Comfrey extract and placebo regarding the primary variable. In the course of the trial the pain intensity on active standardised movement decreased on average (median) about 95.2% in the Comfrey extract group and 37.8% in the placebo group. After one hour the pain intensity was already decreased about 33.0% in the Comfrey extract group (104.8 to 60.4 (mean VAS sum)) and 12.0% in the placebo group (100.00 to 86.5 (mean VAS sum)) indicating an early onset of the treatment effect. The results of this clinical trial were clear-cut and consistent across all primary and secondary efficacy variables. Comfrey root extract showed a remarkably potent effect in reducing acute back pain. Reduction of pain and impaired movement was fast and correlated with each other. For the first time a fast-acting effect of the ointment (1 hour) was also witnessed in this trial.

SL50 Active compounds from *Fraxinus excelsior* L. seeds: Anti-diabetic and body weight control effects

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Fraxinus excelsior L. seed extract (FE) is recognized as an anti-diabetic agent [1]. Two new secoiridoid glucosides, excelside A (**1**) and excelside B (**2**) were isolated and elucidated from FE: (2*S*, 3*E*, 4*S*) 2*H*-Pyran-4-acetic acid-3-ethylidene-2-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy]-3,4-dihydro-5-(methoxycarbonyl) methyl ester and (2*S*, 3*E*, 4*S*) 2*H*-Pyran-4-acetic acid-3-ethylidene-2-[(6-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl) oxy]-3,4-dihydro-5-(methoxycarbonyl) 2-(4-hydroxyphenyl) ethyl ester, respectively. Eight known isolated compounds were identified as nuzhenide (**3**), GI3 (**4**), GI5 (**5**), ligstroside (**6**), oleoside-11-methyl ester (**7**), oleoside dimethyl ester (**8**), 1'''-O-β-D-glucosylformoside (**9**), and salidroside (**10**) [2]. Compounds **1-9** showed inhibitory activity against adipocyte differentiation in 3T3-L1 cells. FE (1:10,000) as well as **1**, **3**, **4**, **5**, and **8** were activating in PPARα-reporter cell system in the range of 10^{-4} M comparable to 10^{-8} M WY14,643. Therefore, PPARα-mediated mechanism is a possible relevant pathway for FE anti-diabetic activity. We also evaluated the effects of a low-fat diet (LFD), high-fat diet (HFD), and high fat diet + 0.5% FE (FED) on C57BL/6J mice during 16-weeks. FED decreased fasting blood glucose (33.2%, $P< 0.001$), plasma insulin level (53.4%, $P< 0.05$), and body weight gain (32.3%, $P< 0.05$) compared to HFD. Finally, we used a screening model against glucose (50 g) to assess the effect of FE on plasma glucose and insulin levels. FE (1.0 g) was used in a double blind, randomized, crossover, placebo (wheat bran) controlled study on 16 healthy volunteers. FE reduced the glycemic AUC ($P= 0.02$), but not the insulinemic AUC. These results encourage conducting long term clinical studies to further evaluate the efficacy and safety of FE. **References:** [1] Maghrani, M. et al. (2004) J. Ethnopharmacol. 91:309 – 316. [2] LaLonde, R.T. et al. (1976). J. Am. Chem. Soc. 98:3007 – 3013.

SL51 Antiadhesive effects of natural extracts out of *Myrothamnus flabellifolia* Welw. and *Rumex acetosa* L. against *Porphyromonas gingivalis*

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Porphyromonas gingivalis (ATCC 33277), a Gram-negative anaerobic bacterium, is strongly associated with adult periodontitis, one of the major public health problems. The bacterium expresses a number of well-characterized virulence factors, including lipopolysaccharides, fimbriae and cysteine proteinases, designated gingipains, which contribute to an ultimately loss of tooth. One of the first steps in initiation of this inflammatory disease is the adhesion process of the bacterium to epithelium pocket and therefore it represents one possible aspect of therapy by blocking the adhesion. Basically, the bacterial adhesion is mediated by fimbriae, proteolytic activity and hemagglutinins. Toxicity of the extracts on *P. gingivalis* (1 mg/mL) and KB cells (0.1 mg/mL) were tested using MTT assay and did not show any toxic effects. Polyphenol-enriched extracts from *M. flabellifolia* Welw. and *R. acetosa* L. showed strong inhibition (two titer steps) of hemagglutinating activity against *P. gingivalis*. The adhesion of *P. gingivalis* on epithelial buccal cells (KB cells, ATCC CCL 17) was investigated and quantified by flow cytometric analysis (FACS). Preincubation of *P. gingivalis* with 1 mg/mL, resp. 0.1 mg/mL extract of *M. flabellifolia* Welw. reduced adhesion by 40%, resp. 20%. Incubation with 1 mg/mL, resp. 0.1 mg/mL extract of *R. acetosa* L. reduced adhesion by 35%, resp. 15%. Preincubation of KB cells with the extracts showed no significant inhibition of adhesion, supporting the thesis that adhesion factors on *P. gingivalis* are affected by the extracts.

Real-time PCR analysis revealed that 10 μg/mL extract of *M. flabellifolia* Welw. did not influence the expression of *Lys*- or *Arg*-Gingipains, but enhanced the expression of *Fim*A gene in *P. gingivalis* threefold compared to an untreated control.

SL52 Changes in caffeic acid derivatives, alkamides/polyacetylenes and phenylalanine ammonia-lyase (PAL) activity in three *Echinacea* species in response to salinity stress

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Echinacea is a native North American medicinal plant widely used as a non-specific stimulant for the immune system [1]. Three species of *Echinacea* (*E. purpurea*, *E. pallida* and *E. angustifolia*) were exposed to 50, 75 and 100 mM NaCl under a hydroponic system to test the hypothesis that salinity stress will cause qualitative and quantitative changes in caffeic acid derivatives, alkamides/polyacetylenes, as well as the activity of phenylalanine ammonia-lyase. Hydrophilic and lipophilic compounds were extracted from the roots by Accelerated Solvent Extractor (ASE) then analysed simultaneously by HPLC. Cichoric acid was the main phenolic compound in *E. purpurea* (35.1 mg g^{-1} dry weight), whereas echinacoside was the major phenolic in *E. pallid*a and *E. angustifolia* (11.8 and 7.5 mg g^{-1} dry weight, respectively). The lipophilic fraction contained mainly alkamide 8/9 in *E. purpurea* and *E. angustifolia*, while in *E. pallida* it was ketone 22 [2]. Low salinity stress increased caftaric acid and cynarin content in *E. purpurea*, and chlorogenic acid, caffeic acid, cynarin and alkamide 8/9 in *E. angustifolia*. However, high salinity stress diminished the content of cichoric acid, alkamides 2 and 8/9 in *E. purpurea* as well as echinacoside in *E. angustifolia*. In *E. pallida*, moderate and high salt concentrations significantly increased cichoric acid and ketones 20 and 21. HPLC-based assay of PAL activity revealed no significant changes in salt-stressed *E. pallid*a or *E. angustifolia*, while in *E. purpurea*, the highest salt concentration significantly increased PAL's specific activity. These changes in marker compounds, in response to salinity stress, will affect the quality of *Echinacea* products. **References:** [1] Hu, C. and Kitts, D.D. (2000) J. Agric. Food Chem. 48:1466 – 1472. [2] Numbers of alkamides and polyacetylenes are according to Bauer R. and Remiger P. (1989) Planta Med. 55:367 – 371.

SL53 Gene expression profiling of blood cells from rats treated with different fractions of a standarized aequeous willow bark extract – evidence for immuno- and neuromodulatory activities

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Willow bark extracts (WB) are known for their anti-inflammatory activities. Their efficacy is primarily attributed to the content of salicin and its derivates. However, WB have a substantial content of polyphenols known to possess also antioxidant, neuroprotective and regulatory effects on signalling pathways. We used the gene microarray technique (Agilent whole Genome Rat array) to analyse the expression of the complete rat genome (~ 41 000 genes) which may be modulated by WB or its different fractions. The WB STW 33-I was sequentially separated into five fractions of different polarity, using toluene, ethyl acetate, n-butanol and ethanol in addition to the aqueous extract. 84 rats were treated with these fractions (30 mg/kg). Blood samples (3 ml) of treated and untreated rats (n = 12) were collected in PAX-gene collection tubes. RNA was isolated and the gene modulation was determined in two to three animals per group. After filtering the data to remove genes showing no differences between the differentially treated samples, an ANOVA analysis was performed. The resulting gene set was clustered both hierarchically and by applying SOTA. The analysis revealed groups with a consistent gene expression according to the treatment. 1143 genes were identified as differentially regulated. They included genes for AMP-activated protein kinases, hyaluronoglucosidase 6, chondroitin sulfate N-acetylgalactosamyltransferase 2, H2-Ea (histocompatibility class II antigen) or Gria2, a glutamate receptor, activated in a variety of normal neurophysiologic processes. WB appears to be more than an anti-inflammatory agent. Microarray expression profiling will support us in understanding the mode of action of phytopreparations.

SL54 Allele-specific primers for diagnostic PCR authentication of *Halenia elliptica*, a traditional Tibetan medicinal plant

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Halenia elliptica D. Don. is a biennial herb belonging to the family Gentianaceae that has a long history of widespread use in traditional Tibetan folk medicine. It has beneficial effects on the liver, and is used to treat gall conditions and various other diseases [1,2]. Based on rDNA ITS sequences of *Halenia elliptica* and the other samples from *Swertia* and *Lomatogonium*, respectively, a pair of allele-specific diagnostic primers, was designed for differentiating *H. elliptica* from its adulterants (*Swertia angustifolia, S. erythrosticta, S. franchetiana, S. punicea, S. macrosperma, S. przewalskii, S. tetraptera* and *Lomatogonium oreocharis*) by PCR. Before the diagnostic PCR, the primer pair, TIS 4 and ITS 5, for amplifying the whole ITS region was used to validate template DNA and to obtain the appropriate template DNA for the diagnostic PCR. Diagnostic PCRs were performed using the diagnostic primers with the total DNAs of the original plants as a template. When the annealing temperature was raised to 60 °C, only the template DNA of *H. elliptica* could be amplified whereas the diagnostic PCRs of the other samples were all negative. The diagnostic PCRs have been repeated many times and have played an important role in authenticating the herbs of *H. elliptica* in China. This is a major diagnostic improvement, as all other methods for the specific identification of *H. elliptica* are more time consuming and practical assay. *Acknowledgements: This research was supported by the Natural Science Foundation of China (NSFC 30770153 to CY Xue) and the Natural Science Foundation of Yunnan (NSFN, 2006C 0050 M to CY Xue).* **References:** [1] Ho, T.N. and Pringle, J.S. (1995) Gentianaceae. In: Wu, Z.Y. and Raven, P.H. eds. Flora of China. Science Press, Beijing and Missouri Botanical Garden, St. Louis, MO., 16:1 – 139. [2] Yang YC. Tibetan Medicines. Qinghai People Press, Qinghai. (in Chinese); 1991. p. 111.

SL55 An ethanol extract from *Alstonia scholaris* is a potent irritation inhibitor in human skins

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Alstonia scholaris is a plant, known for having bitter tonic and astringent properties in India and the Philippines; it is particularly useful for chronic diarrhea and dysentery [1]. These potentials allow us to investigate the applicability of *A. scholaris* extract as a cosmetic ingredient; basically we have measured its ability to inhibit the release of pro-inflammatory cytokines, including MCP-1 (monocyte chemotactic protein-1), IL-6, and IL-8, thus providing anti-inflammation effect on the human keratinocytes. In an anti-inflammatory assay, measuring the degree of inhibiting inflammatory cytokines release in human normal keratinocyte HaCat cells, we have observed that the ethanol extract of *A. scholaris* significantly inhibit cytokine production in 1 μM retinoic acid treatment. We have also found that our extract, whose concentration is 50ppm, inhibits the release of MCP-1 and IL-6 by 81.2% and 79.7%, respectively. Moreover, our extract has inhibited the release of IL-8 by 72.2% at concentration of 100ppm, which is remarkably comparable to the result of non-treated condition. In our further human clinical tests, we have demonstrated that the extract of *A. scholaris* inhibited skin irritation against retinol at concentration of 0.1%. Moreover, it does not reveal the skin primary irritation, which allow us to rule out the possibility to primarily irritate human skin through 24 h occlusive patch test in lower back skin (n = 40). These results show that extract of *A. scholaris* has sufficient irritation inhibitory effect as well as skin safety, thereby

providing us with a useful cosmetic ingredient for anti-irritation cosmetics that target to sensitive skins. **References:** [1] Satyavati, G. and Gupta, A. (1987) In: Medicinal Plants of India [2] Indian Council of Medical Research. India.

SL56 Biotransformation of menthol by sporulated surface cultures of *Penicillium* sp. and study of the pathways involved

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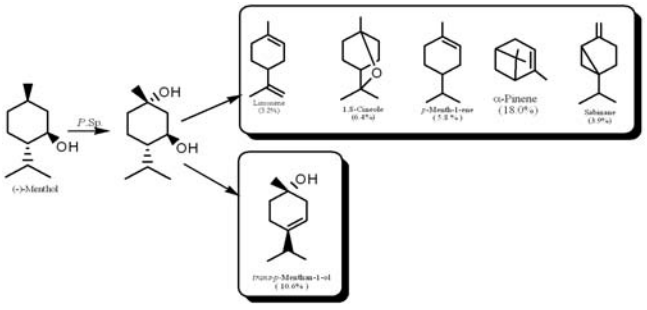
A simple and efficient method was developed to carry out biotransformation reactions on terpenoid compounds. For these experiments, sporulated surface culture of *Penicillium* sp. was inoculated on solid media in conical flasks. After a short incubation period, the spores germinated and a mycelia culture was formed. After 1 week, the cultures had completely sporulated and bioconversion reaction was started. For this purpose, known volume of menthol was added onto the sporulated surface culture. After 7 days, a period during which transformation took place, menthol was extracted with Et_2O three times and after evaporation, recognition by GC and GC/MS was followed. The main bioconversion product obtained from menthol by surface *Penicillium* sp. was α-pinene (18.0%), trans-p-Menthan-1-ol (10.6%), p-Menth-1-ene (5.8%), sabinene (3.9%) 1,8-Cineole (6.4%), and limonene (3.2%) using sporulated surface culture. The pathways involved in the biotransformation of menthol by *Penicillium* sp. to main products are also discussed.



References: [1] Demyttenaere, J. and De Kimpe, N. (2001) J. Mol. Catal. B Enzym. 11:265 – 270.

SL57 Bioassay-directed isolation of hypotensive alkaloid from *Holarrhena pubescens*

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Holarrhena pubescens belongs to the family Apocynaceae, commonly known as "kurchi" is highly reputed in traditional medicine as a remedy for amoebic dysentery and other intestinal ailment. Bioassay-directed fractionation [1] of the ethanolic extract of *Holarrhena pubescens* resulted in the isolation of steroidal alkaloids i.e. holamide and pubscinine. Holamide showed a three proton doublet at 1.45 (J= 6.56 Hz) and two AB doubles at 3.17 and 3.00 each for on proton (J= 12.06 Hz) in the 1 H NMR spectrum suggested that it belongs to conanine series of alkaloid (a class of compounds with the steroid nucleus and a five member heterocyclic ring with nitrogen). In contrast pubscinine showed one methyl at 1.28 while the doublet is missing, a three proton singlet was observed at 2.28 due to a vinylic methyl indicated a double bond in the 18,20 – epimino ring of the conanine series of alkaloids. In anaesthetized rats, the holamide and pubscinine caused a fall in blood pressure in a dose-dependent manner. Pretreatment of animals with atropine completely abolished the hypotensive response of acetycholine; whereas hypotensive effect of holamide and pubscinine were not modified by atropine [1]. Similarly, acetylcholine produced contractile effect in guinea-pig ileum, which was antagonized by atropine, however both holamide and pubscinine failed to produced any stimulant response on guinea-pig ileum. These data indicate that the steroidal alkaloids i.e. holamide and pubscinine from *Holarrhena pubescens* mediated hypotensive response through a mechanism different to that of acetycholine. **Reference:** [1] Aftab, K. et al. (1996) Adv. Exp. Med. Biol. 404:429 – 442.

SL58 Saffron and its constituents: new pharmacological findings

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Crocus sativus L. commonly known as saffron is a perennial stemless herb of the Iridaceae family and widely cultivated in Iran. Commercial saffron comprises the dried red stigma. Compounds considered pharmacologically active and important in saffron are volatile agents (e.g. safranal), bitter principles (e.g. picrocrocin) and dye materials (e.g. crocetin and its glycoside, crocin). In this review, new pharmacological effects of saffron and its constituents on topic such as some new clinical trials on saffron, effect on sexual activity, genoprotective effect, and prevention of cerebral, renal and skeletal muscle ischemia will be discussed. Saffron tablets (200 – 400 mg) changed some hematological and biochemical parameters. However, these alterations were in normal ranges and they were not important clinically. Saffron (200 mg) showed a positive effect on sexual function with increased number and duration of erectile events seen in patients with erectile dysfunction after taking it for ten days ($P < 0.001$). The aqueous extract of *C. sativus* stigmas and its constituents, crocin and safranal repressed the genotoxic potency of methyl methane sulfonate (MMS)-induced DNA damage in multiple mice organs (liver, lung, kidney and spleen), using comet assay. Saffron extract and its constituents showed some protective effects on different markers of oxidative damage in hippocampal tissue from ischemic rats and against lower limb ischemia-reperfusion in rats.

SL59 Discovery of impact taste molecules in natural products by an integrated analytical and chemosensory platform

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Consumers demand for healthy and tasty foods which challenges researchers to identify flavour molecules capable to impart and/or modulate gustatory as well as somatosensory sensations (e.g. sweet and salt enhancers, bitter-masking, cooling, tingling, etc.). In addition, flavour and food scientists face other challenges such as ingredient performance (e.g. unique taste signature, stability, and solubility), applicability, and the request of products without artificial ingredients. Therefore products from plant materials are screened in order to identify and deliver naturally occurring ingredients. The presentation will highlight some challenges in the development of screening tests and some of the different scientific assays developed to identify and characterize new active molecules in natural products. Some of the recent discoveries will be presented as examples of the success of the use of such an integrated platform which include sensory screenings and advanced analytical tools among others.

SL60 Mushroom tyrosinase activity of phenolic compounds isolated from *Greyia flanaganii* (bolus)

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Pigmentation has become an important phenotypical characteristic in the pharmaceutical, medicinal as well as in the cosmetic field. Plants are inexpensive resource that can be utilized to inhibit tyrosinase activity as well as melanin production [1]. Ethanolic leaf extract of *Greyia flanaganii* (Bolus) showed significant ($p < 0.05$) anti-tyrosinase activity when L-tyrosine was used as a substrate exhibiting the 50% inhibitory concentration (IC_{50}) of 32.62 μg/ml. The extract exhibited significant ($p < 0.05$) 9.89% reduction of melanin content at 6.25 μg/ml and no toxicity on melanocyte cells was observed at the highest concentration (400 μg/ml) tested. Seven phenolic compounds; 2',4',6'-trihydroxydihydrochal-

cone (**C1**), 2',6',4-trihydroxy-4'- methoxydihydrochalcone (asebogenin/lyonogenin) (**C2**), 2',6'-dihydroxy-4'-methoxydihydrochalcone (**C3**), 5,7-dihydroxyflavanone [(2*S*)-pinocembrin] (**C4**), 2',6'-dihydroxy-4',4-dimethoxydihydrochalcone (**C5**), (2*R*,3*R*)-3,.5,7-trihydroxy-3-*O*-acetylflavanone (pinobanksin-3-acetate/dihydrogalangin-3-acetate) (**C6**) and a possible novel compound (**C7**) were isolated from the ethanolic leaf extract of *Greyia flanaganii*. The isolated compounds were tested for their antioxidant, cytotoxicity and tyrosinase activities. **C1** resulted in significant ($p < 0.01$) antityrosinase activity exhibiting an IC_{50} value of 17.86 µg/ml as compared to the other compounds and the positive control Kojic acid. **C1** also showed low toxicity effect on melanocytes cells (IC_{50} of 95.56 µg/ml) and caused 10% reduction of melanin content at 1.5 µg/ml. **C1, C2, C3** and **C4** showed good antioxidant activity (IC_{50} values ranged from 0.895 ± 0.04, to 19.5 ± 0.11 µg/ml). The leaf extract, **C1, C7** and **C2** exhibited a minimum inhibitory concentration of 250, 250, 500 and 12.5 µg/ml against *Propionibacterium acnes*. The leaf extract of *Greyia flanaganii* can be considered as effective tyrosinae inhibitors and can be used for treating dermatological disorders such as age spot, melasma, actinic damage etc. **Reference:** [1] Momtaz, S. et al. (2008) J. Ethnopharmacol. 119:507 – 512.

SL61 Spectral aliasing in 2D-NMR. A straightforward method to considerably increase the resolution of signal clusters and facilitate identification of two cubebin epimers in *Drimys winteri*

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Drimys winteri J.R. et G. Forster (Winteraceae) is a well-known plant in South America used as an insecticide and in the treatment of various inflammatory diseases. Phytochemical studies have shown the presence of diverse secondary metabolites such as terpenoids, sesquiterpenes, flavonoids and lignans [1]. In the latter, epimers of cubebin are difficult to distinguish in complex mixtures. In typical 2D NMR experiments, the width of signals in the carbon dimension of HSQC, HMBC, etc. is much larger than what is observed in a 1D carbon spectrum. This makes it difficult to resolve signals that are only a few Hertz apart, rendering assignment and structure determination difficult or impossible. The brut-force method of increasing the number of time increments is impractical as it imposes too long acquisition times especially when many spectra are required or when series of analyses have to be made. Recording spectra with a 10-ppm carbon window [2] is a powerful method to improve the spectral resolution by a factor of 20 – 25 without increasing experimental time and approaches the resolution of 1D carbon spectra. Many applications have been developed among which diffusion measurements of complex mixtures [3] and kinetic measurements [4]. We illustrate the power of the Computer-Optimized Spectral Aliasing (COSA) by recording 10-ppm HSQC and HMBC spectra of a mixture of two epimers of cubebin extracted from the leaves of *D. winteri*. **References:** [1] Muñoz, O. et al. (1999) Plantas medicinales de uso en Chile. Editorial Universitaria. Santiago, Chile. [2] Vitorge, B. et al. (2009) Chem. Commun. 950 – 952. [3] Vitorge, B. et al. (2006) Anal. Chem. 78:5601 – 5606. [4] Gasparini, G. et al. (2008) Chem. Commun. 26:3034 – 3036.

SL62 Rapid quantification of 14 saponins of *Maesa lanceolata* by UPLC-MS/MS

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Triterpene saponins are a class of plant natural products with a wide range of bioactivities, and therefore they are a promising research subject. In previous work a triterpenoid saponin mixture was isolated from the leaves of *Maesa lanceolata* and the compounds were identified [1,2]. These compounds showed virucidal, haemolytic, molluscicidal and anti-angiogenic activity [3,4]. Maesasaponin II displays the highest anti-angiogenic activity, but is only present in very small amounts in the plant. To increase this amount, a platform of combinatorial biosynthesis in the plant was developed. By introducing genes involved in saponin biosynthesis we are attempting to identify new active compounds, and a higher production of the known compounds. In the first phase of the project, only small amounts of transgenic plant material are available. Therefore it is important to use very sensitive analytical methods. For the fast and sensitive analysis of the extracted and purified plant samples, ultra-performance liquid chromatography (UPLC) was coupled to a triple-quad mass spectrometer for MS/MS detection (TQD). The intensity of the signal obtained by fragmentation of the sodium adducts of the saponins was optimized by addition of sodium acetate to the mobile phase. The method was linear over the investigated concentration range with a correlation coefficient higher than 0.99. Furthermore the method was shown to be repeatable and accurate and therefore suitable for screening of the saponin production of plants transformed with genes involved in saponin biosynthesis. References: 1. Apers, S. et al. (1998) J. Pharmaceut. Biomed. 18:737 – 743. 2. Apers, S. et al. (1999) Phytochemistry 52:1121 – 1131. 3. Apers, S. et al. (2001) Planta Med. 67:528 – 532. 4. Apers, S. et al. (2002) J. Pharm. Belg. 57 (Hors-série) 1:47 – 48.

SL63 Rapid identification of hydroxycinnamate esters in *Miscanthus giganteus* and *Miscanthus goliath* as substrates for polyphenol oxidase using ESI-MS/MS

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Miscanthus giganteus and *Miscanthus goliath* are perennial grasses that are native to subtropical and tropical regions of the world. In the UK and Europe, *Miscanthus* is being extensively trialed as a biofuel. We have recently demonstrated polyphenol oxidase (PPO) and laccase activity in this temperate grass; however, no endogenous substrates have yet been identified. Here we describe studies on the isolation of PPO substrates and PPO and laccase activity from two *Miscanthus* varieties. The free phenol fraction of *Miscanthus* leaves and stems were extracted with cold aqueous methanol and extracts were partially purified on a C_{18} reversed-phase extraction cartridge. Phenols were separated on a HPLC C_{18} column under a methanol gradient, detected with a photo diode array (PDA) detector and individual peaks were collected for analyses by electrospray ionization-tandem mass spectrometry (ESI-LC-MS/MS). Four caffeoylquinic acids (1-CQA, 3-CQA, 4-CQA and 5-CQA), four feruloylquinic acids (1-FQA, 3-FQA, 4-FQA and 5-FQA) and three coumaroylquinic acids (1-*p*-CQA, 3-*p*-CQA and 5-*p*-CQA) were identified in the leaves and stems of *Miscanthus giganteus*. Furthermore, free ferulic acid and *p*-coumaric acid were detected in the stem fraction. In *Miscanthus goliath*, four caffeoylquinic acids (1-CQA, 3-CQA, 4-CQA and 5-CQA), four feruloylquinic acids (1-FQA, 3-FQA, 4-FQA and 5-FQA), 4-coumaroylquinic acids (1-*p*-CQA, 3-*p*-CQA and 4-*p*-CQA and 5-*p*-CQA), free caffeic acid and *p*-coumaric quinic were detected in both leaf and stem extracts. Caffeoylquinic acid diastereoisomers and feruloylquinic acid diastereoisomers were also detected in these fractions. In addition, dicaffeoylquinic acid and dicoumaroylquinic acid and 2-*o*-caffeoylglucarate were also detected in the leaves.

SL64 Highly efficient, sensitive and selective molecular screening of acetylcholinesterase inhibitors of natural origin by SPE-LC/ESI-TOF-MS and novel TLC-based bioauthography

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Compounds with acetylcholinesterase (AChE) inhibitory properties play an important role in therapy of mild and moderate Alzheimer's disease (AD) [1]. Among others *Amaryllidaceae* alkaloids with galanthamine-like skeleton possess their strong reputation. In clinical trials symptomatic improvements in some patients taking galanthamine prescribed drug

were observed [2]. New findings suggest also a role of galanthamine as an adjunctive treatment in major depression [3]. In the presented paper significant improvements in screening methodology of AChE inhibitors among *Amaryllidaceae* alkaloids were elaborated. It comprised optimized pressurized liquid extraction (PLE) of plant materials followed by highly selective solid-phase extraction (SPE) using Oasis HLB cartridges. Pure alkaloidal fractions were analyzed by a newly developed high-performance liquid-chromatography (HPLC) on a 3 µm Atlantis HILIC silica stationary phase combined with recently introduced electrospray ionization (ESI) octopole -orthogonal acceleration time-of-flight (oa TOF)-mass spectrometry (MS) with high mass accuracy (about 2 ppm) and high sensitivity [absolute limit of detection (LOD) for galanthamine was about 43 fg at signal-to-noise 13:1]. Moreover, a newly developed and validated TLC-bioautography permit galanthamine sensitivities at pg levels. In this way, more potent than galanthamine AChE inhibitor namely 1,2-dihydrogalanthamine in *Narcissus jonquilla* 'Pipit' extract could be found (with IC_{50} value 0.19 µM) lower of about 42% than that of galanthamine) [4]. *Acknowledgments: I would like to thank Ms. Dominika Dyk for her assistance in laboratory work, and The Polish Ministry of Science and Higher Education for funding the new LC/ESI-TOF-MS system and financial support of this research project (Grant No 2 P05 F 006 29).* **References:** [1] Hostettmann, K. et al. (2000) Curr. Org. Chem. 4:973 – 1010. [2] Assal, F. (2008) J. Am. Geriatr. Soc. 56:946 – 947. [3] Elgamal, S. and MacQueen, G. (2008) J. Clin. Psychopharm. 28:357 – 359. [4] Mroczek, T. (2009). J. Chromatogr. A 1216:2519 – 2528.

SL65 A new pyridine cysteine-sulphoxide identified in *Allium stipitatum*

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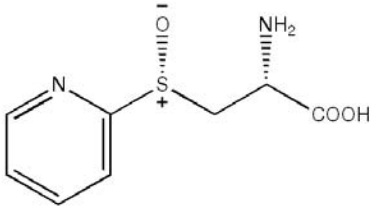
Allium stipitatum Regel is known as ornamental plant in Europe. In Central Asia it is used as spice and in folk medicine and known as "Musir" or "Anzur". *Allium stipitatum* belongs to subgenus *Melanocrommyum section Megaloprason*. A newly developed HPLC-MS/MS method was used for screening on amino acids, amines and sulphoxides. Amino acid derivatives were analysed as corresponding *o*-phthaldialdehyde derivatives. Besides the already described cysteine sulphoxide methiin, a new compound was identified, which is a 2 pyridyl-S-*L*-cysteine sulphoxide (Figure). Structure elucidation was performed by HRESI, NMR, IR and polarometric measurements. This compound could also be found in further members of the section *Megaloprason* and is probably useful as chemical marker for this group. Interestingly, the recently described 3 pyrrol-S-(+)-*L*-cysteine sulphoxide [1], which is also characteristic for the subgenus *Melanocrommyum*, could not be found in these species carrying the corresponding pyridine derivative. It can be assumed that both biogenetic pathways are excluding each other. Additionally to the above described structure elucidation, the alliinase of *A. stipitatum* could be partially characterized.



(*R*)-2-amino-3-((*R*)-pyridin-2-ylsulfinyl)propanoic acid

Reference: [1] Jedelská, J. et al (2008) J. Agric. Food Chem. 56:1465 – 1470.

SL66 Jacaranone derived glucosidic esters from *Jacaranda glabra*

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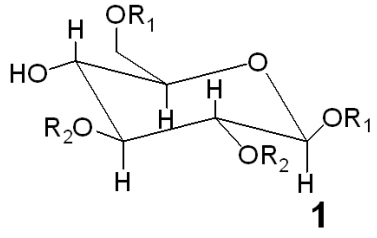
The genus *Jacaranda* (Bignoniaceae) native to the New World, but also widely cultivated in the Old World, contains 49 species. Recently, a review of the ethnobotanical and pharmacological uses of *Jacaranda* species has pointed out interesting biological and chemical perspectives with regard to skin illnesses and protozoa related diseases [1]. In our current project on the validation of anti-protozoal activity of plants traditionally used in Ecuador, the dichloromethane extract of the leaves of *Jacaranda glabra* (DC.) Bureau & Schumann has shown promising activity against *Plasmodium falciparum* K1 strain. Activity guided isolation yielded 4 novel glucosidic esters (**1 – 4**) containing quinolacetic acid (R_1), phenylacetic acid (R_2) and para-hydroxy-phenylacetic acid (R_3) moieties. The compounds identified by NMR experiments and MS techniques exhibited activity against *Pl. f.* K1 strain (IC_{50} **1**: 1.1, **2**: 0.6, **3**: 0.6 and **4** 0.5 µg/mL) and low cytotoxicity on L-6 cells, except for compound **1** (IC_{50} **1**: 2.6, **2**: > 90, **3**: 87 and **4** 85 µg/mL). In addition, 4 ethnobotanical preparations were found active. Similar structures have been previously reported in literature [2,3].



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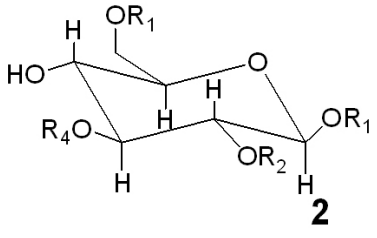

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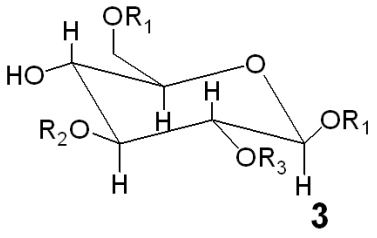

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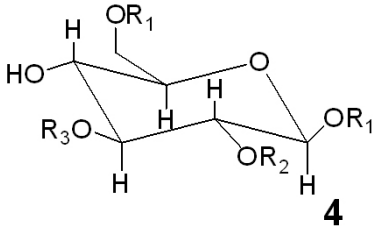

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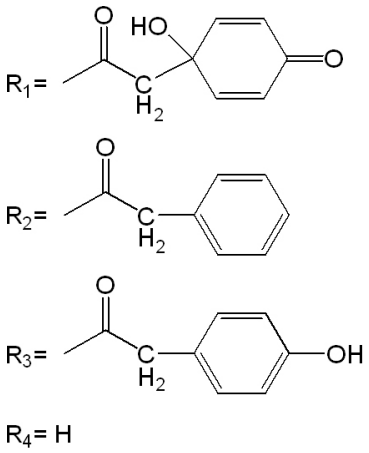

Acknowledgements: This research is part of a dissertation funded by the Austrian Exchange Service (ÖAD). **References:** [1] Gachet, M.S. and Schühly, W. (2009) J. Ethnopharmacol. 121:14 – 27. [2] Tian, X.Y. et al. (2006) J. Asian Nat. Prod. Res. 8:125 – 132. [3] Ming, D.S. et al. (1998) J. Nat. Prod. 61:377 – 379.

SL67 Quality control and standardisation of phytomedicines – From cultivation of medicinal plants to its clinical application

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In the recent years with ever growing commercialization in the field of herbal medicines, there has been an instant demand for quality control of the drugs used in this system. The studies on the identity, purity and quality of the genuine drug will enhance information in checking the adulteration. A set of standards would not doubt be detterent on substitution and adulteration and also an aid both for 'Drug law Enforcement' as well as for Safety Assessment of the Finished Herbal Products. In the present paper an attempt has been made for a sequential study of the Quality Control of Phytomedicines starting from -Selection of Medicinal Plants, Good Agricultural Practices (GAP), Cultivation, Good Field Collection Practices (GFCP), Organized and Unorganized Drugs, Source and Period of Collection, Identification and authentication, Storage, Chemical Standardisation, Assay, Good Manufacturing Practices (GMP), Pre Clinical studies up to Clinical Approach, with special reference to maintain Standardisation at each and every stage. Besides above protocols, this study deals with approaches towards establishing the Safety & Quality starting from preliminary examination of a medicinal plant, its morpho-anatomical, pharmacognostic, physicochemical and analytical parameters, foreign organic matter, pesticide residue, radioactive and microbial contamination, chemical assay, finger printing of different extractives using modern extractors, Chromatographic and Spectroscopic techniques, phytochemical screening, quantitative analysis of inorganic constituents and standardisation with special reference to marker compounds in plant species and their fingerprinting along with its modern perspectives. Different stages, i.e Quality Control Studies of Raw medicinal plant, Controlled Studies on Method of Processing, Quality Control Studies of Finished Phytomedicines and Standardisation Proceedures at each stage from birth of the medicinal plant up to clinical application of herbal medicine have been described. An emphasis has been given on the protocols which are required for Registration of phytomedicines. An example is cited with standardisation and quality control studies of *Salvadora persica* carried out in our laboratories.

SL68 Models of organic certification in herbal sector of Western Balkan

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Transition from central economy to market economy in Balkan region has left visible marks on natural resources. At times when clear legal basis for property over natural resources and public spaces still doesn't exist, we are facing with specific chaos in herbal sector that result in extensive exploitation of medical plants and their illegal export [1,2]. One of models for sustainable picking of wild medical plants and their turnover on local and international market is organic certification. Actual approaches and experiences in this sector are presented in this study. Organic certification is aimed toward safety, sustainability and better administration of plants. Process of organic certification is very complex. Evaluation and identification of safety includes the following: application to company in charge for organic certification; visiting places of future exploitation (site visit with representatives of certification company); mapping of territory for gathering medical plants, mushrooms, forest fruits; and ensuring ecological certificate, issued by ministry in charge, that would guaranty safety of the territory in terms of pollution, use of pesticides, artificial fertilizers, global influences. In administration, it is necessary to conduct permanent education of harvesters and other people employed in herbal sector, to conduct accreditation of people, development of more solid legislation and more effective monitoring and inspections. In order to reach sustainability in use of natural resources, it is necessary to have on disposal the following: list of wild medical plants, their biomass, models of reproduction, affiliation with living forms, conservation status, etc. Even though many international certification houses are active on the territory of Western Balkan (KRAV Sweden, IMO Switzerland – Germany, SOIL Association Great Britain, NOP – National Organic Program for the US Market, BIO SUISSE Switzerland and others) this segment still suffers from serious problems in reaching sustainability [3]. Study is demonstrating experience from various geographical areas of western Balkan, particularly in Bosnia and Herzegovina. **References:** [1] Redzic, S. (2006) Proc 1st IFOAM Intern. Conf. Organic Wild Production, 117 – 141. [2] Redzic, SS (2007) Collegium Antropol. 31:869 – 890. [3] Redzic S (2006) Proc 4th Balkan Botanical Congress, 42.

SL69 More clinical studies with herbal medicines: A requirement for the future

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Within the last 20 years the use of herbal medicine, especially in the European countries, was steadily growing. However, in the recent years the sales volume remains static, or at least the growth figures were lower. This development goes in line with the number of published clinical trials with these herbs and products. The frequency distribution demonstrates that since 1990 the number of clinical trials in this field is steadily growing until 2005. After a two-year stagnation the number of published clinical trials falls by 45.9% from 2007 to 2008, with a tendency to further decrease in 2009 (see figure 1). On the other side the number of clinical trials in the functional food area is constantly increasing. The reason for this tremendous decline is manifold. However, to strengthen the economic position of phytomedicine, and to further increase their acceptance also by consumer, pharmacists, and physicians, high quality clinical studies are necessary. Without clinical trials there will be no further success stories and PR opportunities like those of *Gingko*, *Saw Palmetto*, *Hypericum*, *Pelargonium*, and many others. Herbals should not avoid the direct comparison with conventional drugs in clinical studies since many of them have the potential to demonstrate that they are as active as conventional drugs, with fewer side effects. Only high quality studies in strict accordance with the GCP guidelines will strengthen the position of herbals in medicine, and will ensure herbals the chance of further expansion and growth.

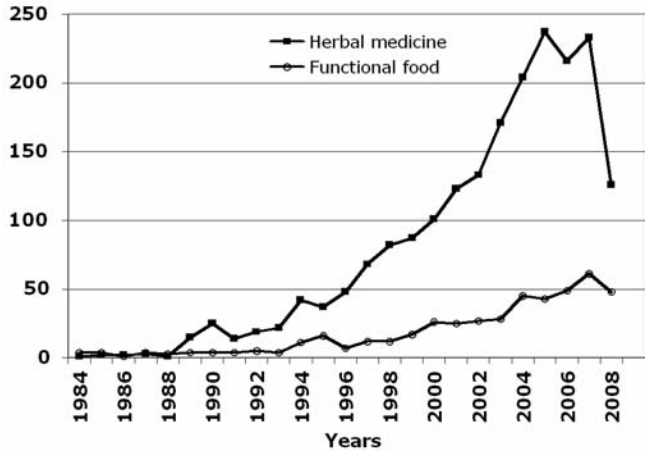

Figure 1: Number of published randomized controlled clinical trials with herbal medicines and functional foods listed in the database PubMed.

SL70

Dry olive leaf extract promotes avant-garde apoptosis in melanoma cells; switch from caspase- dependent to caspase- independent pathway

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Aim of work: It is well known that malignant melanomas respond poorly to chemotherapy. Having in mind efficacy of some traditional approaches in treatment of apoptotic unresponsive cancers, it was of interests to evaluated sensitivity of melanomas to compounds present in total dry olive leaf extract (DOLE) with known antimicrobial, anti-inflammatory and antioxidative properties. Applied methods: Crystal violet and MTT viability assays, analysis of cell cycle distribution of PI stained cells, Anex/PI double staining for detection of apoptotic/necrotic cell death, Western blot analysis of relevant protein and Real time PCR analysis of gene expression; solid melanoma were induced by subcutaneous inoculation of B16 cells in singeneic C57BL/6 strain. Main results: DOLE strongly abrogated growth of B16 cells *in vitro* as well as *in vivo*. Its intraperitoneal application resulted in significant reduction of tumor size. Decreased B16 cell viability was associated with its morphological transformation, Go/G1 arrest and delayed apoptosis with recognizable signs of early and late phases. Surprisingly, key executioner molecules – caspases were continuously inhibited at both- protein and gene levels while the expression of caspase-independent endonuclease – Endo G was exaggerated, thus indicating its involvement in finalization of apoptotic process. Time limited upregulation of cell protective Bcl-2 within first two hours of incubation with DOLE, followed with its rapid and permanent decrease, possibly contributed to switch from caspase-dependent to caspase-independent pathway. **Conclusions:** Compounds present in DOLE are capable to overcome insensitivity of melanoma cells to classical apoptosis by unconventional mechanisms, indicating that data presented are worthy of further investigation. *Acknowledgements: This work was supported by Serbian Ministry of Science and Technological Development (Grant Nº 143029)*

Poster

Topic A: Lead finding from Nature

PA1

Extracts of *Leonorus cardiaca* L. influence the histone deacetylase activity

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Recently, histone deacetylase modulators (HDACm) are studied as potential new treatment of cardiovascular diseases [1]. However, progress in developing new drugs is largely dependent on the existence of bioassays well-suited for modulator screening. Herein, we are presenting a modified fluorimetric enzyme assay for HDAC modulation to study the influence of plant extracts on histone deacetylase activity. Nuclear extracts from HeLa cells served as source of HDAC protein. The assay was checked for interferences with different classes of primary plant metabolites such as sugars, chlorophylls, acids, and applied on extracts from plants used in European folk medicine against heart problems. The herb *Leonorus cardiaca* L. (Laminaceae) was selected for a detailed study. We have tested extracts of different polarity for their effect on HDAC activity. The plant material was extracted with dichloromethane, ethyl acetate, methanol and water. The methanol extract was most active and fractionated further by solid phase extraction on RP-18 cartridges. The initial results showed that the extracts obtained with 20% and 40% methanol have a potential to inhibit HDAC activity. HPLC fingerprinting showed flavonoids and caffeoylquinic acid derivatives as major components. The effects were confirmed by a cell based assay. **References:** [1]Kong, Y. et al. (2006) Circulation 113:2579 – 2588.

PA2

Proteolytic activity in the genus *Euphorbia*

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Over 110 latices of different plants are known to contain at least one proteolytic enzyme. Proteolytic enzymes from plant latex have received special attention in the pharmaceutical industry and biotechnology due to their property of being active over wide range of temperature and pH. In the latex of Euphorbiaceae only proteolytic endopeptidases of the subclass serine proteases (EC 3.4.21) are mentioned in the literature [1]. For detecting proteolytic activity in the genus *Euphorbia* we collected latex of 30 different plants which are located in the Botanical Garden Berlin. To determine proteolytic activity we used the fluorogenic substrate BODIPY FL- casein (Moleculare Probes, Inc., USA) [2]. The change in fluorescence was compared to that produced by trypsin as standard; with a volume of 100 µl of each trypsin concentration. The plants were divided into two groups; group I has its activity in the range between 5 µg/ml and 2.5 mg/ml trypsin (27 plants) and group II has a very strong proteolytic activity higher than 2.5 mg/ml trypsin (3 plants). Proteolytic activity of latices in the genus *Euphorbia* could be expect as a chemotaxonomic characteristic because every tested sample was able to make a significant change in fluorescence compared to the control. To investigate if serine proteases are the active endopeptidases, the latex samples were pre-incubated with serine protease specific inhibitors (AEBSF (4-(2-Aminoethyl)-benzenesulfonyl fluoride hydrochloride) and Aprotinin) and the remaining activity was determined. 9 plants are not influenced by the inhibitors, 19 plants were influenced but had still a remaining proteolytic activity, and by 2 plants the residual activity was negligible. **References:** [1] Domsalla, A., Melzig, M.F. (2008) Planta Med 74:1 – 13. [2] Menges, D.A. et al. (1997) Anal. Biochem. 251:144 – 147.

PA3

Inhibition of xanthine oxidase activity by *Filipendula* species

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Filipendula species, in particular *F. ulmaria* (meadowsweet), have traditionally been used for the treatment of gout [1,2]. One of the primary targets for the treatment of gout is xanthine oxidase (XO), an enzyme responsible for the oxidation of hypoxanthine and xanthine to uric acid

[3]. The scope of this research was to analyze the XO-inhibitory activity of *F. ulmaria* and a related species, *F. vulgaris*, in order to rationalize the traditional use of these plants. Extracts were tested for their capacity to inhibit XO by spectrophotometrical determination of the rate of uric acid formation. The anti-gout drug allopurinol and its active metabolite oxypurinol were used as positive controls. Methanolic extracts of the flowers of *F. ulmaria* and *F. vulgaris* were demonstrated to have high inhibitory activity towards the XO enzyme with IC50-values of 6.2 ± 0.6 μg/ml and 8.9 ± 0.8 μg/ml, respectively. In comparison, IC50-values of allopurinol and oxypurinol were 2.6 ± 0.9 μg/ml and 1.0 ± 0.2 μg/ml, respectively. Thin-layer chromatographic analysis showed flavonoids to be present as the main constituents in the methanolic extracts. This is in line with literature data reporting *F. ulmaria* to contain 5.1 – 7.3% of flavonoids in the flowering tops [4]. Since flavonoids have previously been described as inhibitors of XO [5], these constituents probably attribute to the activity of the methanolic extracts of *F. ulmaria* and *F. vulgaris*. The XO-inhibitory activity of *F. ulmaria* and *F. vulgaris* extracts further substantiates the traditional use of these medicinal plants in the treatment of gout. **References:** [1] Madaus, G. (1938) Lehrbuch der biologischen Heilmittel. Georg Thieme Verlag, Leipzig. [2] Gessner, O., Orzechowski, G. (1974) Gift- und Arzneipflanzen von Mitteleuropa. Carl Winter Universitätverlag, Heidelberg. [3] Schlesinger, N. (2004) Drugs. 64:2399 – 2416. [4] Lamaison, J.L. et al. (1992) Pharm. Acta Helv. 67:218 – 222. [5] Cos, P. et al. (1998) J. Nat. Prod. 61:71 – 76.

PA4 Bioassay guided isolation of antiplasmodial constituents from *Ormocarpum kirkii*

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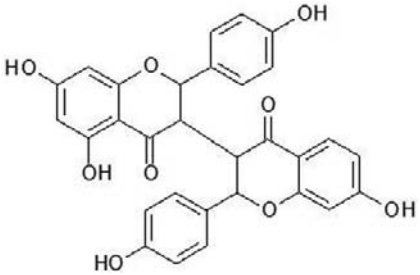
Ormocarpum kirkii (Papilionaceae) is a shrub or small tree that grows in South West Africa [1]. It is used in traditional medicine against fever-like symptoms, such as in malaria [2]. Previous screening of medicinal plants used in Tanzania showed that the extract of the root of *O. kirkii* has high *in vitro* antiplasmodial activity (IC_{50} of 15.6 – 31.3 μg/ml against *Plasmodium falciparum*) [3]. Since this genus is used in traditional medicine and limited phytochemical information is yet available, further investigation by means of bioassay guided isolation was performed. After extraction and liquid-liquid partitioning, ten fractions were obtained from the ethyl acetate layer by means of column chromatography. Using HPLC, several compounds were obtained that were identified according to their NMR- and mass spectra and optical rotation measurements, and evaluated for their antiplasmodial activity. Four new compounds were identified: 5,5''-dimethoxy-diphysin (IC_{50}= 15.8 μM), 4''-hydroxydiphysolone (IC_{50} = 16.4 μM) and two biflavonoids, liquiritigeninyl-(I-3,II-3)-naringenin (IC_{50} = 30.3 μM) and apigeninyl-(I-3,II-3)-naringenin (IC_{50} > 64.0 μM).



Liquiritigeninyl-(I-3,II-3)-naringenin

References: [1] Gillet, J.B. et al. (1971) Flora of Tropical East Africa. Kew Publishing. Richmond, UK. [2] Maregesi, S.M. et al. (2007) J. Ethnopharmacol. 113:457 – 470. [3] Maregesi, S.M. (2008) Ph.D. Thesis, University of Antwerp, Belgium.

PA5 Immunopharmacological potential of the leading chemical constituents from *Leuzea carthamoides*

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Pharmacological potency and utilization of the medicinal plant *Leuzea carthamoides* DC (Wild.) Iljin is so far attributed to ecdysteroids [1], although the plant contains also other interesting biologically active compounds: lignans, stilbenoids, flavonoids and sesquiterpene lactones. Effects of the three above indicated characteristic types of secondary metabolites were tested on immunobiological responses triggered by lipopolysacharide and interferon-γ under *in vitro* conditions using murine resident peritoneal macrophages. Namely, production of nitric oxide was investigated. The series of test agents encompassed ecdysteroids occurring as major components of the plant extract: 20-hydroxy-ecdysone, polypodine B, ajugasterone C, makisterone A, ponasterone A and inokosterone [1, 2] supposed to be significant for the often reported pharmacological activities of preparations derived from this species [1, 3]. However, the tested ecdysteroids did not interfere with the immunobiological activity. A small activity was recorded only in high concentrations of inokosterone and ponasterone A [4]. The efficacy of ecdysteroids was compared with lignans: tracheloside, carthamoside and their aglycones, and with N-feruloylserotonins isolated from the species, exhibiting higher immunomodulatory activity [5]. Searching for relations with the significant immunomodulatory activity of sesqui-terpene lactones from *Laser trilobum* (L.) Borkh. (Apiaceae), it has been tested also the main sesquiterpene constituent of *L. carthamoides*: cynaropicrin (a guaianolide type α-exomethylene γ-lactone), known as a strong immunoactive compound [6]. The results indicate that phenylpropanoids and sesquiterpenoids are more competent for the immuno-pharmacological feature of the species than the ecdysteroids. Acknowledgement: Supported by Grant Agency of the Czech Republic, grant No. 305/07/0061. **References:** [1] Lafont, R. et al. (2002) The Ecdysone Handbook. [2] Vokáč, K. et al. (2002) Collect. Czech. Chem. Commun. 67:124 – 139. [3] Lafont, R., Dinan, L. (2003) J. Insect. Sci. 3:7. [4] Harmatha, J. et al. (2008) Steroids 73:466 – 471. [5] Harmatha, J. et al. (2004) In: Polyphenols Communications 2004, University of Helsinki. [6] Cho, J.Y. et al. (2004) Biochem. Biophys. Res. Commun. 313:954 – 961.

PA6 Antiprotozoal activities of *Melampyrum arvense* and its secondary metabolites

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The genus *Melampyrum* (Scrophulariaceae) consists of annual semiparasitic plants and represented by two species, *M. arvense* and *M. pratense* in the flora of Turkey [1]. In the continuation of our efforts to find natural antiprotozoal compounds from the Scrophulariaceae family [2,3], the crude MeOH extract of *M. arvense* was found to show *in vitro* activity against parasitic protozoa *Trypanosoma brucei rhodesiense* and *Leishmania donovani* (IC_{50} values 8.8 and 30.5 μg/ml). The MeOH extract was suspended in H_2O and partitioned against $CHCl_3$. Both aqueous and $CHCl_3$ phases showed activity against two parasites and possessed no cytotoxicity against mammalian L6 cells (IC_{50} > 90 μg/ml). An activity guided fractionation of the H_2O extract using MPLC and repeated column chromatography techniques afforded ten pure compounds, whose structures were elucidated by spectroscopic methods (^1H and ^{13}C NMR, ESIMS) to be iridoid glucosides aucubin **(1)**, melampyroside **(2)**, mussaenoside **(3)**, mussaenosidic acid **(4)**, 8-*epi*-loganin **(5)**, flavonoids, apigenin **(6)**, luteolin **(7)**, luteolin 7-*O*-glucopyranoside **(8)**, lignan glycoside dehydrodiconiferyl alcohol 9-*O*- β-glucopyranoside **(9)** and benzoic acid **(10)**. All compounds showed moderate to remarkable trypanocidal activity (IC_{50}s 3.8 – 60.8 μg/ml), with compound **7** being the most potent one. The majority of the metabolites also possessed leishmanicidal properties and again compound **7** was the most active constituent (IC_{50} 3.0 μg/ml). Except for compounds **6** and **7**, all compounds lacked cytotoxicity towards mammalian cells (IC_{50} > 90 μg/ml). This is the first detailed phytochemical study on Turkish *M. arvense* and first report on the

antiprotozoal effect of the plant and its constituents. **References:** 1. Hedge, I.C. (1967) *Melampyrum* L. in: Flora of Turkey and East Aegean Islands Vol. 6, Ed. Davis P.H., University Press. Edinburgh. 2. Tasdemir, D. et al. (2005) Phytochemistry 66:355 – 362. 3. Tasdemir, D. et al. (2008) Phytomedicine 15:209 – 215.

PA7 Oviposition deterrent effects of *Scutellaria brevibracteata* Stapf. essential oil

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Scutellaria is a genus of the Lamiaceae family with about 300 species widespread in temperate regions and on tropical mountains, whose essential oils possess ecological properties such as anti-feedant [1]. *Scutellaria brevibracteata* Stapf. is a species typical of Lebanon whose chemical composition or biological activity were never previously studied. Therefore, in this communication we report on the study of the chemical composition of the essential oil of this plant collected in June 2007 on Mont Chabrouh in Lebanon, on rocky ground at 1500 m a. s.l., and on its activity against the feeding and oviposition behaviour of *Spodoptera littoralis*, a polyphagous insect attacking a number of plant species. The oil was isolated by hydrodistillation [2]. GC and GC/MS analyses evidenced the presence of 48 compounds; the most abundant components were sesquiterpenes (24.9%), particularly sesquiterpenes hydrocarbons (17.3%). Caryophyllene (14.4%) was recognized as the main constituent together with hexadecanoic acid (12.6%), phytol (10.7%) and 4-vinylguaiacol (10.2%). Binary choice bioassays were undertaken to investigate if the essential oil could modulate the feeding behaviour of final stadium larvae of *S. littoralis*; data show that *S. brevibracteata* essential oil did not significantly modulate larval feeding behaviour but deterred the ovoposition behaviour of adult moths. **References:** [1] Rosselli, S. et al. (2007) Biochem. Syst. & Ecol. 39:797 – 800. [2] European Pharmacopoeia 5th ed. (2004) Council of Europe, 217.

PA8 Bioactivity guided and random isolation of polyacetylenic compounds from *Seseli praecox*

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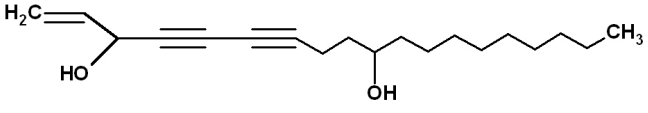
Seseli praecox (Gramisans) Gramisans, (Apiacaee) is an endemic chamaephyte species from Sardinia. The lipophilic extract of *S. praecox* stems was subjected to a bioactivity guided fractionation and isolation process with the binding affinity towards human CB receptors as a lead. The activity guided isolation process afforded (R)-falcarinol (panaxynol), which accounted for 12 – 15% of total crude lipophilic extract. Random isolation afforded the new polyacetylenic compound heptadeca-1-ene-4,6-diyne-3,10-diol (dihydroseselidiol) (fig). With respect to seseldiol reported by [1] dihydroseselidiol differs by the missing double bond between C-8 and C-9.



Falcarinol is known for its notorious instability. In order to identify the main degradation products sunlight exposed and freezer stored falcarinol was subjected to HPLC isolation. Falcarinone and *E*-Heptadeca-1,8-diene-4,6-diyne-3,10-diol were found to be the main oxidation products of sunlight exposed falcarinol, while 4,5-dihydrofalcarinol was found in freezer stored falcarinol. It was shown that only falcarinol elicits allergic contact dermatitis in patch tests, while the degradation products have no allergenic potential [2]. We found that only freshly isolated falcarinol showed CB receptor affinity and that this might be the mechanism of action for its pro-allergenicity. **References:** [1] Hu, C.Q. et al. (1990) J. Nat. Prod. 53:932 – 935. [2] Hansen, L. et al. (1986) Phytochemistry 25:285 – 293.

PA9 Antioxidant in the Red Kwao Krua (*Butea superba* Roxb.)

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Red Kwao Krua (*Butea superba* Roxb.) is a protected medicinal plant of Thailand. Red substances are released when the tuberous roots of Red Kwao Krua (RKK) are wounded. This study aimed to find the amount and capacity of an anthocyanin in the tuberous roots of RKK. Anthocyanin was determined from the extracted solution of the tuberous roots of RKK with two mobile phases (HCl: formic acid: H_2O, 25: 24: 51 and 7: 51: 42 v/v) using TLC technique [1] and the absorbent wavelength of visible light. The amount of anthocyanin was determined by pH differential technique [2]. The antioxidant capacity was determined by using DPPH [3], ABTS [4] and the inhibition of LDL oxidation techniques [5] in comparison with standard trolox. The R_f values of extracted solution were 0.12 and 0.34 and they absorbed wavelength at the peak of 519 nm also their color changed from red to brown when the pH had been changed from 1 to 14 which are characteristics of anthocyanin [6,7,8]. The diameter of cortex of tuberous root of RKK was correlated with the amount of anthocyanin. Tuberous roots of RKK contained anthocyanin between 69 – 144 μg/g fresh weight. The RKK crude extract had approximately the same IC_{50} as trolox. *Acknowledgements: Suranaree University of Technology and the National Research Council of Thailand (NRCT)* **References:** [1] Bonillard, R., Delaport, B. (1977) J. of Am. Chem. Soc. 99:26. [2] Wrolstad, R.E. et al. (2005) Trends Food Sci. Technol. 16:423 – 428. [3] Chaovanalikit, A . (2004) The 30th Congress on Science and Technology of Thailand. [4] Miller, N. J., Pagan, G. (1998) Free Radical and Antioxidant Protocols 108:325 – 335. [5] Ambra, R. et al (2004) Eur. J. Nutr. 95:23 – 28. [6] Sherma, J., Fried, B. (2003) Handbook of Thin Laler Chromatography. Marcel Dekker, USA. [7] Longo, L., Vasapolla, G. (2006) Food Chem. 94:226 – 231. [8] Adrian, A. et al. (2004) J. Food Compos. Anal. 17:1 – 35.

PA10 Immunosuppressive effects of sesquiterpene lactones from *Laser trilobum* (L.) Borkh

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Sesquiterpene lactones (SLs) are plant metabolites, widely distributed within the families of Asteraceae and Apiaceae. A number of them have proved to inhibit the immune-stimulated secretion of cytokines including the proinflammatory cytokines IL-1β, IL-6 and TNF-α, and the production of nitric oxide (NO). SLs have received ever increasing attention for their promising anti-inflammatory, anticancer, and anti-infectious activities. However, due to their alkylation capabilities, they are generally toxic. Searching for compounds with preserved immunobiological properties and decreased cytotoxicity is therefore a challenge for immunopharmacological research. We have investigated the immune activity of the SLs laserolide, isolaserolide, eudeslaserolide, archangelolide and 2-deangeloyl-archangelolide isolated from *Laser trilobum* (L.), a C. and E. European plant not included in pharmacopoeias. Acetylisomontanolide from *Laserpitium siler* (L.) and the recognized SLs costunolide and helenalin were used for comparative purposes. Their immunobiological effects were screened *in vitro* using rat peritoneal cells primarily activated with lipopolysaccharide (1 μg/mL). The cells were cultured at a density of 2×10^6/mL in complete RPMI-1640 medium for 24 h. The supernatant levels of interferon-gamma (IFN-γ), IL-1β, IL-6, and vascular endothelial growth factor (VEGF) were determined by ELISA. The production of NO was assayed using Griess reagent. In sharp contrast to costunolide and helenalin, the tested SLs were found to be free of cytocidal effects up to a concentration of 50 μM. The highest potential to inhibit cytokine and NO production is possessed by laserolide, which is effective at an IC_{50} of approximately 5 μM. Laserolide also inhibits VEGF, a factor known to be

associated with tumour growth. *Acknowledgements: The work was supported by the grant 305/07/0061 from GACR.*

PA11 Thapsigargin and trilobolide – sesquiterpene lactones with immunostimulatory properties

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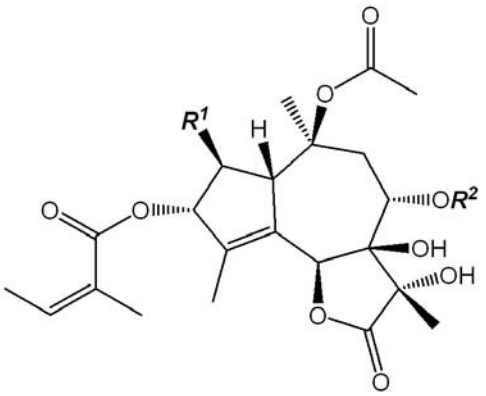
Thapsigargin (TG) and trilobolide (TB) are sesquiterpene lactones of guaianolide type isolated from *Thapsia garganica* L. and *Laser trilobum* (L.) Borkh., respectively. TG is widely used experimentally as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) leading to rapid elevation of intracellular calcium.



	R^1	R^2
Thapsigargin	O-octanoyl	butyryl
Trilobolide	H	(*S*)-2-methylbutyryl

We have investigated effects of TG and TB on secretion of interferon-gamma (IFN-γ). The experiments were done under conditions *in vitro* using rat and mouse peritoneal cells (PECs) and human peripheral blood mononuclear cells (hPBMCs). The concentrations as low as 40 nM and 1 μM were effective ($P < 0.001$) in rat PECs and hPBMCs, respectively, to induce IFN-γ. It was associated with enhanced production of NO by rat PECs. The immunostimulatory effects are mediated by transcription factor NF-κB and depend on the activation of MAP kinases p38 and ERK1/2. The Ca^{2+}-chelating agent BAPTA-AM was unable to suppress the enhancing effects of TG and TB on IFN-γ production. *Acknowledgements: The work was supported by the Grant Agency of the Czech Republic, no. 305/07/0061.*

PA12 New antibacterial terpenes from Cretan propolis

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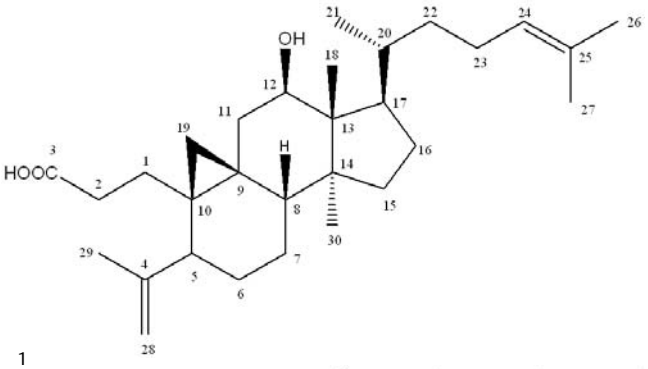
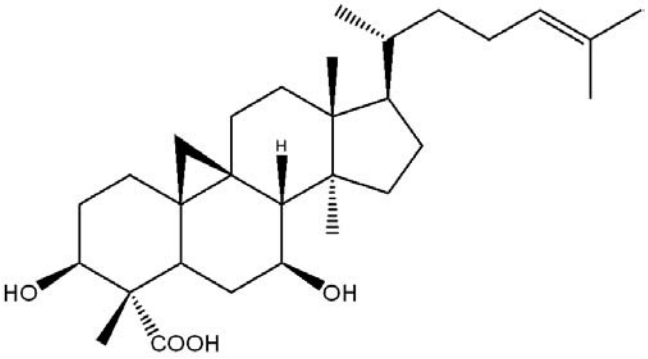
Propolis (bee glue) is a well known natural product with healing properties. Chemical composition of propolis is highly variable and depends mainly on the local flora. Mediterranean region and Greece are characterized by high biodiversity flora, assuming different propolis chemical composition. In this study, propolis from the island of Crete, which demonstrated significant antibacterial activity, was studied. Twenty two compounds, mainly diterpenes, were isolated and their structure elucidated by means of modern spectral methods. Out of them, four were new natural compounds: two cycloartane triterpenes (**1** and **2**) and two diterpenes (**3** and **4**), while another eight compounds were found for the first time as propolis constituents. The majority of the isolated compounds showed significant antibacterial activity against all assayed human pathogenic bacteria and fungi.



1



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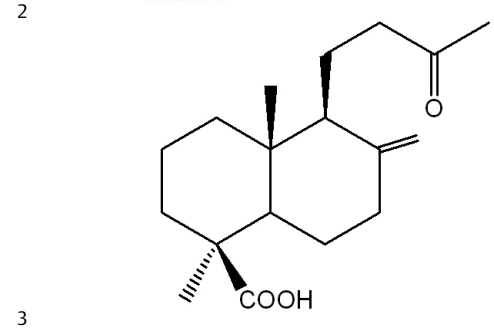

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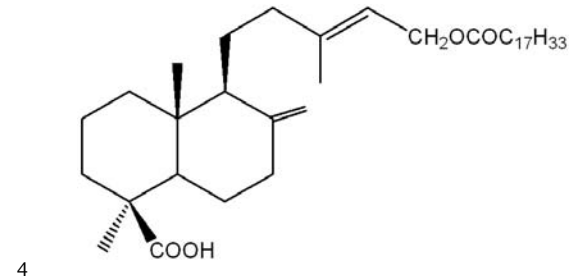

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Acknowledgements: M.P. is grateful for PostDoc grant from the Ministry of Science and Education of Bulgaria (PD-3).

PA13 Shikonin pigments from cultures of *Lithospermum canescens* and *Arnebia euchroma*

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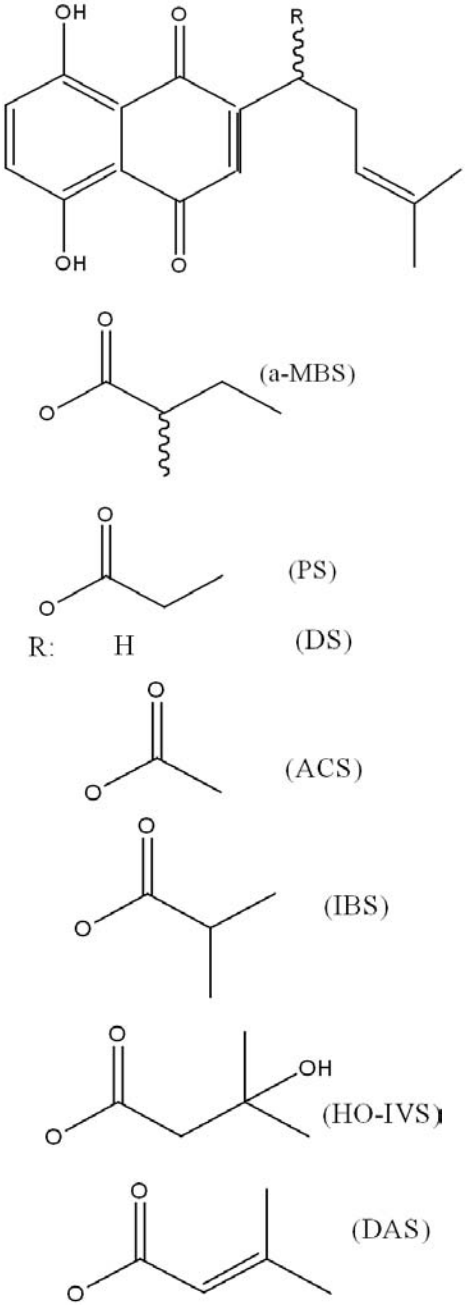
Two plants from Boraginaceae family *Lithospermum canescens* (Michx.) Lehm., a common plant in northern America also known as Indian paint and *Arnebia euchroma* (Royle) Jonst., a perennial plant of the alpine region, were investigated. Shikonin and its derivatives are well known since ancient times and have been used for food products, as dyes for silk [1] and have been reported to possess antimicrobial, antiinflammatory and antitumor activities [2]. In the present study, hairy root cul-

tures of *L. canescens* were established using three strains of *Agrobacterium rhizogenes*: ATCC 15834, LBA 9402 and NCIB 8196. In the n-hexane extract of the hairy root culture of *L. canescens* the presence of acetylshikonin (ACS), isobutylshikonin (IBS), β,β-dimethylacrylshikonin (DAS), β-hydroxyvalerylshikonin (HO-IVS), shikonofuran C and D has been confirmed. The callus culture and cell suspension culture of *A. euchroma* yielded likewise the pigments ACS, IBS, HO-IVS as well as shikonin, α-methylbutyrylshikonin (α-MBS), propionylshikonin (PS) and deoxyshikonin (DS). All chemical constituents have been determined by modern spectral means. Moreover, all isolated compounds showed a very interesting antimicrobial spectrum of activity against all assayed human pathogenic microogranisms.



References: [1] Manjkhola, S. et al. (2005) In Vitro Cell. Dev. Biol. Plant 41:244. [2] Couladouros, E. et al. (1997) Tetrahedron Lett. 38:7263.

PA14 Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania

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Guided by ethnobotanical literature and availability from natural sources, in the framework of our research on odoriferous Tanzania plants, used as edibles or spices, and their biological activities, we report herein the analysis of six samples of essential oils from four *Ocimum* species (*O. basilicum* (A and B), *O. kilimandscharicum*, *O. lamiifolium, O. suave* (A and B). Leaves and flowering tops of these *Ocimum* species were collected from the wild, in Mbeya region, Tanzania. The samples were analyzed by GC and GC-MS. Eighty-one compounds, corresponding to 81.1 – 98.2% of the chemical components of the oils, were identified. Major compounds were either, phenyl propane derivatives or terpenoids, including methyl eugenol, 1,8-cineole, camphor, bornyl acetate, germacrene-D, E-myroxide, germacrene-B, caryophylene oxide and *p*-cymene. The oils were also evaluated for antimicrobial activity against eight bacterial strains and three fungi. The oil of *O. suave* (B), showed the strongest antibacterial activity; *O. suave* (A), O. *kilimandscharicum*, *O. lamiifolium* were moderately active, while *O. basilicum* oil was weakly active. However, none of the oils was active against the fungi species. The study has shown that, *Ocimum* oils could be used potentially as antimicrobial agents, as well as accordingly, as food preservatives against food spoilage microorganisms. *Acknowledgements: This study was partially supported by a grant from the Directorate of Research and Publications, Muhimbili University of Health and Allied Sciences, as well as by a grant from National Kapodistrian University of Athens (70/4/8807), which are gratefully acknowledged.* **References:** [1] Vagionas, K. et al. (2007) Food Chem. 105:1711.

PA15 *In Vitro* antigenotoxic activities of the aqueous extract from Thai Noni's Leaves (ANL) against chemotherapeutic agent, mitomycin C

Thitiorul S[1], Ratanavalachai T[2], Nandhasri P[3], Tanuchit S[4], Jansom C[4]
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The leaves from Noni (*Morinda citrifolia* L.; Rubiaceae) has been increasing popular usage as food supplement and therapeutic medicine especially in form of tea, powder and serum. Their therapeutic effects have been known for various treatments such as malaria, diabetes and topical inflammation [1]. In our previous study, we found that Noni fruit juice has some antigenotoxic effects as demonstrated by significantly decrease in the sister chromatid exchange (SCE) level induced by mitomycin C (MMC) ($p < 0.05$) [2]. This study was focused on antigenotoxic activities of the aqueous extract from Thai Noni's leaves (ANL) against a chemotherapeutic drug, MMC. Chromosomal aberration and SCE assays in human lymphocytes *in vitro* were conducted. The method was performed by pretreatment of ANL at concentrations of 0.8 – 25 mg/ml for 2 h followed by MMC at 3 μg/ml for 2 h. Our result showed that ANL pretreatment could not significantly reduce chromosomal aberration and SCE levels induced by MMC ($p < 0.05$). Combination usage of ANL and MMC also leads to cell cycle toxicity as shown by significantly decrease in mitotic index and proliferation index (compared to that of the negative control). We concluded that ANL pretreatment followed by MMC did not show antigenotoxic potential. However, other form of Noni's leaf extract such as ethanolic extract containing high antioxidant activities would be investigated further to verify the antigenotoxic activities. *Acknowledgement: This study was supported by Research Fund, Faculty of Medecine, Thammasat University, Thailand.* **References:** [1] Wang, M.Y. et al. (2002) Acta pharmacol. Sin. 23:1127 – 1141. [2] Ratanavalachai, T. et al. (2008) Songklanakarin J. Sci. Technol. 30:583 – 589.

PA16 Oleanane saponins from *Guapira graciliflora*

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The Brazilian biodiversity represents a particularly rich source of new biologically active compounds. *Guapira graciliflora* (Mart. Ex J. A. Schmidt) Lundel (Nyctaginaceae) is an endemic small tree found in Atlantic forest and Cerrado which is used in folk medicine for cicatrisation [1]. Despite its medicinal use, there are no reports on its chemical composition. In the present work, we have investigated the constituents of the methanolic extract of *G. graciliflora* leaves collected at Itapetininga, São Paulo State, Brazil. The powdered dried leaves were percolated with methanol. A portion of MeOH extract was partitioned between *n*-BuOH and water. The *n*-BuOH portion was chromatographed on a Sephadex LH-20 gel column, eluted with MeOH. Fractions collected were checked by TLC for the presence of saponins and subsequently purified by HPLC to afford several oleanane saponins including the new derivative 3-*O*-[β-D-xylopyranosy-(1→3)-β-D-galactopyranosyl-(1→3)-β-D-glucuronopyranosyl] oleanolic acid 28-*O*-β-D-glucopyranosyl ester. The compounds were identified by detailed spectroscopic analysis including 2D NMR and ESI-MS as well as acid hydrolysis. These results represent the first data on the chemistry of plants of the genus *Guapira*. The isolated compounds are being currently evaluated in various biological test systems including cytotoxic and antimicrobial assays. *Acknowledgments: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico Tecnológico (CNPq) for financial support and a fellowships to Severi JA.* **References:** [1] Rocha-Coelho, F.B. et al. (2005) Rev. Eletron. Farm. 2:52 – 55.

PA17 Profiling of *Iris germanica* extracts by LC-PDA-MS and off-line microprobe NMR

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The roots of German iris (*Iris germanica* L., Iridaceae) have been traditionally used for various topical applications including the treatment of sores and freckles [1]. Characteristic constituents of the root are isoflavones which reportedly show anti-inflammatory and anti-oxidative properties [1] [2]. For these reasons iris root extracts are used as cosmetic ingredients. Lipophilic and polar extracts of iris root were submitted to a phytochemical profiling by semi-preparative HPLC and off-line NMR measurements in a 1 mm TXI microprobe (active volume 5 µL) [3]. A total of 18 compounds were purified in sub-milligram to milligram amounts via two successive chromatographic steps on a SunFire column (10 x 150 mm; 5 µm, Waters) with a gradient of acetonitrile in water containing 0.1 % HCOOH. The compounds were identified as isoflavones, isoflavone glycosides and acetovanilllone by analysis of on-line MS and PDA, and off-line NMR data including HSQC and HMBC spectra. The activity of the isolated compounds on the proliferation of endothelial cells is currently being investigated. The example demonstrates the applicability of the off-line HPLC microprobe NMR approach as a robust means for a rapid chemical and biological characterization of the constituents of plant extracts. **References:** [1] Rahman, A.U. et al. (2003) J. Ethnopharmacol. 86:177 – 180. [2] Wollenweber, E. et al. (2003) Planta Med. 69:15 – 20. [3] Griffin, J.L. et al. (2002) Analyst 127:582 – 584.

PA18 Bioactivity-guided isolation of acetylcholinesterase inhibiting constituents of the flowers of Bride's Feathers (*Aruncus dioicus*)

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Acetylcholinesterase (AChE) inhibition is the main strategy in the clinical management of Alzheimer's disease. Natural products have already established themselves as an excellent source for AChE inhibiting compounds (e. g. galantamine, huperzine A), but new substances with better efficacy and less side effects are still demanded. The aim of the performed study was a bioactivity-guided isolation of constituents of the aerial parts of Bride's Feathers (*Aruncus dioicus* (Walter) Fernald, Rosaceae) in order to obtain novel AChE inhibitors. The activity of the obtained extracts and sub-fractions was monitored by an *in vitro* enzyme inhibition assay based on the method of Ellman [1]. Investigations of extracts of different polarity and from varying plant parts identified a methanolic extract of the flowers as ideal starting material. Activity guided isolation afforded several active principals with moderate activity e. g. quercetin-3-O-β-D-galactopyranoside (= hyperin), quercetin-3-*O*-β-D-glucopyranoside (= isoquercitrin), 4'-O-methylquercetin-3-*O*-β-D-galactopyranoside (= tamarixetin-3-*O*-β-D-galactoside), 3'-*O*-methylquercetin-3-*O*-β-D-glucopyranoside (= isorhamnetin-3-*O*-β-D-glucopyranoside) as well as a mixture of 3,4-dicaffeoyl-α-D-glucopyranoside and 3,4-dicaffeoyl-β-D-glucopyranoside. Among them the isomer-mixture of caffeic acid glucosides showed the highest activity with an IC_{50} value of 67.8 µM (CI_{95}: 50.8 – 90.2 µM). Isolation of two further inactive but prominent compounds resulted in the identification of two new monoterpene lactone glucosides: aruncolactonoside (= 4*R**-hydroxy-5*S**-(2-methylprop-1-enyl)-3-(2-(β-D-glucopyranosyloxy)-ethylid-*E*-en)-dihydrofuran-2(3 H)-one and isoaruncolactonoside (= 4*R**-hydroxy-5*S**-(2-methylprop-1-enyl)-3-(2-(β-D-glucopyranosyloxy)ethylid-*Z*-en)-dihydro-furan-2(3 H)-one). *Acknowledgements:* This work was supported by the Austrian Science Fund (P18379). **References:** [1] Ellman, G.L. et al. (1961) Biochem. Pharmacol. 7:88 – 95.

PA19 Role of phenolic compounds release by *Peganum harmala* L. on germination and growth suppression of *Convolvulus arvensis* L.

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Peganum harmala L. (Zygophyllaceae) is a medicinal herb with a wide range of pharmacological properties [1]. In traditional medicine, various parts of the plant are used to treat several diseases [2]. In order to search for new integrated strategies to improve weed management, we investigated potential herbicidal activity of *P. harmala* against *Convolvulus arvensis.* Sixteen g of fresh *P. harmala* leaves were soaked in 100 ml distilled water for 24 h. After filtering and centrifuging, the extract was diluted with sterile distilled water to concentrations of 4, 8, 12 and 16 % (w/v). Fifteen seeds of *C. arvensis* were placed in Petri dishes containing 5 ml of each *P. harmala* extract (or distilled water for control). Results indicate that in 8, 12 and 16 % extract concentrations, a significant reduction in germination, seedling length, seedling dry weight and total chlorophyll content of *C. arvensis* was obtained when compared to control. In general, the effect was concentration-dependent whereas there was a significant correlation between each parameter and extract concentration. The adverse effect on *C. arvensis* indicates the presence of some water-soluble inhibitory substances in *P. harmala* aqueous extract. Upon HPLC analysis, seven phenolic compounds were identified in the extract. Between these phenolics, 4- hydroxybenzoic was present in maximum amount followed by caffeic acid and ferulic acid. The study concluded that *P. harmala* aqueous extract exerted phytotoxicity effect on germination and growth of *C. arvensis,* possibly by releasing water-soluble phenolic acids. *Acknowledgements: This research was supported by project MSM 6046070901.* **References:** [1] Kartal, M. et al. (2003) J.

Pharm. Biomad. Anal. 31:263 – 269. [2] Agedilova, M.T. et al. (2006) Chem. Nat. Comp. 42:226 – 227.

PA20 Discovery of benzofuran derivatives in Ratanhiae radix as novel inhibitors of NF-κB activation

Baumgartner L[1], Fakhrudin N[2], Atanasov AG[2], Heiss E[2], Schwaiger S[1], Ellmerer EP[3], Rollinger JM[1], Dirsch VM[2], Stuppner H[1]
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The roots of Red Rhatany (*Krameria triandra* Ruiz et Pavon), listed in several pharmacopoeias, have been used in traditional medicine for their anti-inflammatory, anti-microbial and astringent potential [1]. Until now the polyphenolic constituents, high molecular weight procyanidines, were held responsible for the activities. The aim of this study was to determine whether the anti-inflammatory activity of Ratanhiae radix may be also due to other constituents. As a general model to assess the anti-inflammatory potential of Ratanhiae radix constituents, the inhibition of TNFα-induced NF-κB activation was chosen. Thus, the dichloromethane root extract of *K. triandra* was analyzed for its ability to inhibit the NF-κB activation in a TNFα-induced NF-κB-luciferase reporter assay in HEK293 cells. Since the crude extract showed a moderate activity (26% inhibition at 10 μg/ml), it was further phytochemically investigated, ending up in the isolation and identification of nine benzofuran and two tetrahydrofuran lignan derivatives. All isolates were analyzed for their ability to inhibit NF-κB activation. Among the tested compounds six benzofuran derivatives showed a significant inhibition of NF-κB activation at a concentration of 10 μM. Half of them, ratanhiaphenol II, 2-(4-hydroxyphenyl)-5-(*E*)-propenylbenzofuran and 2-(2,4-dihydroxyphenyl)-5-(*E*)-propenylbenzofuran, inhibited NF-κB activation to the level of unstimulated control cells with IC_{50} values of 8.9 μM ± 0.9, 1.9 μM ± 0.3 and 2.9 μM ± 0.6, respectively. The mode of action of the isolated compounds within the NF-κB pathway remains to be elucidated. *Acknowledgements: This work was granted by the Austrian Science Foundation (NFN: Drugs from Nature Targeting Inflammation, B89-B03).* **References:** [1]Carini, M. et al. (2002) Planta Med 68:193 – 197.

PA21 eNOS-activating polyphenol fractions from Austrian red wines

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Red wine polyphenol extracts (RWPE) lead to an increased endothelial nitric oxide synthase (eNOS) activity in EA.hy926 endothelial cells [1]. Trans-resveratrol seems to partly contribute to this effect by inducing eNOS expression [1]. This study aims to identify further major active components by bio-assay guided fractionation. The principal components of 60 representative red wines from Austria were quantified. Two samples (Blaufränkisch and Merlot) were selected for bio-assay guided fractionation using EA.hy926 endothelial cells and the [^{14}C]L-arginine/[^{14}C]L-citrulline conversion assay measuring eNOS activity. Dealcoholised concentrates were separated by polystyrene column chromatography to obtain the first eluate (FE) and the red wine polyphenol extract (RWPE). Further partition of RWPE of the two samples was done by liquid-liquid dispersion with ethylacetate and water resulting in a polar fraction (PF) and an apolar fraction (AF). Subsequently, the AF of both wines was fractionated using solid-phase extraction with increasing MeOH concentrations into five solid phase fractions (SPF1 – SPF5) which showed differences in their HPLC-ELSD fingerprints. The RWPE of both red wine samples showed enhanced eNOS activity at a concentration of 600 μg/ml. The FE, tested in equal concentration, were inactive. AF of the samples revealed an effect on eNOS activation at 200 μg/ml, whereas the complementary PF did not increase enzyme activity at 400 μg/ml. SPF1, SPF2 and SPF3 were completely inactive but SPF4 of both wines was activating the enzyme significantly. SPF5 only of the Merlot, containing one single peak as detected by an evaporative light scattering detector, enhanced NO release. This peak was identified as Quercetin. Ongoing fractionation of SPF4 will lead to the identification of further eNOS activating compounds. **Reference:** [1] Räthel, T.R. *et al.* (2007) J. Hypertens. 25(3):541 – 549.

PA22 Anthraquinones from the Roots of *Rennellia elliptica* Korth. (Rubiaceae)

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Rennellia elliptica Korth. (Rubiaceae) is a Malaysian tropical shrub. Decoction of the roots of *R. eliptica* Korth. is taken by the locals for general good health and also claimed to be antidiabetic [1]. The powdered roots of *R. elliptica* Korth. collected from Kuala Keniam, National Park, Pahang were successively extracted with hexane, dichloromethane and methanol. The dichloromethane crude extract was fractionated using column chromatography packed with acid-washed silica gel eluted with various compositions of hexane-dichloromethane and dichloromethane-methanol in increasing polarity. The isolation of anthraquinones was accomplished following repeated column chromatography and preparative thin layer chromatography. The chemical structures were established on the basis of spectral data. As a result, two new anthraquinones, 1-hydroxy-2-methoxy-6-methyl-9,10-anthraquinone and 1,2-dimethoxy-6-methyl-9,10-anthraquinone were isolated and characterized along with eight known anthraquinones which were nordamnacanthal, damnacanthal, rubiadin, rubiadin-1-methyl ether, lucidin-ω-methyl ether, 2-formyl-3-hydroxy-9,10-anthraquinone, 3-hydroxy-2-methyl-9,10-anthraquinone and 3-hydroxy-2-hydroxymethyl-9,10-anthraquinone from the roots of this plant. One of major the anthraquinones, 2-formyl-3-hydroxy-9,10-anthraquinone has been shown to posses *in vitro* antileishmanial and antiplasmodial activities[2]. This is the first phytochemical report on *Rennellia elliptica* Korth. *Acknowledgements: 1. Universiti Teknologi MARA 2. Mr Shamsul Khamis* **References:** [1] Mat Salleh, K., Latiff, A. (2002) *Tumbuhan Ubatan Malaysia*: Universiti Kebangsaan Malaysia & Kem, Sains, Teknologi dan Alam Sekitar. [2] Sittie, A.A. et al. (1999) Planta Med. 65:259 – 261.

PA23 Isoliquiritigenin isolated from the roots of *Glycyrrhiza uralensis* attenuates glutamate- and LPS-induced oxidative stress of neuronal and microglial cells

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In neuronal cells, excessive glutamate stimulation leads to accumulation of reactive oxygen species (ROS) which ultimately contribute to cell death in stroke, trauma and other neurodegenerative disorders. Activated microglia produce inflammatory mediators, including nitric oxide (NO) and proinflammatory cytokines such as interleukin (IL)-1β, (IL)-6, and tumor necrosis factor (TNF)-α as well as neurotoxic substances, which are thought to be responsible for brain injuries and various neurological diseases, including stroke, Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis, and cerebral ischemia [1,2]. Thus, inhibition of oxidative stress and production of proinflammatory mediators would be an effective therapeutic approach to alleviate the progress of neurodegenerative diseases. In this study, we examined whether the isoliquiritigenin (**1**) of *G. uralensis* would protect HT22-immortalized hippocampal cells and BV2-microglial cells against glutamate- LPS-induced oxidative stress, respectively. The 5 μM isoliquiritigenin reduced ROS (67.13%) and increased the viability (17.6%) in glutamate-treated HT22 cells by FACS and MTT assay. The protective action of **1** is mainly due to its antioxidative effect. In using microglial BV2 cell, **1** is an effective inhibitor accompanied by the decrease in expression of inducible NO synthase (iNOS) as one of important proinflammatory mediators. The inhibition of iNOS was evident by the reduction of NO. In addition, **1** also effectively inhibited LPS-induced cytokines, such as IL-1β, IL-6 by regulating the transcriptional levels. These results suggest that **1** inhibits microglial cell activation by decreased NO and proinflammatory cytokines. These results represent new insights about protection of neuronal and microglial cell by the **1** after oxidative stress stimulation. *Acknowledgements:1. Biogreen 21 program from RDA. Korea, 2. BK21 program from MEST, Korea.* **References:** [1] Tan, S. et al. (1998) J. Neuro-

chem. 71:95 – 105. [2] Gonzalez-Scarano, F., Baltuch, G. (1999) Annu. Rev. Neurosci. 22:219-240.

PA24 Protein fraction from three genus of *Passiflora* seeds cause red blood cell lysis *in vitro*

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The use of herbal treatments within veterinary medicine is not new, however, the knowledge in this area is not so wide, when compared with human herbal medicine. We know that plants may contain many different chemical constituents and some of them have strong pharmacological activity. Secondary metabolites clearly provide most of the therapeutic activity of medicinal plants, although it showed that some small peptides rich in cysteine (defensins) could be responsible for antimicrobial activity. In this work, we looked for antibacterial peptides with potential use in veterinary medicine. With this goal, protein fraction from seeds of *Passiflora setacea, Passiflora gibertti* and *Passiflora mucronata* were used to determinate antibacterial activity against Gram-negative and Gram-positive bacteria, using tests *in vitro*. Seeds flour were obtained and utilized to produce protein fraction by precipitation with ammonium sulphate salt. After the dialysis against water using membrane of very small pore (3,000 Daltons), this material was used in several *in vitro* assays employing different bacteria and culture media. When the culture media blood agar was utilized, the red cell lysis halos between 10 and 15 mm were observed in all samples analyzed. Banerjee and Sen [1] reported the purification of a protein called lectin from *C. tiglium* seeds with haemagglutinating activity towards erythrocytes of sheep and cow as well as haemolytic activity towards rabbit erythrocytes. Thus, probably, some proteins such as lectin could be one of the compounds present in the protein mixture used in this work. Although this protein fraction showed to be an effective antibiotic *in vitro*, the characterization of this haemolytic molecule is necessary, as well as its inactivation or isolation to ensure safe and proper use *in vivo*. **References:** [1] Banerjeek, K., Sen, A. (1983) J. Biosci. 5:121 – 129.

PA25 Volatile components and antifeedant activity of the essential oil from *Scutellaria hastifolia* L.

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The genus *Scutellaria* comprises about 300 species of herbs or subshrubs and rarely shrubs, some of which with ecological properties such as anti-feedant and anti-fungal [1]. These properties are often due to the presence of essential oils [2]. *Scutellaria hastifolia* L. (leafy skullcap) is a perennial gramineous plant with the running rhizome; it is a rare species in Lithuania, where is called *Ieçialape kalpoke* and grows in meadows (often in the water-meadows) and riversides [3]. No report on the essential oil of this species has been found in the literature so far, therefore in this communication we describe the volatile compounds of *S. hastifolia* collected in Lithuania on June 2007 and its activity against the feeding and egg laying behaviour of *Spodoptera littoralis*, a polyphagous insect attacking a number of plant species. The oil was isolated by hydrodistillation [4]. The GC and GC/MS analyses evidenced the presence of 50 compounds, accounting for 92.1% of the oil that consisted mainly of terpenoids, particularly sesquiterpenes (61.5%), among which sesquiterpenes hydrocarbons (44.9%) prevailed over oxygen containing sesquiterpenes (16.6%). The most representative compounds were caryophyllene (12.9%), germacrene D (7.7%), caryophyllene oxide (6.9%), hexadecanoic acid (6.3%) and hexahydrofarnesylacetone (5.6%). Binary choice bioassays were undertaken to investigate if the essential oil could modulate the feeding behaviour of final stadium larvae of *S. littoralis*. **References:** [1] Bruno, M. et al. (2002) Biochem. Syst. Ecol. 30:793 – 799. [2] Rosselli, S. et al. (2007) Biochem. Syst. Ecol. 39:797 – 800. [3] Galinis, V. (1976) *Scutellaria* L. – In: Natkevičaitė-Ivanauskienė, M. (ed): Flora of the Lithuanian SSR, No 5. Vilnius. [4] European Pharmacopoeia 5th ed. (2004). Council of Europe, 217.

PA26 Semisynthesis and pharmacological investigation of lipo-alkaloids prepared from aconitine

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Processed aconite drugs are widely used in Eastern medicine, principally as painkillers and antirheumatic agents. Active constituents of these drugs are aconitine-type diterpene alkaloids, which exhibit a broad spectrum of pharmacological activities, including antinociceptive and anti-inflammatory effects [1,2]. Unfortunately, most of the aconitine-type alkaloids (aconitine, hypaconitine, mesaconitine) are highly toxic, in contrast to the lipo-alkaloids, which possess far more less toxicity due to the presence of long chain fatty acid moiety in the molecules at C-8. The traditional processing (usually boiling) of the crude aconite drugs afforded the increasing of the concentration of lipo-alkaloids. There is a presumption that these compounds possess anti-inflammatory activity depending on the type of the ester group. Therefore, the aim of our work was to prepare a series of lipo-alkaloids, investigate their anti-inflammatory activity, and establish the correlation between the effect and the quality of the esterifying fatty acids. The present paper reports the semisynthesis of aconitine-derived lipo-alkaloids, prepared according to the modified method of Bai et al [3]. In the reactions, aconitine was esterified by palmitic, stearic, lauric, myristic, palmitoleic, oleic, α- and γ-linolenic, linoleic, eicosapentaenoic and docosahexaenoic acids resulting the corresponding 14-benzoylaconin-8-*O*-esters and pyroaconitine. The reaction mixtures were purified of necessity by gelfiltration, preparative TLC and centrifugal planar chromatography. The structures were proved with the aid of ^{1}H-NMR, JMOD spectra, further, ^{1}H and ^{13}C NMR assignments were determined for all compounds on the basis of ^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC experiments. The COX-1, COX-2 and LTB4 formation inhibitory activity of the lipo-alkaloids were investigated, and found that especially compounds having unsaturated ester groups demonstrated remarkable COX-2 inhibitory activities. **References:** [1] Ameri, A. (1998) Prog. Neurobiol. 56:211 – 223. [2] González-Coloma, A. et al. (2004) J. Chem. Ecol. 30:1393 – 1408. [3] Bai, Y. et al. (1994) J. Nat. Prod. 57:963 – 970.

PA27 Anti-inflammatory and toxicity evaluation of *Maytenus heterophylla* and *M. senegalensis* extracts

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Medicinal extracts of *Maytenus heterophylla* (Eckl & Zeyh.) Robson and *M. senegalensis* (Lam.) Exell used in Mozambican traditional medicine to treat painful and inflammatory conditions were evaluated for their anti-inflammatory potential. Thus, leaf, stem, and root extracts of *M. heterophylla* and the leaf and stem extract of *M. senegalensis* were tested *in vivo* by using the carrageenan-induced paw oedema model in rats. Previous studies report the anti-inflammatory activity of the maitenoic acid, isolated from leaf extracts of *M. senegalensis*. However, there is no reference to the activity of the hydroalcoholic extract of both species, complemented by its toxicological evaluation [1,2]. Two doses were tested in this model, specifically 120 and 240 mg/Kg. In adjuvant-carrageenan-induced inflammation, orally administered extracts, inhibited the acute phase of this experimental model of inflammation. Some of these extracts exhibited potent anti-inflammatory activity throughout the experiment, and were compared against effective NSAID reference drugs, such as indomethacin (10 mg/kg body wt.). The results were expressed as the mean ± SEM and were compared using a one-factorial ANOVA test, followed by a Bonferroni's post-hoc test. A P value less than 0.05 was considered to be statistically significant. The most interesting plant extracts were the leaf hydroalcoholic extract of *M. heterophylla*, and the leaf hydroalcoholic extract of *M. senegalensis.* Furthermore, toxicological data is reported for all five extracts. Acute toxicity tests were tested at 1200 mg/Kg, in mice. At this dose, extracts of *M. heterophylla* revealed to be non-toxic and extracts of *M. senegalensis* have shown to be toxic. These data confirm the traditional use of *M. heterophylla* and *M. senegalensis* for painful and inflammatory conditions, contributing to the pharmacological validation. **References:** [1] Sosa, S. et al. (2007) *Phyto-*

medicine 14:109 – 114 [2] Mueller, M., Mechler, E. (2005) Medicinal Plants in Tropical Countries. Thieme Stuttgard.

PA28 Effects of MSM (methylsulfonylmethane) on SNP (sodium nitroprusside) and H_2O_2 (hydrogen peroxide) induced RAW 264.7 macrophages

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MSM is an organic sulfur compound with therapeutic potential that occurs naturally in various fruits, vegetables, grains and humans [1]. SNP is a direct donor of nitric oxide (NO); high NO concentrations induce cell death [2]. H_2O_2, a non-radical molecule that leads to the generation of highly toxic hydroxyl radicals, could also participate in cell death and various disease processes [3].In this study, we investigated the effects of MSM on SNP- and H_2O_2 -induced RAW264.7 cells. Cell viability and nitrite levels were determined with MTT and Griess assays respectively. For this purpose, after co-incubation with MSM (6mM,12mM,16mM,20mM) for one hour, the cells were incubated with different concentrations of SNP (500 µM,1000 µM,2000 µM) and H_2O_2 (0.5 µM,5 µM,50 µM,500 µM) for 24 h. Our results showed that 500 µM H_2O_2 decreased cell viability significantly and MSM partially restored the effect of H_2O_2 only at a concentration of 6mM. However, MSM potentiates the anti-proliferative effect of H_2O_2 at doses higher than 12mM. Among the doses tested, the SNP concentration which causes less than 80% viability was found to be 2000 µM. MSM could not also restore the anti-proliferative effect of SNP and decreased cell proliferation for all the doses tested ($p < 0{,}001$).There was no significant change in nitrite levels among H_2O_2 treated cells and MSM did not exert any effect on nitrite levels. SNP treatment increased nitrite levels dose dependently ($p < 0{,}001$). 6mM concentration of MSM weekly decreased nitrite levels of 500 µM SNP treated cells. In our previous work we have shown that MSM boosts cell viability and decreases nitrite levels in lipopolysaccharide activated RAW264.7 cells. In this study we tested the same concentrations of MSM. However it does not exert the same strong effects in this stress model. Further research is needed to test the effective dose or mechanism of action of MSM. **References:** [1] Methylsulfonylmethane Monograph (2003) Altern. Med. Revi. 8:4. [2] Espey, M.G. et al. (2000) Ann. NY Acad. Sci. 899:209 – 221. [3] Fridovich, I. (1997) J. Biol. Chem. 272(18):515 – 517.

PA29 Antinociceptive activity of *Ficus deltoidea* aqueous extract in experimental animals

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The aqueous extract of leaves of *Ficus deltoideae* (AFD) was evaluated for antinociceptive activity. The analgesic activity was studied by measuring nociception by formalin, acetic acid and hot plate methods in several *in vivo* experimental models. These methods investigated both the peripheral and central antinociceptive mechanisms [1,2]. The results showed that intraperitoneal administration of AFD at doses of 100, 300, and 1000 mg/kg indicated the present of both peripherally and central mediated activities. In the formalin test, highest inhibition at late paw licking responses caused by the formalin-induced pain was displayed by 100 mg/kg AFD (71.2%) followed by 300 mg/kg (70.0%) and 1000 mg/kg (65.7%) AFD respectively. In contrast, 300 mg/kg AFD demonstrated highest % inhibition at early response (61.4%) followed by 1000 mg/kg (50.9%) and 100 mg/kg of AFD. At early phase, 1000 mg/kg AFD (50.9%) exhibited equal inhibition as compared to acetylsalicylic acid (ASA 100 mg/kg) 50.8%. Animals treated with ASA showed significant inhibition at the late phase (72.7%) and reduced inhibition at early phase (50.8%). Morphine (5 mg/kg) displayed very strong inhibition both at early (92.1%) and late (91.0%) phases. In the acetic acid induced abdominal test, both 300 and 1000 mg/kg AFD significantly reduced the number of writhes in mice with 67.3% of inhibition. 100 mg/kg of AFD however showed low rate of inhibition at 17.4%. In the hot plate test, all extracts exerted significant prolong in the response latency time to heat stimulus. Both 300 and 1000 mg/kg AFD demonstrated early effect at 60 min after administration of AFD and persists until the following fifth hour. The results demonstrate that *F. deltoideae* presents potent antinociceptive activity in mice and rats, which supports its folkloric use as an analgesic. **References:** [1] Ridtitid, W. et al. (2007) J. Ethnopharmacol. 118:226 – 230. [2] Mahmoudi, M. et al. (2008) Fitoterapia 79:361 – 365.

PA30 New labdane diterpenes from *Solidago canadensis*

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Solidago canadensis L. (Asteraceae), commonly known as Canadian goldenrod is a medicinal plant native to North America. Today the plant is widespread and invasive all over Europe and in East Asia, as well. Due to its invasiveness, it is considered a threat to biodiversity. *S. canadensis* has been used by Canadian Indians as traditional anti-inflammatory, antiflogistic, antispasmodic, and antirheumatic medicine. The aim of this study was to investigate the chemistry of roots of Canadian goldenrod. Fractionation and isolation were performed by using VersaFlash CC (normal phase Si-gel and reverse phase C 18), centrifugally accelerated TLC (Si-gel), and preparative HPLC (reverse phase C 18). Chemical structures were determined using 1 D and 2D NMR techniques and MS analysis. The ethanol extract of the roots of *S. canadensis* yielded six labdane-type diterpenes. Three of them were new natural compounds (15,16-epoxy-labdane-7,13-diene-6,16-dione; 15-ethoxy-9,13,15,16-bisepoxy-labdane-7-ene-6-one; 9-hydroxy-15,16-epoxy-16-cyclo-9-friedolabdane-7-ene-6,15-dione), while solidagenone, deoxysolidagenone and *ent*-16-hydroxy-6-oxo-labdane-7,13-diene-15 acid lactone are previously known diterpenes.

PA31 Effect of *Nelumbo nucifera* on nitric oxide production and co-stimulatory molecules

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Nelumbo nucifera Gaertn. (Nymphaeaceae) is a well known aquatic plant which has been used for the treatment of several disorders including inflammation, fever, cough etc. [1]. The hydro-alcoholic extract of *Nelumbo nucifera* rhizome (HENN) showed potent immunomodulatory effect on delayed type hypersensitivity (DTH), phagocytic response and neutrophil adhesion test [2]. The aim of this study is to evaluate the mechanism of immunomodulation involved for the extract and its three solvent fractions viz. ethyl-acetate (EANN), n-butanol (BUNN) and water (AQNN) using the *in vitro* models like nitric oxide (NO) production, expression of co-stimulatory molecules, e.g. CD 40, CD 80 and CD 86 [3]. HENN, EANN, BUNN and AQNN inhibited *in vitro* NO production induced with lipopolysaccharides (LPS, 100 ng/ml). The most significant ($P < 0.001$) inhibition was observed for BUNN (5 µg/ml) compared to control. Expression of CD 40, CD 80 and CD 86 were observed based on the fluorescent intensity produced by the extract and its fractions. The mean fluorescent intensity (MFI) on treatment with CD 40, CD 80 and CD 86 were observed but the maximum reduction of MFI with BUNN ($P < 0.001$) were 19.57, 12.84 and 7.45 respectively. The results supports that BUNN was the most effective fraction of HENN and it acts similarly to that of dexamethasone, a standard immunosuppressive drug. *Acknowledgements: Council for Scientific and Industrial Research (CSIR), Govt. of India, for financial assistance [(file no.-[9/96(0531)2K8-EMRI, 2008] to the School of Natural Product Studies, Jadavpur University.* **References:** [1] Mukherjee, P.K. et al. (2009) J. Pharm. Pharmacol. 61:407-422. [2] Mukherjee, P.K. et al (2009) International Herbal Conference SNPS-09127, 67. [3] Ludger, L. et al. (1999) Am. J. Pathol. 154:1711 – 1720.

PA32 Bio-active secondary metabolites from two Malaysian Clusaceae: *Calophyllum flavo-ramulum* and *C. wallichianum*

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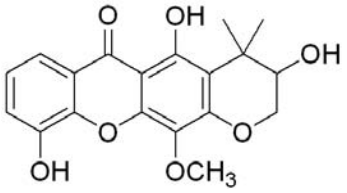
Calophyllum species (Clusiaceae) are known as a rich source of various secondary metabolites and prenylated phenolic derivatives [1] are very common among them. As part of our continuing phytochemical investigation on *Calophyllum* species from Malaysia [2] we report here our results on the fractionation of different crude extracts obtained from two endemic species – *Calophyllum flavo-ramulum* and *C. wallichianum* – which exhibited significant anti-oxidant activities. This study mainly resulted in the identification of phenolic derivatives among which prenylated xanthones and biflavonoids (amentoflavone) appeared as the active principles. The structure of flavoramulone, a new xanthone exhibiting a quite unusual α-dimethyl β-hydroxy tetrahydropyrane was determined through extensive NMR studies.



Finally, biological results dealing with anti-oxidant, antifungal and antileishmanial activities will be discussed. In this matter the leaves of *C. flavo-ramulum* appeared as an interesting renewable source of amentoflavone with a yield exceeding 3 $g.kg^{-1}$ (dried weight). Indeed, this biflavonoid which is found in major medicinal plants such as *Ginkgo biloba* or *Hypericum perforatum* (St John Wort's) has a recognized therapeutic potential [3]. **References:** [1] Hay, A.-E. et al (2003) Planta Med. 69:1130 – 35. [2] Raad, I. et al (2006) Bioorgan. Med. Chem. 14:6979 – 87. [3] Pan, X. et al (2005) Bioorgan. Med. Chem. 13:5819 – 25.

PA33 *Malassezia* spp. extracts and metabolites induce the AhR dependent genes in HaCaT cells

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In previous works [1,2] we compared *Malassezia furfur* isolates from healthy and seborrhoic dermatitis skin for the production of indole derivatives and identified the preferential biosynthesis of malassezin, indolo[3,2-*b*]carbazol (ICZ) and indirubin by *M. furfur* strains isolated from diseased skin. These compounds, which are known highly active Aryl hydrocarbon receptor (AhR) inducers, were synthesized and used as standards for their quantification in extracts of *M.furfur* strains grown on L-tryptophan agar. HPLC analysis revealed that ICZ (1.2 – 6.0 μg/mg), indirubin (0.1 – 1.7 μg/mg), malassezin (2.3 – 40.9 μg/mg) are produced in significant quantities especially in the extracts of the clinical strains. The extracts were normalized according to ICZ content and cytotoxicity tests (MTS assay) were performed after exposure of HaCaT cells to different concentrations for 24, 48 and 72 hours. Subsequently, expression of the AhR battery of enzymes was assessed by Reverse Transcriptase Real-Time PCR (exposure for 24 h). The results showed statistically significant alterations in AhRR, CYP1A1, CYP1B1, GSTT1 and GSTP1 mRNA. In addition to this, malassezin (0.04 – 1.5 μg/mg), indirubin (0.03 μg/mg) and ICZ (0.2 μg/mg) were identified and quantified for the first time, in extracts of other species like reference strains of *M.japonica*, *M. sympodialis* and *M.yamatoensis* grown on L-tryptophan agar. The concentration of the measured compounds was significantly lower than that of *M.furfur* clinical strains but their widespread presence implies the importance of these metabolites in the skin physiology and disease development. Finally, in an effort to identify the biosynthetic pathway for indirubin which is the most active AhR ligand among the studied metabolites we have found a new reaction for the one-step transformation of indol-3-carboxaldehyde (the major *Malassezia* metabolite of tryptophan) to indirubin using hydrogen peroxide in acid medium or selenium based catalysts. **References:** [1] Gaitanis, G. et al. (2008) J. Invest. Dermatol. 128:1620 – 1625. [2] Giakoumaki, D. et al. (2008) Planta Med. 74:1081.

PA34 Identification of antimicrobial agents from *Drosera intermedia* using HPLC-MS/HPLC-SPE-NMR

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Drosera intermedia (H.) is a carnivorous plant species that is known for its use in traditional medicine. A recent study showed that extracts (methanol, water and hexane) prepared from *in vitro* grown *D. intermedia* plants have remarkable antimicrobial activity [1]. Despite the fact that the hexane extract showed much greater activity than the methanol and water extract, the methanol extract showed activity against one particular microbial strain (*Pseudomonas aureginosa*), which was tolerant to the hexane extract. This work describes the chemical investigation of these extracts in order to identify the antimicrobial agents produced by *D. intermedia*. The methanol, water and hexane extracts were cleaned from apolar compounds using a SPE column before being analyzed by HPLC-MS/SPE-NMR. All major peaks of the HPLC chromatogram were trapped using MS as triggering signal for the SPE unit and subsequently analyzed by 1D and 2D NMR spectroscopy [2]. Noteworthy, the HPLC-MS measurements showed that the hexane extract was constituted by a high purity single compound, the naphtoquinone plumbagin. Plumbagin has been reported to have potent antimicrobial activity, corroborating the good results of the hexane extract in the antimicrobial assays [3]. The main constituents of the methanol extract were flavonoids which are likely responsible for the antimicrobial activity, as plumbagin was only extracted in residual amounts. The water extract showed a low content in secondary metabolites, which explains its relatively low antimicrobial activity. *Acknowledgements: This research was supported by the European Community activity Large-Scale Facility Wageningen NMR Center (FP6 – 2004 – 026164 (2006 – 2009). T. Grevenstuk acknowledges a grant from Portuguese Science and Technology Foundation (SFRH/BD/31777/2006)* **References:** [1] Grevenstuk, T. et al. (2009) Nat. Prod. Commun. (submitted). [2] Grevenstuk, T. et al. (2008) Planta Med. 74:1101. [3] Gonçalves, S. et al. (2009) Nat. Prod. Res. 23:219 – 229.

PA35 Structural investigation of phytochemical compounds from Algerian plant *Convolvulus tricolor* (Convolvulaceae)

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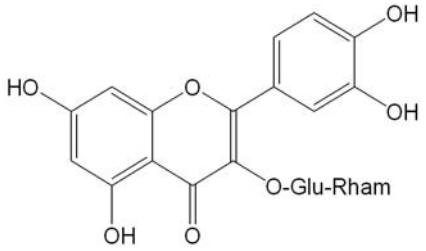
The convolvulaceae family composed of about 50 genera and 1500 species is distributed across the world's tropical and subtropical regions [1], although some species also reach temperate zones. *Convolvulus tricolor* a member of the convolvulaceae family, commonly called dwarf morning glory (local name "Souçane berri"), occurs in the region of Tell in Algeria [2]. Up to now, a few studies have been reported on *C. tricolor.* Analyses of the genus have demonstrated the presence of polyhydroxy alkaloids [3] and polysaccharides (galactomannans) [4]. The purpose of this present work is to phytochemically investigate some constituents of its leaves, flowers, stems, seeds, roots. The preliminary study of methanolic

extracts of seed coats from *C. tricolor*, exhibited *in vitro* a very good antileishmanial activity (99.51% inhibition against *L. amazonensis*) with percentage of cytotoxicity equal to zero. In addition, a strong antioxidant activity has been shown in a DPPH TLC assay for the methanol extract of flowers. The latter was chromatographed on silica gel 70 – 230 mesh using as gradient eluant containing increasing ratio of MeOH in $CHCl_3$. The isolation and purification were performed by preparative TLC on silica gel and Sephadex LH-20 column, leading six compounds which major compound was identified as the flavonol quercetin-3-O-rhamnoglucoside by using spectral analysis (UV, ^1H NMR, ^{13}C NMR, 2D NMR, and MS).



References: [1] Yoneda, Y. (1989) Convolvulaceae In: Grand dictionary of horticultural plants vol. 4:149 (edited by Youtarou Tukamoto). Shougakkan. [2] Quezel, P., Santa, S. (1963) In: Nouvelle Flore de l'Algérie et des Région Désertiques Méridionales, vol. 1 – 2:756. CNRS, Paris. [3] Schimming, T. et al. (2005) Phytochemistry 66:469 – 480. [4] Kooiman, P. (1971) Carbohydrate Research 20: 329 – 37.

PA36 Fractional Extraction of Plant Biomass: Generation of Botanical Extract Libraries

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A novel protocol has been developed to improve the detection and discovery of biologically active substances present in plant extracts. There has been an increased interest in revisiting the ancient concept of botanical therapeutics [1] and development of new therapies from natural complex mixtures [2]. We believe the process will amplify the rate of detection of biologically active constituents and accelerate the efforts to find molecules of biological interest. *Juglans nigra, Artemesia annua* (plants known to produce phytotoxins), *Bacopa monnieri, Actaea racemosa* and *Caulophyllum thalictroides* were among several species extracted by employing a method which pre-wets the plant material with aqueous methanol solution followed by extraction with a series of organic solvents of increasing solvent polarities. A sample set of the extracts were tested by a quick and reliable pre-emergence herbicide assay which demonstrated selective concentration of biological activity into specific extract fractions. Extracts were also tested by a Bioluminex™ assay that is extremely sensitive and showed selective concentration of constituents into specific extract fractions. Bioluminex™ is a unique and patented bio-analytical tool that combines the simple analytical technique of thin-layer chromatography (TLC) with a biosensor detection method providing a unique and effective way of analyzing complex mixtures. We have created extract libraries of authenticated botanical reference materials which can be used as probes for biological assays and accelerate the possibility of creating new therapies based on medicinal herbs. **References:** [1] Schmidt, B. et al. (2007) Nat. Chem. Biol. 3:360 – 366. [2] Chen, S. et al. (2008) Nat. Biotechnol. 26:1077 – 1083.

PA37 Determination of penta- and tetra- cyclic triterpenes in *Pistacia lentiscus* resin

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Oleoresins from *Pistacia* species are mainly composed of triterpenes (penta- and tetra- cyclic) and essential oil constituents (mainly terpenes), as have been recently reviewed [1]. Several studies on oleoresins from *Pistacia* species have revealed that their biological properties are mainly attributed to their triterpene and essential oil constituents. *P. lentiscus* var. Chia oleoresin (mastic gum) has been established to possess antimicrobial, antifungal, anticancer, antioxidant and radical scavenging activity. The ability of this resin to inhibit *in vitro* LDL oxidation, its antiatherogenic effect and gastric and duodenal antiulcer activity have been recently reported. Mastic gum was also established to kill *Helicobacter pylori* and to reduce dental plaque during chewing. The structure elucidation and determination of penta- and tetra-cyclic triterpenes in *P. lentiscus* has been performed so far by GC-MS [1 – 3]. In the present study, a RP-HPLC-DAD method was developed to separate and detect the constituents of *P. lentiscus* resin. Different stationary and mobile phases were applied and optimized and detection was performed at several wavelengths. Furthermore, high-performance liquid chromatography combined to mass spectroscopy was developed for the identification of triterpenes in *P. lentiscus* resin. Additionally, the mass spectrometric behaviour of triterpenoids under HPLC-MS/MS both APCI and ESI was studied. Oleanonic, moronic, masticadienonic and isomasticadienonic acid were identified among other triterpenes by LC-MS/MS in *P. lentiscus* resin. **References:** [1] Assimopoulou, A.N., Papageorgiou, V.P. (2007) Oleoresins from *Pistacia* species: Chemistry and Biology in Recent progress in Medicinal Plants, Natural Products II, Vol.18, CH 7: Common and chronic diseases, Studium Press LLC, USA. [2] Assimopoulou, A.N., Papageorgiou, V.P. (2005) Biomedical Chromatography 19:285 – 311. [3] Assimopoulou, A.N., Papageorgiou V.P. (2005) Biomedical Chromatography 19:586 – 605.

PA38 Rapid log *P* determination of natural products in crude plant extracts from UHPLC-TOF-MS profiling data – an additional parameter for dereplication and bioavailability

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In phytochemical analysis, HPLC metabolite profiling methods provide a large amount of data on the composition of given crude plant extracts for both dereplication and rapid on-line structural determination of given natural products (NPs) [1]. In this respect, the on-line spectroscopic data are mainly exploited for metabolite identification, while chromatographic data are only scarcely considered. It is however known that important physico-chemical properties can be extracted from the retention behaviour of the analytes in RP-LC. Lipophilicity, described by log *P*, a key-parameter involved in many pharmacokinetic and pharmacodynamic processes, can be determined by this way. With the concomitant introduction of ultra high pressure liquid chromatography (UHPLC) systems and new generation of columns packed with sub-2μm particles with very stable chemistries, the determination of log *P* on synthetic libraries has been considerably improved in term of throughput and pH range [2]. In the present study, the exploitation of generic UHPLC profiling gradients for rapid and robust log *P* determination has been estimated on a representative library of NPs, selected by cluster analysis using different molecular descriptors. The relations log *P* – log *k* have been established at different pH and using various UHPLC conditions, compatible with crude extract profiling studies. This strategy is expected to provide a rapid estimation of NPs lipophilicity prior isolation. The use of this parameter in dereplication database is foreseen to ideally characterize NPs retention independently from chromatographic conditions in well characterized systems. **Reference:** [1] Wolfender, JL.

et al. (2006) Exp. Opin. Drug Discovery 1:237 – 260. 2. Henchoz, Y. et al. (2008) J. Med. Chem. 51:396 – 399.

PA39 The norepinephrine transporter as a target for natural products and derivatives

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The Norepinephrine (= noradrenaline) transporter (NET) belongs besides serotonin and dopamine transporters to the family of monoamine transporters, regulating the re-uptake of norepinephrine released from neurons [1]. Several drugs binding to the norepinephrine transporter have been utilized therapeutically for the treatment of various disorders of the central (CNS) and peripheral nervous system (PNS), or cardiovascular disorders. In particular, norepinephrine re-uptake inhibitors are useful drugs in the therapy of depression, attention deficit disorder, obsessive compulsive disorder and panic disorder. Natural products as lead substances in the synthesis of bioactive therapeutics are an emerging field. In order to assess the inhibitory potential of natural products on the norepinephrine transporter, we have recently established a new screening assay based on COS-7 cells, transiently transfected with human norepinephrine transporter cDNA. Norepinephrine uptake studies were carried out using tritium labelled norepinephrine by quantifying radioactivity via liquid scintillation counting. The known selective inhibitor nisoxetine and the tricyclic antidepressant desipramine were used as positive controls with IC_{50} values comparable to literature data [2]. Heterocyclic and biphenyltype skeletons with different kinds of saturated and unsaturated, generally unbranched alkyl side chains showed moderate activity. These natural products as well as a set of derivatives thereof were investigated at different screening concentrations ranging from 10 to 100 µM. Promising candidates are currently under further investigation. **References:** [1] Mandela, P., Ordway, G. A. (2006) J. Neurochem. 97:310 – 333. [2] Olivier, B. et al. (2000) Prog. Drug Res. 54:59 – 119.

PA40 Traditional use, chemical analysis and antinociceptive effects of *Hyptis crenata* Pohl

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Species of the genus *Hyptis* are used in traditional medicine in South America and Africa. The medicinal effects and chemical profile of *Hyptis crenata* were evaluated in order to identify potential bioactive compounds. In December 2007 a survey was conducted regarding traditional preparation and use of extracts of *H. crenata* in Porto Esperidiao and Vila Bela regions of Brazil (20 regular users). The methods for extraction reported were: (i) decoction (boiling in water); (ii) infusion (tea); (iii) cold extraction (water); (iv) cold extraction (15% alcohol); and (v) cold extraction (40% alcohol). Chemical analyses of extracts prepared by these methods showed that camphor and eucalyptol are the major volatile compounds. Regarding phenolic compounds, decoction and infusion extracts were similar, while the cold extractions had a markedly different composition. The most commonly reported traditional uses were for forms of mild pain (11/20 – headache, stomach discomfort, menstrual pain) or treatment of flu/fever (6/20). Based on this traditional use, a study was conducted on the antinociceptive effects of the decoction of *H. crenata* in 8 C57/BL6 mice per treatment. Orally administered doses of 15 and 150 mg/kg b.w. were compared with water and a positive control (indomethacin 10 mg/kg b.w) for effects in a model of phasic pain (Hargreave's test of cutaneous thermal stimulation) and tonic pain (acetic acid-induced abdominal writhing). The treatment effects were significantly different from water, but not from indometacin, in both models. There was no mortality among the mice treated with *Hyptis* extract or water, while 38% of indometacin-treated mice died.

PA41 Anti-allergic activity of principles from the roots and heartwood of *Caesalpinia sappan* on antigen-induced β-hexosaminidase release

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The dichloromethane extract of the roots and heartwood of *Caesalpinia sappan* exhibited potent inhibitory activity against β-hexosaminidase release as marker of degranulation in rat basophilic leukemic (RBL-2H3) cells, with inhibition of 98.7% and 87.5% at concentration of 100 µg/ml, respectively. These extracts were further separated by chromatographic techniques to give two chalcones and seven homoisoflavones. Among the compounds tested, sappanchalcone (**2**) possessed the most potent effect against allergic reaction in RBL-2H3 cells with an inhibitory concentration (IC_{50}) value of 7.6 µM, followed by 3-deoxysappanchalcone (**1**, IC_{50}= 15.3 µM), whereas other compounds showed moderate and mild effects. The results suggested the following structural requirements of chalcones (**1** and **2**) and homoisoflavones (**3**-**9**) for anti-allergic activity: (i) chalcone exhibited higher activity than homoisoflavone (ii) vicinal hydroxylation at B-ring of chalcone conferred higher activity than one hydroxylation; and (iii) for homoisoflavone, the hydroxyl groups at C-3 and C-4 positions decreased the activity. This is the first report of *Caesalpinia sappan* for anti-allergic activity. *Acknowledgments: We are grateful to the Thailand Research Fund through the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), the Commission on Higher Education (CHE-RES-RG), MRG5080164 and Prince of Songkla University through Natural Products from Mangrove Plants and Synthetic Materials Research Unit (NSU) and the Graduate School for financial support.*

PA42 Studies of acetophenones from *Acronychia laurifolia* with HPLC, FCPC and LC-MS techniques

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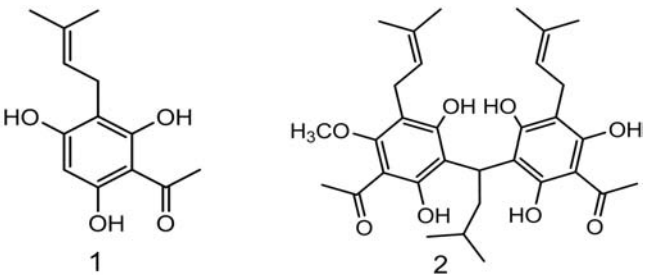
Acronychia laurifolia Bl. (Rutaceae) is a tree growing in tropical regions [1]. It is well documented for the occurrence of phenolic compounds and alkaloids. The diethylether extract of the plant bark was analyzed qualitatively using HPLC-DAD and LC-MS methods. From the same extract numerous of acetophenones, monomers and dimers, among them acronyline (**1**) and acrovestone (**2**) were isolated via classical approach (LC) and FCPC techniques [2]. The method used for FCPC analysis resulted to the separation of acetophenone monomers from dimmers; moreover two acetophenone dimers were isolated almost quantitatively and in high purity. All the isolated metabolites were structurally identified via spectroscopic methods (IR, MS, and NMR 1D & 2D). Furthermore, the coupling of semi-preparative-HPLC-DAD with microprobe 1 mm NMR (off-line) resulted to the isolation and structural determination of minor acetophenones monomers and dimmers contained into the extract.



References: [1] Hartley, T.G. (1974) A revision of the genus *Acronychia (Rutaceae),* J. Arnold Arbor. Harv. Univ. 55. [2] Shung, T.S. et al (1989) J. Nat. Prod. 52:1284 – 1289.

PA43 Microbiological screening of African indigenous plants for antibacterial activity against *Escherichia coli* and *Listeria monocytogenes*

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The use of subclinical doses of conventional antibiotics in food animals results in improved feed efficiency and better meat quality in food production. However, such practice has been linked to increased level of resistance among pathogens isolated from food animals. There is also evidence that the resistant bacterial gene can be transferred to humans through the food chain[1]. These resulted in a complete ban on the use of antibiotic growth promoters within the EU member states since January 2006. This new regulation has stimulated a worldwide research interest in exploring alternatives from natural resources. The aim of this study is to screen compounds from some African plants for antibacterial activity against known animal food pathogens. Two microorganisms were chosen for this study. *Escherichia coli* is a food-borne pathogen which has overtaken *Staphylococcus aureus* as the most frequent cause of bacteraemia in the UK recently. *Listeria monocytogenes NCTC 11994* is among the most virulent food-borne pathogens and can cause miscarriages in pregnant women. Fifty-five plant specimens were extracted with various solvents and screened for sensitivity against the chosen organisms using a microbiological microtitre plate procedure. Results indicated that nine of the extracts tested inhibited *Listeria monocytogenes* as compared to the positive control- Ampicillin. Bioactivity against *E.* coli was also observed in a number of extracts screened. Isolated potent compounds will be subjected to different spectroscopic and chromatographic analysis for the purpose of identification and characterisation. Further work will explore the interaction between isolated compounds themselves and with known antibiotics. **References:** [1] Endtz, H. et al. (1991) J. Antimicrob. Chemot. 27:199 – 208.

PA44 Evaluation of antioxidant activity and phytochemical analysis of *Mentha microphylla* C.Koch extracts

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The genus *Mentha* belongs to the Lamiaceae family and it consists of ca. 25 – 30 species of subcosmopolitan distribution, of which 7 are found in the Greek territory. Members of the genus *Mentha* are known for their spasmolytic, antibacterial and antigenotoxic properties[1]. Extracts (dichloromethane, methanol, water) of *M. microphylla*, *M. aquatica*, *M. longifolia* and *M. pulegium* were obtained with A.S.E (Accelerated Solvent Extraction). Methanolic and aqueous extracts were tested for DPPH scavenging and Total Phenolic Content and showed very strong antioxidant activity. Among them *M. microphylla* C.Koch was chosen for further phytochemical investigation. The Total Phenolic Content for *M.microphylla* C.Koch was 315.5 mg GAE/g of ethanolic extract and 274.5 mg GAE/g of aqueous extract. The DPPH %ΔA values were 100% for the ethanolic and 98% for the aqueous extract relatively. Dichloromethanic, ethanolic and aqueous extracts were received with conventional extraction. From the dichloromethanic extract several monoterpenes (i.e. piperitone, piperitenone oxide), triterpenes (i.e. β-amyrin), phenolic compounds (i.e. thymol) and a luteolin derivative were isolated by classic column chromatography. On the other hand, the ethanolic and aqueous extracts were fractionated with F.C.P.C (Fast Centrifugal Partition Chromatography) technique and were proved to be rich sources of rosmarinic acid and flavonoid compounds (i.e. diosmin). Structural elucidation of all compounds was determined by spectroscopic methods (NMR 1D, 2D). **References:** [1] Dorman, D. et al. (2003) J. Agr. Food Chem. 51:4563 – 4569.

PA45 Antihyperalgesic activity of verbascoside in the chronic constriction injury of the sciatic nerve (CCI) and intra-articular injection of sodium monoiodoacetate (MIA) models of neuropatic pain

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Verbascoside (acteoside) is a phenylpropanoid glycoside widely spread in nature. Different biological activities have been reported for such compound: anti-inflammatory, anti-ulcerogenic and antispasmodic activity [1], antiproliferative properties and inhibition of telomerase [2], immunomodulatory [3], antioxidant and photoprotective [4] and analgesic activities [5]. This study reports on the rapid isolation of verbascoside from *Lippia citriodora* H.B.K. (Verbenaceae), a not expensive and widespread source and its antihyperalgesic activity. Size exclusion chromatography with Sephadex LH-20, using hydroalcoholic solution (50% EtOH) is proposed as a fast and efficient method for the isolation and purification of verbascoside (purity > 98% determined by HPLC/DAD/ESI MS). The antihyperalgesic activity of verbascoside was tested by *in vivo* assay, using the Paw-pressure test, in two animal models of neuropathic pain: a peripheral mononeuropathy produced either by a chronic constriction injury of the sciatic nerve (CCI), or by an intra-articular injection of sodium monoiodoacetate (MIA). Verbascoside administered intraperitoneally (i.p.) at the dose of 100 mg/kg, reverted the mechanical hyperalgesia in both CCI and MIA treated rats, evaluated in the Paw-pressure test. The antihyperalgesic effect started 15 min. after administration and persisted for 30 – 45 min. Verbascoside was also effective against mechanical hyperalgesia after oral administration. At the doses of 300 and 600 mg/kg p.o. reverted the hyperalgesia induced by both CCI and MIA injection: the antihyperalgesic effect started 15 min after administration and was still significant at 60 min. **References:** [1] Hausmann, M. et al. (2007) Clin. Exp. Immunol. 148(2):373. [2] Efferth, T. et al. (2007) Trends Mol. Med. 13(8):353. [3] Akbay, P., Calis, I., et al. (2002) Phytoter. Res. 16(6):593 – 595. [4] Bilia, A.R. et al. (2008) J. Pharmaceut. Biomed. 46:463. [5] Calvo, M.I. (2006) J. Ethnopharmacol. 107:380.

PA46 *In vitro* NF-kappa B inhibitory and antioxidant effects of *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter & Burdet and its phytochemical profiling

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Several species of the genus *Cistus* (Cistaceae) have been reported to exhibit a variety of pharmacological activities including anti-inflammatory and anti-viral potential [1]. *In vitro* pharmacological activities of *Cistus* extracts were previously shown to be in strong correlation with the samples' total phenol content [2]. In order to obtain an extract enriched in phenolic compounds, a new ethanolic-water extract containing 60% polyphenols was developed (Patent application, Finzelberg GmbH & Co. KG). This extract ECce60 was further investigated for *in vitro* anti-inflammatory activity focusing on NF-κB inhibition in the luciferase-reporter gene assay [2] and for radical scavenging capacity (DPPH-TLC). The extract fractionation resulted in the separation of polyphenolic and terpenoid constituents and overall 7 fractions were obtained and characterized using 1D and 2D TLC techniques, HPLC as well as NMR. Fraction 5 demonstrated considerable anti-inflammatory effect (IC50 59.2 μg/ml) which was stronger than that of the ECce60 (IC50: 77.5 μg/ml). This fraction 5 represents only 2,1% of the ECce60 extract and contains relatively 1.4% Catechin, 0.7% Epigallocatechin and 3.9% of flavonoids. No terpenoids were detected in the active fraction. Other factions were inactive up to a concentration of 100 μg/ml. Consequently, fractionation led to enrichment of active constituents in the sample. All the samples exhibited antioxidant potential and fractions 4, 5 and 6 are considered to be good candidates for isolation of individual actives. Overall, this study provides further *in vitro* evidence for the potential anti-inflammatory effects of *Cistus*-derived extracts and especially of ECce60. **References:** [1] Pomponio, R. et al. (2003) J. Chromatogr. A

990:215 – 223 [2] Taila, S. et al. (2008) J. Pharm. Pharmacol. 60:A62-A63. [3] Obolskiy, D. (2008) Phytochemical and *in vitro* NF-kappa B inhibitory/Antioxidant Profiling of *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter Burdet; MSc dissertation, The School of Pharmacy, University of London. [4] Bremner, P. et al. (2004) Planta Med. 70:914 – 918.

PA47 New cyclobutane-containing tropane alkaloids from the aerial parts of *Schizanthus grahamii*

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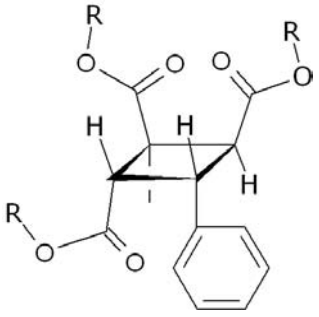
Schizanthus grahamii Gill. (Solanaceae) is a native Chilean plant which possesses large butterfly-like purple flowers and grows up to 60 cm high. Numerous alkaloids have been isolated from this plant, mainly tropane ester derivatives with isomeric C_5 acids (angelic, senecioic, tiglic, itaconic and mesaconic acids) [1] including grahamine, an unusual tropane alkaloid with a 2-methyl-4-phenyl-cyclobutane 1,2,3-tricarboxylic ester as a central structure (Figure 1) [2]. In this study, the alkaloid extract of this species was investigated by LC-MS and was found to accumulate several tropane alkaloids which also encompass this cyclobutane unit. Among them, seven new tropane alkaloids were isolated by semi-preparative liquid chromatography, namely four isomers of 638 Da, two isomers of 777 Da and one of 889 Da, and their structures elucidated by NMR.



References: [1] Lounasmaa, M., Tamminen, T. (1993) The Alkaloids. Cordell G. A. Ed. San Diego. [2] Hartmann, R. et al. (1990) Angew. Chem. 102:441 – 443.

PA48 α-Glucosidase inhibitors from the leaves of *Machilus Philippinensis* (II)[1]

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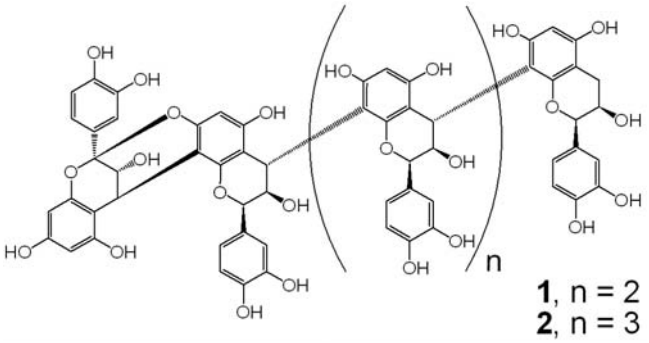
A preliminary screening indicated the EtOH extract of the leaves from *Machilus Philippinensis* to be active against α-glucosidase [1], which is one of the therapeutic targets in the treatment of diabetes mellitus [2]. Following the bioassay-guided fractionation and separation, five proanthocyanidin oligomers (**1-5**) and 12 flavonoids were characterized. Of these, two proanthocyanidin oligomers, named machiphilitannins A (**1**) and B (**2**) after the plant origin, are new compounds, with respective IC_{50} values of 31.28 μM (**1**) and 18.40 μM (**2**) against α-glucosidase. Their structures were elucidated based mainly on elaborate 2D NMR spectral analysis.



References: [1] For the first report see Lee, S. S. et al. (2008) Phytochemistry 69:2347 – 2353. [2]iCheng, A. et al. (2005) Can. Med. Assoc. J. 172:213 – 226.

PA49 Screening anti-AChE constituents from the leaves of *Annona glabra* Linn using HPLC micro-fractionation hyphenated with bioassay system

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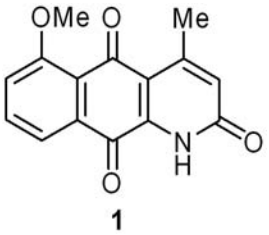
In this study, an efficient method for screening of the bioactive constituents of a complex mixture was developed. The EtOH extract of the leaves of *Annona glabra* Linn was analyzed by the following procedure including the general acid-base treatment, common chromatographic methods, and HPLC micro-fractionation hyphenated with acetylcholinesterase (AChE) inhibition assay [1]. The analytical-scale sample was separated by HPLC-DAD and fractionated into a 96-well microplate. The AChE inhibition assay was followed after evaporation of the microplate via a speedVac concentrator. The fractions possessed better anti-AChE activity were scaled up and separated by semi-preparative HPLC. In total, four bioactive alkaloids, 1-aza-4-methyl-5-methoxyl-2-oxo-1, 2-dihydro- 9, 10-anthracenedione (**1**), and three oxoaporphines were isolated and characterized. Compound **1** is a new natural product, and its IC_{50} value was 23.4 μM.



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References: [1] Ellman, L.G. et al. (1961) Biochem. Pharmacol. 7:88 – 95.

PA50 Chemical Constituents from *Alnus formosana* Burk. III. Polar Constituents from Leaves by HPLC-SPE-NMR and Conventional Methods

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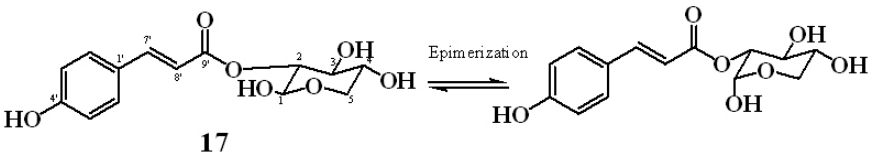
This continual study was aimed to investigate thoroughly the polar constituents present in the *n*-BuOH- soluble fraction of the leaves of *Alnus formosana*. [1] The HPLC-SPE-NMR hyphenated technique was applied for such purpose. This effort led to the characterization of 44 compounds, including thirty diarylheptanoids, ten flavonoids, and a coumaroylxyloside. Of these, seventeen compounds (**1-17**) are new. These compounds were further isolated and the structures were confirmed by general spectroscopic analysis.



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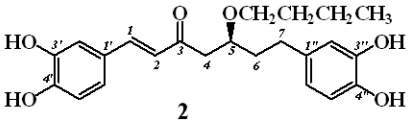

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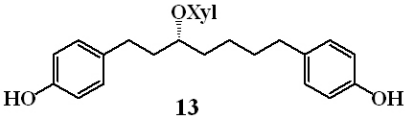

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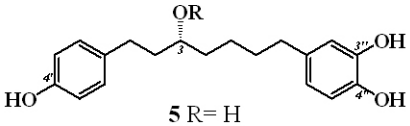
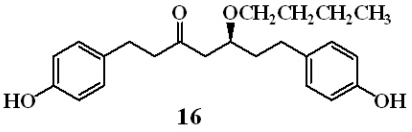


5 R= H
11 R= Glc



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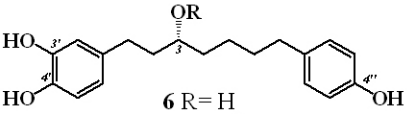
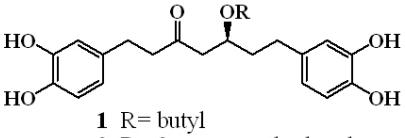
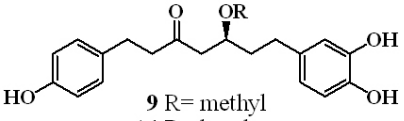


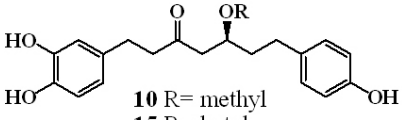
6 R= H
12 R= Glc



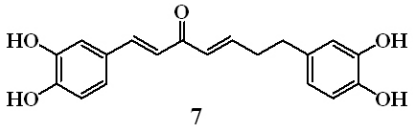
1 R= butyl
3 R= 2-coumaroylxyloxyl
4 R= 2-benzoylxyloxyl



9 R= methyl
14 R= butyl



10 R= methyl
15 R= butyl



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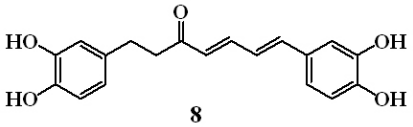

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References: [1] Lee, S.S. et al. (2006) Nat. Prod. Communications 1:461 – 464.

PA51 Inhibition of inducible NO synthase, cyclooxygenase-2 and interleukin-1beta by torilin is mediated by mitogen-activated protein kinases in microglial BV2 cells

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Background and purpose: Traditionally, the stem and root bark of *Ulmus davidiana* var. *japonica* (Ulmaceae) have been known to be anti-inflammatory in Korea [1]. Anti-inflammatory effects of torilin, isolated from this plant [2] and the underlying mechanisms were examined by using lipopolysaccharide (LPS)-stimulated microglial BV2 cells. Experimental approach: The cells were treated with torilin prior to LPS exposure and the effects on pro-inflammatory enzymes, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), and a pro-inflammatory cytokine, interleukin-1beta (IL-1beta) were analysed by RT-PCR, Western blot or elisa. To reveal the mechanism of action of torilin we investigated the involvement of mitogen-activated protein kinase (MAPK) cascades and their downstream transcription factors, nuclear factor-kappaB (NF-kappaB) and cyclic AMP-responsive element (CRE)-binding protein (CREB). Key results: Torilin significantly reduced the LPS-induced expression of iNOS, COX-2 and IL-1beta, and the subsequent release of NO, prostaglandin E_2 and IL-1beta into culture medium. LPS stimulation of extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 MAPK was inhibited by torilin. In addition, the inhibitory effect of torilin on NF-kappaB and CREB was shown by torilin-mediated recovery of LPS-induced degradation of inhibitor kappaB-alpha and suppression of LPS-induced phosphorylation of CREB respectively. Conclusion and implications: This study indicates that torilin inhibited LPS-induced iNOS, COX-2 and IL-1beta via down-regulation of ERK1/2, p38 MAPK, NF-kappaB and CREB and suggests that torilin has a potential as an anti-inflammatory drug candidate. **References:** [1] Lee, S.J. (1966) Korean Folk Medicine. Seoul National University Press. Seoul. [2] Kim, Y.C. et al. (2007) Fitoterapia 78:196 – 199.

PA52 Brine shrimp toxicity and anti-inflammatory properties of the methanolic extract of *Zingiber officinale* var. *rubrum* (Zingiberaceae)

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Rhizomes of *Zingiber officinale* var. *rubrum* have been used as poultice in the treatment of joint pains and swelling in the traditional medicine of indigenous peoples of Malaysia. The concentrations of phenolic ketones,

namely, 6-, 8- and 10-gingerols in the methanolic extract of *Zingiber officinale* var. *rubrum* were found to be higher than in *Zingiber officinale* var. *officinale*. The former was also found to exhibit greater toxicity toward *Artemia salina*. Toxicity is directly proportional to polarity of phenolic ketone. Inhibitors of nitric oxide production have been considered as potential anti-inflammatory agents. In this study, we also evaluated the methanol extracts of *Zingiber officinale* var. *rubrum* and *Zingiber officinale* var. *officinale* for nitric oxide formation (for iNOS inhibitors) in lipopolysaccharide (LPS)-induced mouse macrophages cells. Preliminary results show the methanolic extract of *Zingiber officinale* var. *rubrum* to be almost 50% more potent as anti-inflammatory agent as compared to dexamethasone. The active extract mediating iNOS inhibitory activities is warranted for further elucidation of active principles for development of new anti-inflammatory agents. *Acknowledgements*: Universiti Malaysia Pahang, Institute for Medical Research. **References:** [1] Endo, K. et al. (1990) Phytochemistry 29:797 – 799. [2] Park, K.K. et al. (1998) Cancer Lett. 129:139 – 144. [3] Sam, T.W. (1993) Toxicity Testing Using The Brine Shrimp: *Artemia salina.* In Colegate, S.M. & Molyneux, R.J. Bioactive Natural Products: Detection Isolation & Structural Determination. London: CRC Press, Inc. Boca Raton. [4] Schmidt, H.H.H.W., Walter, U. (1994) Cell 78:919 – 925.

PA53 Chemical profile characterization of biologically active extracts obtained from *Salvia* species

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[1]University of Athens, School of Pharmacy, Division of Pharmacognosy and Chemistry of Natural Products, Zografou, GR-15771, Athens, Greece; [2]University of Thessaly, Department of Biochemistry & Biotechnology, Ploutonos 26 & Aiolou, Larissa, GR-41221, Greece

Salvia is one of the largest and most important genus of the Labiatae family. In Greek soil, twelve species can be found. We chose to investigate the chemical profile of six taxa (*Salvia officinalis, S. fruticosa, S. sclarea, S. argentea*, *S. pomifera ssp. pomifera* and *S. pomifera ssp. calycina*). The aerial parts of these plants were extracted with Accelerated Solvent Extraction (ASE) technique. All methanolic extracts were screened using *in vitro* assays for possible chemopreventive activity. An evaluation was held for their protective activity against $OH^{\cdot}$ Radical induced DNA damage. From the results obtained, the methanolic extracts were more potent (IC_{50}= 170 – 230 μg/ml) compared to the aqueous extracts. Different techniques, both conventional and novel, resulted in the isolation of phenolic compounds, from the methanolic extract of *Salvia sclarea,* such as rosmarinic acid, salvianolic acid K and flavonoid glycosides (luteolin 4'-O-glucuronide, luteolin 7-O-glucuronide and apigenin 7-O-glucuronide). The chemical characterization of the other methanolic extracts was achieved with HPLC-DAD technique, using the isolated compounds from *S. sclarea* as standards. All methanolic extracts were very rich in rosmarinic acid, which indicated that this constituent is responsible for the extracts' chemopreventive activity. The separation of rosmarinic acid was possible using the FCPC technique.

PA54 Bioassay-guided isolation studies on mesophilic Actinomycete M-33 – 5 possessing antibacterial and cytotoxic activities

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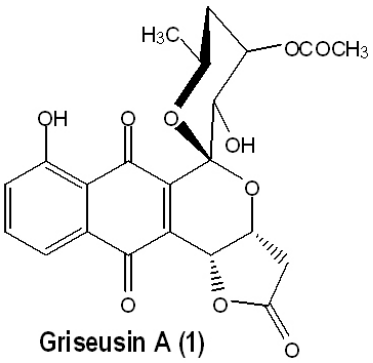
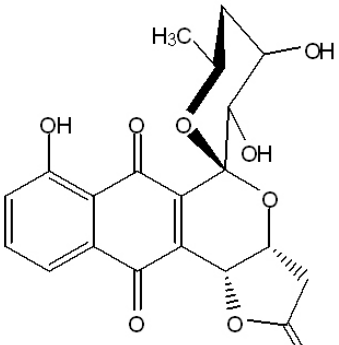
One hundred and twenty six mesophilic Actinomycete cultures were isolated from Aegean Region of Turkey. The antimicrobial activities of pure isolates were tested by using agar-plaque method. Based on high antimicrobial activity versus methicillin resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* 232 (E. coli), the isolate M-33 – 5 was selected for bioactivity-guided isolation. Fermentation followed by solvent partition studies (H_2O-EtOAc, H_2O-n-BuOH) showed that the highest activity was present in EtOAc extract. By using chromatographic methods, two bioactive compounds were isolated. Structures of the active metabolites were determined to be griseusin A (1) and 4′-deacetyl griseusin A (2) by spectral methods [1]. MIC values of 4′-deacetyl griseusin A were less than 1 μg/ml versus MRSA and E. coli. The cytotoxicities of the EtOAc extract and 4′-deacetyl griseusin A were also evaluated by the MTT assay using two human cancer cell lines (L-929, HeLa). Both showed potent cytotoxic activities against aforementioned cell lines.



Griseusin A (1)



4'-Deacetyl griseusin A (2)

L-929 (IC_{50}): 0.18 μg/ml

HeLa (IC_{50}): 0.41 μg/ml

Acknowledgement: This study was supported by Turkish Scientific and Technological Research Council of Turkey (Project No: SBAG-2746). **References:** [1] Tsuji, N. et al. (1976) Tetrahedron 32:2207 – 2210.

PA55 PAF-antagonist 7.3',8.5'-connected bicyclo[3.2.1]octane neolignans obtained from three colombian Lauraceae species and their synthesis

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[1]Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Química, Laboratorio de Investigación en Productos Naturales Vegetales, AA 14490, Cra 30 45 – 03, Bogotá, D.C., Colombia; [2]Universität Potsdam, Institut für Chemie, Karl-Liebknecht Straβe 24 – 25, 14476 Golm, Germany. Present address: Caprotec bioanalytics GmbH, Volmerstrasse 5, 12489 Berlin, Germany

Bicyclo(3.2.1)octane neolignans are a biologically important class of neolignan-type compounds which are further subdivided in 7.1',8.3'-connected (or guianin-type) and 7.3',8.5'-connected (or macrophyllin-type) [1]. Although the guianin-type is the most known bicyclooctanoids, the macrophyllin-type has shown a relevant PAF-antagonistic activity [2]. A set of thirteen bicyclooctanoids (twelve of the macrophyllin-type) were isolated from the leaves of three Lauraceae species (*P. cinereum, O. macrophylla* and *N. amazonum*). Ten of them were found to have novel structures which were fully determined by spectroscopic methods as well as the absolute configuration by chirooptical methods. All bicyclooctanoids showed a significant inhibitory activity in the PAF-induced aggregation of rabbit platelets assay [3]. The new bicyclo(3.2.1)octane neolignan named as cinerin B (IC_{50} 1.5 μM) was found to be one of the most potent PAF-antagonist. For this reason, a valuable route toward its total synthesis was accomplished through dihydrobenzofuran intermediate which was obtained *via* a new, efficient Pd-catalysed oxyarylation reaction, starting from resorcinol and 5-methoxy-piperonal, affording the desired product in seven steps. **References:** [1] Sefkow, M. (2003) Synthesis-Stuttgart 17:2595 – 2625. [2] Han, G. (1995)

Progr. Nat. Sci. 5:299 – 306. [3] Koch, E. (2005) Phytomedicine 12:10 – 16.

PA56 Evaluation of antifertility activity of isolated fraction of *Anthocephalus indicus* stem bark in male albino rats

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The present study was carried out to evaluate the effects of indigenous plant *Anthocephalus indicus* (family- Rubiaceae) on reproductive functions of male albino rats, as literature shows that plant is rich in saponins. Shade dried stem bark of *A. indicus* extracted with 70% methanol, further chromatographed with different solvent systems (Fr. I 75:25 $CHCl_3$:CH_3OH, Fr. II 50:50 $CHCl_3$:CH_3OH, Fr. III 25:75 $CHCl_3$:CH_3OH and Fr. IV CH_3OH) and fed to male rats at the dose level of 50 mg/rat/day for 60 days. After drug treatment the weight of testes and accessory sex organs were significantly decreased ($P < 0.001$), whereas the body weight did not reveal any significant changes. A marked decline was observed in sperm motility in cauda epididymides and in density of cauda epididymal as well as testicular spermatozoa. Serum testosterone levels were also declined significantly. Protein, sialic acid, glycogen in testes and seminal vesicular fructose content were decreased significantly, whereas testicular cholesterol contents were elevated. *A. indicus* treatment caused reduction in spermatogenic cell counts. A declined in number of mature Leydig cells were also found with regressed size of nuclear area, which evident of inhibition of androgen production. Fertility examination resulted in 66.66, 71.36, 84.52 and 100% negative fertility in Fr. I 75:25 $CHCl_3$:CH_3OH, Fr. II 50:50 $CHCl_3$:CH_3OH, Fr. III 25:75 $CHCl_3$:CH_3OH and Fr. IV CH_3OH, respectively. In conclusion *A. indicus* produced infertile state in male rats and showed antispermatogenic and antiandrogenic activity. On the other side hematological parameters remained unaltered, which shows its non-toxic nature.

PA57 Wound healing effects of cycloartane-type triterpenes isolated from *Astragalus* species

Sevimli-Gür C[1], Onbaşılar I[2], Atilla P[3], Çakar N[3], Deliloğlu-Gürhan I[1], Bedir E[1]
[1]Department of Bioengineering, Faculty of Engineering, Ege University, Bornova, 35100 İzmir, Turkey; [2]Experimental Animal Research and Husbandry Unit, Faculty of Medicine, Hacettepe University, 06100 Ankara, Turkey; [3]Departmant of Histology and Embryology, Faculty of Medicine, Hacettepe University, 06100 Ankara, Turkey

In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins [1]. Based on the claims that *Astragalus* has several important therapeutic properties, including wound healing and immunomodulation [2,3], our team has decided to focus on *Astragalus* cycloartanes as a lead to discover new wound healing agents. Thus we made an attempt to characterize wound healing effects of four major cycloartanes isolated from *Astragalus* species [Cycloastragenol (**1**), astragaloside IV (**2**), cyclocephaloside I (**3**) and cycloconthoside E (**4**)]. Effects of cell viability and proliferation of the isolated compounds were evaluated by the MTT assay on primary human skin epithelium cells. The wound healing activity was studied by using the *in vitro* wound healing, proliferation and migration method. Compared to the other compounds and positive control EGF (0.01 μg/ml), the compound **1** showed stronger wound healing activity at 1 μM concentration. In order to see *in vivo* efficiency of the compounds, an animal study with Sprague-Dawley male rats at the age of 12 weeks was carried out, and then the main histological outcomes were investigated to observe reepithelization, neovascularization, presence of inflammatory cells, granulation tissue amount and maturation. Among the compounds, 5% cycloastragenol preparation showed remarkable *in vivo* wound healing activity. **References:** [1] Tang, W., Eisenbrand, G. (1992) Chinese Drugs of Plant Origin, Springer-Verlag, Berlin. [2] Calis, I. et al. (1997) Planta Med. 63:183 – 186. [3] Bedir, E. et al. (2000) Biol. Pharm. Bull. 23:834 – 837.

PA58 Microbial control agent containing a Sri Lankan *Bacillus thuringiensis* isolate for control of Lepidopteran pests

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A complex of Lepidopteran pests is a worldwide problem in crucifers, including cabbage and cauliflower. This pest complex includes mainly diamondback moth (DBM), semilooper and cutworm. Many control measures rely on the intense use of synthetics, which has led to the development of pest resistance and caused adverse environmental consequences. The bacterium, *Bacillus thuringiensis* (*Bt*) has been identified as the most widely used microbial control agent. The objectives of this study were to isolate, identify and formulate indigenous *Bt* strains and test insecticidal activity against Lepidopteran pests of crucifers. In search of insecticidal indigenous *Bt* strains, the insecticidal activity of *Bt* AB142 and AB125 was identified against DBM, semilooper and cutworm. There were no previous records on the investigation of indigenous *Bt* and their *cry* proteins for insecticidal activity against these pests. Environmental samples were collected from different climatic zones of Sri Lanka and isolation was carried out according to standard protocols (1). Isolates were propagated in a fermenter using molasses-based medium (2). Insecticidal activity of *Bt* primary powders were tested against laboratory reared DBM, semilooper and cutworm following the leaf-dip bio-assay (2). Field assay was conducted to test bio-efficacy of oil emulsion formulation containing *Bt* isolates AB125 and AB142. In laboratory assays, *Bt* isolate AB142 showed more activity against semilooper and *Bt* AB125 against DBM and cutworm. According to field data, *Bt* isolates AB142 and AB125 were found to be highly toxic against Lepidopteran pests (DBM, semilooper and cutworm) found in cabbage and cauliflower. The present study provides valuable susceptibility data for the deployment of *Bt*-based control methods for Lepidopteran pests of crucifers grown in Sri Lanka. *Acknowledgements: National Research Council (Grant No 05:10) and National Science Foundation (Grant No RG/2006/AG/08).* **References:** [1] Collings, C.H. et al. (2001) Microbiological Methods. Oxford University Press. [2] Lisansky, S. et al. (2004) CPL Press Science Publishers.

PA59 Wound healing effects of c-phycocyanin isolated from *Spirulina platensis*

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As part of our continuing search for wound healing from medicinal sources, we analysed *Spirulina platensis* crude extract (cyanobacteria) and c-phycocyanin. The blue-green microalgae spirulina a non-nitrogen fixing, consumed in daily diets of natives in Africa and America, has been found to be a rich natural source of proteins, carotenoids and other micronutrients [1]. Recent studies have demonstrated antioxidant, antimutagenic, antiviral, anticancer, anti-allergic, immune enhancing, hepato-protective, blood vessel relaxing and blood lipid-lowering effects of spirulina extracts [2, 3]. The aim of the study is to identify and characterize wound healing effects of compound (c-phycocyanin) isolated from *Spirulina platensis*. Effects of cell viability and proliferation of spirulina crude extract and c-phycocyanin were evaluated by the MTT assay on primary human skin epithelium cells. The wound healing activity was studied by using the *in vitro* wound healing method, which is cell layer in a circular zone of 5 mm diameter, making use of a sterile teflon bar that removes cells, a wound was formed by scratching carefully [4]. Compared to the positive control EGF (0.5 μg/ml), spirulina crude extract and c-phycocyanin showed stronger wound healing activity at 33.5 μg/ml concentration. In order to see in vivo efficiency of the spirulina crude extract and c-phycocyanin, an animal study with Sprague-Dawley male rats at the age of 12 weeks was carried out, and then the main histological outcomes were investigated to observe reepithelization, neovascularization, presence of inflammatory cells, granulation tissue amount and maturation. It is observed that c-phycocyanin 1.25% has a better

effect on the 7th day compared to other preparations. **References:** [1] Muhammed, F. S. et al. (2004) Clin. Chim. Acta 348:199 – 205. [2] Kim, H.M. et al. (1998) Biochem. Pharmacol. 55:1071 – 1076. [3] Subhashini, J. et al. (2004) Biochem. Pharmacol. 68:453 – 462. [4] Arikan, F. et al. (2007) Braz. J. Oral Sci. 6 (23):1432 – 1437.

PA60 Isolation and structure elucidation of new antioxidants from leaves of *Populus ussuriensis*

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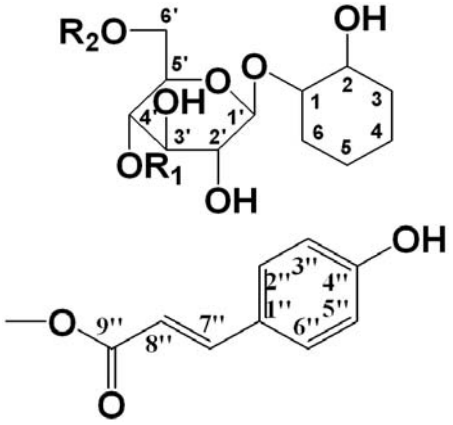
Populus ussuriensis Kom. (Salicaceae) leaves have been widely used in folk medicines to treat various diseases [1 – 2]. A 70% acetone extract of *P. ussuriensis* leaves was analyzed for antioxidant activity by $ABTS^{\cdot+}$ and DPPH free radical scavenging assays. After partitioning with several solvents, the EtOAc soluble fraction, which showed strong antioxidant activity, was further purified by Sephadex LH-20 column chromatography. The known phenolic acids ρ-coumaric acid (**I**) and caffeic acid (**II**), 5 known phenolic glucosides, salireposide (**III**), populoside (**IV**), suwonpopuloside (**V**), salicortin (**VI**) and grandidentatin (**VII**), and 2 new phenolic glucosides, 2-hydroxycyclohexyl-4'-*O*-*p*-coumaroyl-β-D-glucopyranoside (**VIII**) and 2-hydroxycyclohexyl-6'-*O*-*p*-coumaroyl-β-D-glucopyranoside (**IX**) were isolated as the active principles and their structures were elucidated on the basis of physicochemical and spectroscopic methods. This is the first report of the chemical composition of *P. ussuriensis*. Compounds **I-VI** and **IX** showed significant antioxidant activities (DPPH IC_{50} values of 6.68, 6.61, 6.75, 6.84, 6.76, 6.79 and 5.92 μM, respectively; $ABTS^{\cdot+}$ TEAC values of 1.21, 1.28, 1.26, 1.05, 1.69, 1.60, and 2.00 mM, respectively), suggesting they may be exploited as biopreservatives in food applications and health supplements in functional food, to alleviate oxidative stress.



R_1=*p*-coumaroyl, R_2= H (**VIII**); R_2=*p*-coumaroyl, R_1= H (**IX**) (*p*-coumaroyl)

Acknowledgements: Financial support from Natural Science Foundation of Tianjin City (09JCYBJC 15800, 09JCZDJC21800). **References:** [1] Zhang, XF. et al. (2006) J. Nat. Prod. 69:1370-1373. [2] Lin, M. et al. (1993) Acta Pharm. Sinic. 28:437-441.

PA61 Phytochemical and antibacterial studies of *Cissus ibuensis*

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Cissus ibuensis L.Hook, family Vitaceae, is a climber found in tropical countries including Nigeria. The plant is used in folkloric medicine of Northern Nigeria to treat bacterial infections and also to relieve pain and inflammation [1]. In our continuing search for bioactive plant metabolites from Nigerian medicinal plants, the aerial parts of *Cissus ibuensis* was investigated. The acetone and the ethanol extracts at concentration of 5 and 10 mg/ml were screened for preliminary antibacterial activity against the test organisms: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* using agar diffusion assay [2]. The results showed that the ethanol extract was more active. This extract was suspended in water and partitioned with ethylacetate and N-butanol and the antibacterial studies showed that the N-butanol extract was more active against all the test pathogens with zones of inhibition ranging from 15 mm to 18 mm at 5 mg/ml comparable to the standard antibiotics gentamycin (10 μg/ml and ciprofloxacin 10 μg/ml). Fractionation of this extract by flash column chromatography, gel filteration over Sephadex LH-20 and preparative thin layer chromatography afforded the flavonoids: kaempferol, kaempferol-3-O-rutinoside, quercetin-3-O-rutinoside and kaempferol 3-O-galactoside. The structures were elucidated by NMR spectroscopy and compared with literature [3]. The observed antibacterial activity might justify the folkloric use of this plant. **References:** [1] Dalziel, J.M (1965) The useful plants of West tropical Africa. A Crown agent for oversea publication. [2] Mendoza, L. et al. (1997) J .Ethnopharmacol. 58:85 – 88. [3] Mabry, T.J. and Markham, K.R (1968) Systematic identification of Flavonoids.

PA62 Activity guided isolation of antioxidants from the stem bark of *Diospyros sanza-minika* A. Chevalier

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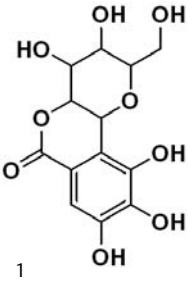
Many species of *Diospyros* (Ebenaceae) are used in African and Chinese traditional medicinal systems for the prevention of gastric ulcers, inflammatory disorders and hepatotoxicity [1]. Recent studies show that some of these species possesses antitumor, antidiabetic and antioxidant effects [2]. The methanol extract from the stem bark of *Diospyros sanza-minika* showed radical scavenging activity against DPPH with IC_{50} values of 1.33 mg/mL. This extract was exhausted with hexane, dichloromethane and ethyl acetate. The strongest active fraction (ethyl acetate, 1.18 mg/mL) was subjected to activity-guided purification to give norbergenin (**1**) and 4-*O*-galloylnorbergenin (**2**). These compounds were isolated for the first time from *Diospyros sanza-minika*. Norbergenin and 4-*O*-galloylnorbergenin, the main components of the plant, showed DPPH scavenging activities with IC_{50} values of 1.12 and 0.61 mg/mL, respectively, which were comparable to that of quercetin (0.74 mg/mL).



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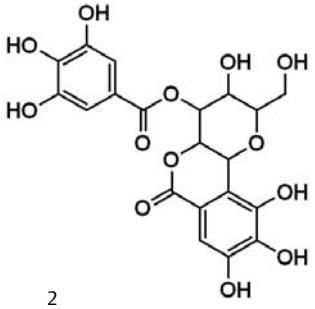

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Acknowledgments: Jean Gustave Tangmouo wishes to acknowledge AUF for "Bourse post-doctorat" in University of Geneva, Switzerland. **References:** [1] Mallavadhani, U.V. et al. (1998) Phytochemistry 49:901 – 951. [2] Chen, X.N. et al. (2008) J. Food Sci. 73:C24 – 28.

PA63 Secondary metabolites of *Centaurea depressa* Bieb

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The genus *Centaurea* L. (Asteraceae) is represented by 178 species in the flora of Turkey, 61.6% of which is endemic [1,2]. In this study, MeOH extract of the aerial parts of *C. depressa* Bieb. was partitioned between water and *n*-butanol. Butanolic extract was fractionated by using several chromotographic methods. 2 flavones (apigenin and luteolin), a flavone glucuronide (scutellarin), a phytosterol (β-sitosterol-3-*O*-β-D-glucopyranoside), a phenylpropanoid glycoside (syringin) and a phenolic acid (chlorogenic acid) were isolated and identified. Structure elucidation of the pure compounds was achieved by using spectroscopic methods (1D and 2D-NMR). Apigenin, luteolin, β-sitosterol-3-*O*-β-D-glucopyranoside, syringin and chlorogenic acid are reported for the first time in *C. depressa* by our work. **References:** [1] Wagenitz, G. (1975) In: Flora of Turkey and the East Aegean Islands, Volume 5, Edinburgh University Press. [2] Davis, P.H., Mill, R.R. (1988) Flora of Turkey and the East Aegean Islands, Volume 10, Edinburgh University Press.

PA64 Comparative antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species in *Juniperus* section from Turkey

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This work was designed to define and compare the biological potential of branches methanol extracts of *Juniperus* species from Turkey: *J. communis* L. var. *communis* (Jcc), *J. communis* L. var. *saxatilis* Pall. (Jcs), *J. drupacea* Labill. (Jd), *J. oxycedrus* L. ssp. *oxycedrus* (Joo), *J. oxycedrus* L. ssp. *macrocarpa* (Sibth. & Sm.) Ball. (Jom). Total polyphenol content (TPC) ranged from 170.43 ± 2.13 mgGAE/g Jcc to 253.29 ± 3.16 mgGAE/g Joo [1]. The antioxidant properties were examined by different *in vitro* systems; BHT, EDTA, and propyl gallate were utilized as standards. In the DPPH test the extracts exhibited strong scavenging activity (IC_{50} from 0.046 ± 0.004 mg/mL Joo to 0.160 ± 0.005 mg/mL Jcc) [2]. The extracts showed marked reducing power (ASE from 1.78 ± 0.04 ASE/mL Joo to 3.56 ± 0.14 ASE/mL Jcc) [3]. *Juniperus* extracts manifested dissimilar trends in the ferrous ion-chelating assay (IC_{50} from 1.41 ± 0.24 mg/mL Jom to 33.11 ± 0.40 mg/mL Jcc) [3]. The extracts showed marked effects in the lipid peroxidation of liposomes assay (IC_{50} from 0.29 ± 0.17 mg/mL Jom to 4.36 ± 2.16 mg/mL Jd) [4]. A strong positive correlation between TPC and each antioxidant test was found ($R^2 > 0.8$), except for ferrous ion-chelating assay ($R^2 = 0.354$). The antimicrobial potential was evaluated by standard methods [5]. *Juniperus* extracts showed activity on the Gram-positive bacteria assayed, and Jd resulted the most effective on *Staphylococcus aureus* (*MIC* 9.76 μg/mL). Concerning Gram-negative tested, a small inhibition was found only on the growth of *Proteus mirabilis*, and Jcc resulted the most active (*MIC* 625.00 μg/mL). No antifungal activity was observed. The results suggest that *Juniperus* branches from Turkey could represent sources of natural antioxidants and antimicrobial agents. **References:** [1] Gao, X. et al. (2000) J. Agric. Food Chem. 48:1485 – 1490. [2] Ohinishi, M. et al. (1994) Phytochemistry 36:579 – 583. [3] Gülcin, I. et al. (2007) Phytother. Res. 21:354 – 361. [4] Güvenç, A. et al. (2005) Pharm. Biol. 43:173 – 177. [5] NCCLS (2001) Document M100-S 11.

PA65 *In vivo* investigation of the wound healing effect of the traditional Hungarian medicinal plant *Centaurea sadleriana*

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The decoction of the aerial parts of *Centaurea sadleriana* JANKA (Asteraceae), a plant native to Hungary, is traditionally used to treat the wounds of sheep in the Southern Great Plain region. Phytochemical and pharmacological studies on this plant have not been performed so far. Our preliminary in vitro pharmacological screening has revealed that the extract of the aerial parts of *C. sadleriana* possess a marked anti-inflammatory effect. The objective of the present work was the *in vivo* investigation of the supposed wound healing effect and the identification of the active fractions of the herbal extract. Aerial parts of *C. sadleriana* were extracted with methanol and water. The concentrated methanol extract was partitioned using *n*-hexane and chloroform. The wound healing effect of different fractions of the methanol extract and the water extract of the plant material was investigated on rats [1]. Extracts (2.5%) incorporated in a Carbomer gel were applied topically to experimental wounds inflicted on healthy rats by means of a branding iron. Wound-healing time is calculated as the number of days required for 50% of the scabs to separate spontaneously from the animals. Two groups served as controls, one was treated with pure gel only, and the other was not treated at all. The third group was treated with 1% salicylic acid gel as positive control.The hexane fraction of the methanol extract accelerated significantly wound healing. This effect was similar to those of the active control. Other fractions exhibited moderate activities. Our present study confirmed the rationale of the traditional ethnomedicinal application of this plant and may serve as the basis for the identification of wound healing compounds of *C. sadleriana*. *Acknowledgements: The financial support of OTKA PD 71724 is gratefully acknowledged.* **References:** [1] Blazsó, G. et al. (2004) Phytother. Res. 18:579 – 581.

PA66 Competitive interactions between fungi: a new source of original bioactive molecules

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An innovative approach is presented for the chemical and biological investigation of confrontation zones formed by the fighting between fungi growing in confined spaces. In these zones the fungi are submitted to intense stress and this may lead to the induction of original defense compounds. In this work, two wood-decaying fungi involved in esca disease, *Eutypa lata* and *Botryosphaeria obtusa*, were selected as a case study and grown in Petri dishes. Metabolic profiles of pure fungal strains and confrontation zones were differentially analyzed by ultra-high pressure liquid chromatography coupled to time-of-flight mass spectrometry (UHPLC/TOFMS). Selected metabolites induced by the confrontation were isolated and characterized by capillary NMR (CapNMR) at the submilligram level. Fungitoxic and phytotoxic assays were applied to the crude extracts and isolated molecules. While the extracts of pure strains were inactive, the extract from confrontation zones exhibited significant activities. A very strongly induced compound, *O*-methylmellein, was found to be involved in these toxic properties. The developed approach [1] demonstrates the use of fungal confrontations as an original source of bioactive molecules, and opens the way for investigations on human pathogens such as opportunistic fungi responsible for skin or nail infections. **Reference:** [1] Glauser, G. et al. (2009) J. Agric. Food Chem. 57:1127 – 1134.

PA67 Secondary metabolites from leaves of *Iryanthera ulei* Warb. (Myristicaceae)

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Iryanthera species are native plants from South America and Panama which are used by indigenous people to treat fungal infections, itching of skin and stomach poisoning [1,2]. *Iryanthera ulei* is widely distributed around of Colombia, but chemical composition of Colombian specie has never been determined, while there are some reports about constituents from Brazilian specie affording five diarylpropanes, two dihydrochalcones, four flavonolignoids, two neolignans and one butanolide [3 – 5]. Hence, the present study shows the results obtained to conventional fractionation from leaves of *Iryanthera ulei*. Following compounds were characterized by means of repetitive chromatography over ethanolic extract: 2',4'-dihydroxy-4,6'-dimethoxydihydrochalcone, engeletin, isoengeletin, afzelin, quercitrin, loliolide, β-sitosterol and iryantherin L together with machilin C and a new enantiomer of 8-*O*-4'-neolignan named as iryantheral. Absolute stereochemistry was assigned to the latter two compounds for the first time by CD measurements in comparison with reported data for related molecules. **References:** [1] Garzón, L. et al. (1987) Noticias Químicas 45:21 – 25. [2] Gottlieb, O.R. et al. (1979) J. Ethnopharmacol. 1:309 – 323. [3] Conserva, L.M. et al. (1990) Phytochemistry 29:3911 – 3918. [4] Conserva, L.M. et al. (1990) Phytochemistry 29:3986 – 3988. [5] Vieira, P.C. et al. (1983) Phytochemistry 22:711 – 713.

PA68 *Tulbaghia alliacea*: A potential anti-tuberculosis phytotherapy

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Tulbaghia alliacea is used in traditional medicine to combat infections. Extracts of *T.alliacea* (0 – 10 mg/ml), were comparatively assessed for *in vitro* activity against *Mycobacterium smegmatis* using a disk diffusion assay, and IFN-γ in human cells using ELISA technology. *T.alliacea* aqueous ($P < 0.002$) and ethanolic ($P < 0.003$) extracts inhibit the pathogen in a dose-dependent fashion compared to controls. More specifically, the 10 mg/ml chloroform extract of *T. alliacea* most potently inhibited the growth of the pathogen ($P < 0.0001$). Developed TLC plates of the *T.alliacea* chloroform extract inoculated with *Mycobacterium smegmatis* were sprayed with 2,5-diphenyltetrazolium bromide. Comparatively, developed TLC plates of the *T.alliacea* chloroform extract were sprayed with vanillin-sulphuric acid reagent. NMR analysis identified the active compound A as Marasmicin, with chemical shift values (ppm) of 2.38, 4.07, 4.18, 4.25 and 2.27, which have been previously reported for this entity[1]. The inhibitory effect of *Tulbaghia alliacea* against *Mycobacterium smegmatis*, is due to three active compounds, observed using TLC. Through NMR, one of these compounds was identified as Marasmicin (R_f 0.44), a potent anti-infective compound previously identified in *T. Alliacea* [1]. In addition, the aqueous extracts of *Tulbaghia alliacea* showed greater potency in stimulating the expression of IFN-γ, when compared with the chloroform extract ($P < 0.05$). *Tulbaghia alliacea* phytotherapy is antimycobacterial and modulates IFN-γ, which is vital in fighting TB infection. **References:** [1] Thamburan, S. et al. (2006) Phytother. Res. 20 (10):844 – 50.

PA69 Tannins with LPS-induced NO production inhibitory activity from *Juglans sigillata* bark

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Juglans sigillata Dode (Juglandaceae), a deciduous tree indigenous to China, has been extensively used in folk medicines to cure esophageal, gastric, cardiac and lung cancer [1 – 3], though its chemical constituents have never been reported. The Inhibitory effect of *J. sigillata* bark constituents against LPS-induced NO production in murine microglial BV2 cells was investigated. Bioactivity-guided fractionation and purification of a 70% acetone extract of the bark led to the isolation of 9 tannins and their structures were elucidated as gallic acid (**1**), ellagic acid (**2**), 1,2,6-tri-*O*-galloyl-β-D-glucose (**3**), 1,3,6-tri-*O*-galloyl-β-D-glucose (**4**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (**5**), 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (**6**), 2,3-*O*-4,4′,5,5′,6,6′-HHDP-(α/β)-D-glucose (**7**), 4,6-*O*-4,4′,5,5′,6,6′-HHDP-(α/β)-D-glucose (**8**), and pedunculagin (**9**) on the basis of spectral and chemical evidence. All the nine tannins exhibited significant inhibition of LPS-induced NO production compared with NAME (positive control). A structure-activity relationship analysis revealed that the introduction of galloyl and HHDP groups increased the inflammation inhibitory effect. Total tannin content of *J. sigillata* bark was 178 mg/100 g which was measured indirectly using Folin-Ciocalteu after being removed with PVPP. *Acknowledgements: Financial support from Foundation for the Development of Science and Technology in Tianjin Universities (Year 2009), Natural Science Foundation of Tianjin City (09JCYBJC15800, 09JCZDJC21800) and China Postdoctoral Science Foundation is gratefully acknowledged.* **References:** [1] Ahmadiani, A. et al. (2000) J. Ethnopharmacol. 72:287 – 292. [2] Ansari, S.A. (1991) Ekhtiyarat Badii. Razi Publication, Tehran. [3] Avecina, A. (1991) Ghanon of Medicine, Vol.2, 5th Edn. Soroush Publication, Tehran.

PA70 Oryzadine, a new alkaloid, attenuates oxidative stress-induced cell damage via a radical scavenging effect

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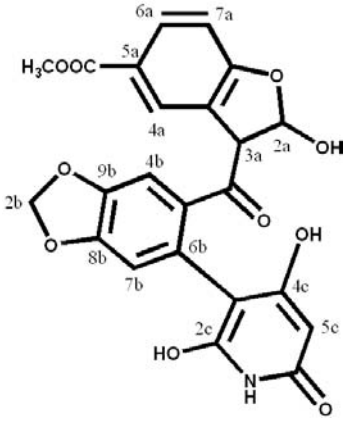
In the course of our search for a new antioxidant, we isolated oryzadine from the aleurone layer of *Oryza sativa* cv. *Heugjinjubyeo* [1].



Oryzadine is a new alkaloid, was investigated by applying various methods based on cell-free and cell experiments [2]. Oryzadine showed scavenging effects of the hydroxyl radical, superoxide radical, and intracellular reactive oxygen species. Oryzadine inhibited the H_2O_2-induced

DNA damage which was demonstrated by DNA tail formation, lipid peroxidation which was demonstrated by the formation of thiobarbituric acid reactive substance, and protein oxidation which was demonstrated by protein carbonyl formation. Based on these results, oryzadine protected H_2O_2-induced cell damage. Our results show that the cytoprotective effects of oryzadine stem from its ability to inhibit H_2O_2-induced apoptosis, as demonstrated by a decrease in apoptotic body formation and the inhibition of mitochondrial membrane potential ($\Delta\psi$) loss. **References:** [1] Kim, E.S. et al. (2009) Bull. Korean Chem. Soc. 30:739 – 741. [2] Shin, J.S. et al (2008) Cell Biol. Int. 32:1099-1107.

PA71 Wheatgrass extract increases proliferation of RAW 264.7 macrophages induced by hydrogen peroxide (H_2O_2) or lipopolysaccharide (LPS)

Özkan T[1], Karabay ZA[3], Koç A[3], Karadag A[2], Aydos S[2], Çalıskan E[1], Öztürk G[2], Ilgaz SN[2], Yükselen I[2], Sunguroglu A[2]
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Wheatgrass, the young grass of *Triticum aestivum* L. contains chlorophyll, amino acids, minerals, vitamins, and enzymes and acclaimed to have antioxidant properties. In RAW 264.7 macrophages, a high level of NO production accompanied by cell apoptosis was achieved with LPS treatment (1). Direct treatment of cells with oxidants such as hydrogen-peroxide(H_2O_2) was thought to exclusively cause necrosis and apoptosis (2). Therapies aimed to inhibit NO-dependent cell apoptosis and oxidative stress mediated cell toxicity may contribute to improving the outcome of various diseases. In this study, the effect of wheatgrass extract on proliferation of RAW 264.7 macrophages induced with H_2O_2 or LPS was tested. RAW 264.7 cells seeded in 96 well plates were incubated with (positive controls) or without (negative controls) different concentrations of wheatgrass extracts dissolved in water, LPS(1 μg/ml and 10 μg/ml) or H_2O_2 (500 μM) for 24 h. To test the effect of wheatgrass extract on proliferation, cells were pre-treated with different concentrations of wheatgrass extract for 1 h and then induced with LPS or H_2O_2 for 24 hours. At the end of the incubation period cell proliferation was estimated by MTT test and the statistical significance of differences was evaluated using one-way ANOVA. After 24 hours of incubation with LPS(1 μg/ml and 10 μg/ml) and H_2O_2 (500 μM) cell proliferation decreased significantly ($p < 0{,}0001$) and wheatgrass extract increased cell proliferation in both LPS and H_2O_2 induced cells. The effective proliferative doses of wheatgrass extract in H_2O_2 and LPS induced cells were found to be 0,5%; 1,5%; 2,5%; 3,5%, 5%, 7,5%, 10%v/v with p values of < 0,0001 and < 0,001 respectively. Our previous research has demonstrated that wheatgrass extract induced apoptosis and decreased proliferation in various cancer cell lines (3). While wheatgrass has an antiproliferative effect on leukaemia cells, it protects macrophages which are one of the immune system cells against death. **References:** [1] Slomiany, B.L. et al. (1998) Mol. Biol. Int. 46:1063 – 1070. [2] Zhang, Y. et. al. (2005) Apoptosis 10:545 – 556. [3] Karadag, A. et al. (2007) Planta Med. 73:991 – 992.

PA72 Topical anti-inflammatory effects of *Ocimum basilicum* leaf extract in the phorbol-12,13-dibutyrate model of mouse ear inflammation

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Indian basil *(Ocimum basilicum* L. Family Lamiaceae) is a well-known traditional medicinal plant in the Indian subcontinent. The wound healing activity of the leaves has been reported in our earlier work [1]. In search of possible mode of action for wound healing activity, the topical anti-inflammatory property of the ethanol-water (25%) extract of leaves in mice has been carried out. Swiss albino mice of 22 – 25 g of weight were used and approved by the ethical committee of the institute. Mice were divided into four groups (n = 5) viz. Normal, toxin control, positive control and treated group. Animals of each group except normal group were applied phorbol-12,13-dibutyrate (1 μg daily) on the right ear for 5 days as toxin. After 30 min of toxin application, the animals of Group III were applied 20 mg of cream formulation of indomethacin and Group IV animals were applied OB extract (4 mg) daily. On the 5th day, all the animals were sacrificed and their ears were separated for the estimation of various parameters viz. ear weight, lipid peroxidation, interleukin1-β, interleukin-6 and tumor necrosis factor-α. OB extract significantly ($p \leq 0.05$) reduced the ear weight variation (difference in the weight of right and left ear of animals), levels of LPO (malonaldehyde), IL-1β, IL-6 and TNF-α when compared with toxin group using ANOVA test and as shown in table.

Parameters	Group I Normal (mean ± SEM)	Group II Toxin control (mean ± SEM)	Group III Positive control (mean ± SEM)	Group IV OB extract (mean ± SEM)
Ear weight variation (mg)	3.47 ± 0.187	34.54 ± 0.39 [a]	19.34 ± 4.56 [b]	17.43 ± 1.54[b]
LPO (pg/ml)	11.05 ± 2.38	63.84 ± 3.196 [a]	19.65 ± 2.09 [b]	20.72 ± 2.10 [b]
IL-1β (pg/ml)	57.04 ± 4.10	606.02 ± 7.37 [a]	292.03 ± 10.67 [b]	362.01 ± 12.11 [b]
IL-6 (pg/ml)	118.79 ± 7.50	1431.84 ± 34.72 [a]	701.88 ± 18.78	644.07 ± 14.59 [b]
TNF-α (pg/ml)	60.14 ± 2.54	221.89 ± 8.05 [a]	166.29 ± 7.18	95.76 ± 3.68 [b]

Values with 'a' exhibit significant difference ($p \leq 0.5$) when compared to normal group and value with 'b' exhibit significant difference ($p \leq 0.5$) from toxin group. Therefore we can conclude that ethanol-water extract of OB has shown significant anti-inflammatory activity against phorbol-12,13-dibutyrate induced topical inflammation in mouse ear. **Reference:** [1] Yadav, N. et al. (2008) J. Pharm. Pharmacol. 60(Sup.1):A-31.

PA73 Phytochemical characterization of *Juniperus* spp. leaves

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Juniperus is the second most abundant genus among the conifers. Numerous folk medicinal uses have been reported for *Juniperus* leaves and fruits, such as their application as antirheumatic, blood cleansing, digestive, diuretic and febrifuge agents; they have also been used in the treatment of arteriosclerosis, bronchitis, colic, common cold, cough, inflammation, tuberculosis, cancer, psoriasis and wounds [1]. The aim of this work was to evaluate the potential application of *Juniperus* leaves from species naturally occurring in Portugal (*J. phoenicea* subsp. *phoenicea*, *J. turbinata*, *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *badia* and *J. navicularis*) against some diseases in which oxidative reactions play a crucial role. To this end, the seasonal evolution of total polyphenols [2], total flavonoids [3] and antioxidant activity for peroxyl radical [4] was determined. All species exhibited minimum polyphenol and flavonoid contents in March/April and July and therefore a reduced antioxidant activity. Maximum concentrations of these compounds were detected in November/December, with the levels of antioxidant activity peaking three times a year, May/June, August/September and November/December. *J. phoenicea* subsp. *phoenicea*, the most widespread species, showed the lowest levels of polyphenols, flavonoids and antioxidant activity. To compare their metabolite composition by HPLC-MS, leaves from all *Juniperus* under study were collected in November/December. The polyphenolic profiles obtained for *J. phoenicea* subsp. *phoenicea* and *J. turbinata* are very similar. Analogous HPLC profiles were also obtained for both *J. oxycedrus* subspecies and for *J. navicularis*. *Acknowledgements: To FCT for financial support of C. Santos (SFRH/BPD/26562/2006) and L. Tavares (SFRH/BD/37382/2007).* **References:** [1] Johnson, T. (1999) CRC Ethnobotany Desk Reference. CRC Press. Boca Raton. [2] Singleton, V.L. et al. (1965) Am. J. Enol. Vitic. 16:144 – 158. [3] Michalska, A. et al. (2007) Eur. Food Res. Technol. 225:545 – 551. [4] Cao, G. et al. (1993) Free Radic. Biol. Med. 14:303 – 311.

PA74 A new flavonol with anti-inflammatory activity from *Boldoa purpurascens* Cav.

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Phytochemical analysis of the leaves of *Boldoa purpurascens* Cav. [1] led to the isolation of two flavonol glycosides [2,3]. The structure of the new compound was determined by mass spectrometry and by 1D and 2D NMR analysis as 4',5-dihydroxy-6,7-methylenedioxyflavonol-3-*O*-α-L-rhamnopyranosyl-(1 – 2)-β-D-xylopyranoside [4].The aglycone 3,4',5-dihydroxy-6,7-methylenedioxyflavonol is known as gomphrenol [5]. The new flavonoid was evaluated for its effects in the acute and chronicle phases of inflammation. For this reason, two experimental techniques were developed: edema induced by dextrans, and granulomas induced by cotton disks. Test doses of 2.5, 5.0 and 10 mg/Kg of weight were used, with a volume of administration of 10 mL/Kg. Indomethacin was the positive control (7 and 5 mg/Kg) and distilled water the negative control. Gonfrenol-3-*O* – [α- L-rhamnosyl-(1 – 2) – β- D-xilósido] presented anti-inflammatory activity in the acute phase to superior dose to 2.5 mg/Kg in the experimental pattern of edema to plant induced by dextrano; also, this activity increased when increasing the dose. In the granulomas pattern induced by cotton disks, the compound presented similar anti-inflammatory activity to the Indometacine to all the evaluated doses, being bigger the effect to the 10 mg/Kg dose. The statistical analysis was carried out by the test of Kruskal-Wallis with an interval of trust of 99% and a level of significance of 0.816. The flavonol; showed significant dose-dependent inhibition of both acute and chronic inflammation. The activity was comparable to that of the standard reference drug, indometacine. The results of the present investigation indicated that the flavonol isolated of *B purpurascens* Cav. shows profound antiinflammatory activity. Keywords: *Boldoa purpurascens*, Nyctaginaceae, flavonol glycosides, gomphrenol glycosides. Reference 1. Roig, J.T (1988) Dictionary of Cuban Common Yams, 3 rd. Editorial Science and Technic 2. Niassy, B. et al. (2004) C. R. Chimie 7: 993 – 996. 3. Jpn. Kokai Tokkyo Koho (1988) JP63203682 A2. 4. Magalhães, A.F. et al. (2007) Acad. Bras. Cienc. 79:351 – 367. 5. Bouillant, M.L. et al. (1978) Phytochemistry 17:2138 – 40.

PA75 Antioxidant iridoid and phenylethanoid glycosides from *Teucrium chamaedrys* (L.)

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Wall Germander (*Teucrium chamaedrys*) is a Mediterranean species used as medicinal herb [1]. A previous *screening* turned to the radical scavenging efficacy determination was carried out on crude extracts from *T. chamaedrys* ipogeal and epigeal components undergoing each one to DPPH radical antioxidative HAT assay. Leaf and root methanolic extracts are responsive of a peculiar DPPH radical scavenging efficacy. The methanolic crude extracts were object of extractive and chromatographic analyses to yield twelve compounds: seven iridoid and five phenylethanoid glycosides, four of them isolated and characterized for the first time on the basis of their spectroscopic features. The DPPH radical scavenging and antioxidant capabilities [2] of the purified metabolites were assessed. The antioxidant capability in cell-free systems of the isolated metabolites was carried out by measuring their capabilities to inhibit the synthesis of thiobarbituric acid reactive in assay media using as oxydable substrates a vegetable fat and the pentose sugar 2-deoxyribose. The inhibiting capacity of isolated metabolites the protein oxidation, defined as the covalent modification of a protein induced either directly by reactive oxygen species or indirectly by reaction with secondary by-products of oxidative stress, was also measured. Compounds from *T. chamaedrys* were tested in increasing concentration (5.0 μM, 10.0 μM and 20.0 μM) in triplicate analysis. The detected activities were compared to those exercised from Trolox®. When DPPH radical scavenging was tested, phenylethanoid glycosides highly reduced the oxidant probe employing an activity strongly dose-dependent. Iridoid glycosides prevent massively the 2-deoxyribose and BSA oxidations in assay media. The results show that oxidation and radical processes are highly complex and involve various mechanisms and targets. In particular the ability to scavenge free radical does not necessarily confer antioxidant properties. **References:** [1] Özel, M.Z. et al. (2006) J. Chromatograph. A 114:164. [2] Piccolella, S. et al. (2008) J. Agric. Food Chem. 56:1928.

PA76 Neuroprotective and MMP-9 inhibitory activity of hydroethanolic extract of *Arbustus unedo* leaves

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Arbutus unedo L., the strawberry tree (Ericaceae family), is an endemic Mediterranean species. Its leaves have been employed for a long time in traditional and popular medicine as an astringent, diuretic, urinary antiseptic, and more recently, in the therapy of hypertension and diabetes [1]. The aim of this work is to evaluate the antioxidant properties of a hydroethanolic extract of *A. unedo* leaves in a neurodegeneration cell model and the inhibitory activity to human matrix metalloprotease (MMP-9), an enzyme involved in cancer invasion. The intracellular radical scavenging activity of the plant extracts in an oxidative stress-induced model of neurodegeneration in SK-N-MC cells was evaluated to the nontoxic range of concentrations. The pre-treatments with the extract protects the cells from the oxidative stress injury as detected by an increase in cell viability up to 42% with 15 μg GAE.mL^{-1} and 86% with 30 μg GAE.mL^{-1}. An enriched polyphenolic fraction, obtained by a SPE, presents an IC50 of 2.88 μg.mL^{-1} for the MMP-9 inhibitory activity, a very interesting result when compared with the value obtained for green tea extract, with already described significant inhibition [2], in the same assay conditions (4.28 μg.mL^{-1}). The HPLC-MS analysis of the leaves reveals several gallic acid derivatives that could be responsible for the observed effects, further analysis should be done to correlate the compounds with the detected bioactivities. *Acknowledgements: To FCT for financial support of C. Santos (SFRH/BPD/26562/2006) and L. Tavares (SFRH/BD/37382/2007).* **References:** [1] Bnouham, M. et al. (2007) Pharmazie 62:630 – 632. [2] Adhami, V.M. et al. (2003) J. Nutr. 133:2417S-2424S.

PA77 Topical anti-inflammatory effect of lipophilic constituents from *Alchornea floribunda* and *Alchornea cordifolia* leaves

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The leaves of *Alchornea floribunda* and *Alchornea cordifolia* are used traditionally as topical anti-inflammatory agents. Several studies have shown that the hexane extracts of the plant materials exhibited significant anti-inflammatory activity [1,2,3]. In the present study, we subjected the hexane extracts of *Alchornea floribunda* and *Alchornea cordifolia* leaves to column chromatographic separation and isolated two highly lipophilic fractions **AFLF** and **ACLF** respectively. The anti-inflammatory effects of these fractions were investigated using xylene – induced oedema as a model of inflammation. **AFLF** and **ACLF** at 5 mg/ear showed significant ($P < 0.001$) topical anti-inflammatory effect with oedema inhibitions of 64.0 and 79.0% at 2 h respectively. These fractions showed significantly higher topical anti-inflammatory effect than 5 mg/ear indomethacin (oedema inhibition of 48% at 2 h). GC/MS analysis of these fractions revealed that **AFLF** is composed mainly of long chain saturated and unsaturated hydrocarbons (18.78%) and their oxygenated derivatives (1.89%), long chain carboxylic (fatty) acids (2.72%) and their esters (5.53%); while **ACFL** is rich in volatile oils euginol (21.26%) and cadinol (4.76%) and other constituents like long chain primary alcohols (4.78%), long chain saturated hydrocarbon, nanocosane (36.86) and steroid derivatives, ethyl iso-allocholate (4.59%) and 3-acetoxy-7,8-epoxyla-

nostan-1-ol (15.86%). These constituents are highly lipophilic and can easily permeate lipoidal layers of skin. They may contribute significantly to the observed topical anti-inflammatory effect of *Alchornea floribunda* and *Alchornea cordifolia* leaf extracts. **Reference:** [1] Osadebe, P.O. et al. (2003) J. Ethnopharmacol. 89:19 – 24. 2. Marva-Manger, H. et al. (2004) J. Ethnopharmacol. 92:209 – 214. 3. Okoye, F.B.C. et al. (2008) Planta Med. 74:90.

PA78 Preliminary fractionation indicates that flavonoids, steroids and terpenoids are the main immunomodulatory constituents of *Loranthus micranthus* (Linn)

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In recent times, we have established the immunomodulatory effects of the Eastern Nigerian mistletoe, *Loranthus micranthus* [1, 2] as well as its antimicrobial property [3,4]. In our continued efforts to isolate the active constituents, five solvents of varying polarity namely; *n*-hexane, chloroform, ethyl acetate, acetone and methanol were respectively and successively employed in the complete fractionation of the crude aqueous methanol extract of *Loranthus micranthus* Linn., harvested from *Kola acuminata*. The fractions were dried *in vacuo* using a rotary evaporator maintained at a temperature of 40 ± 5 °C. The different fractions were screened for immunomodulatory activity using a well established model;- the cellular- mediated delayed type hypersensivity test in experimental mice. This was performed by administering intraperitoneally, two different dose levels: 250 and 500 mg/kg of each fraction against standard positive and negative controls. Results of the study established dose dependent immunostimulatory (upregulatory) effects. The five fractions of the extract exhibited different percentage stimulations compared to controls ($p < 0.05$). At the dose levels of 500 and 250 mg/kg body weight, the percentage stimulation observed were as follows; chloroform fraction-311.11% and 122.22%, ethyl acetate fraction-193.38% and 95.56%, *n*-hexane-155.56 and 3.50%, acetone fraction-95.56% and 51.11% and methanol fraction-68.89% and 24.44% respectively. Levamisole, a known potent immunostimulant, afforded a stimulation of 68.89% at a dose of 2.5 mg/kg. The phytochemical evaluation of the fractions carried out showed that the most active fractions were either chiefly steroidal, terpenoidal or flavonoidal. This work suggests that the main constituents of our local mistletoe responsible for immunostimulation are the flavonoids, steroids and terpenoids. Glycosides, carbohydrates, tannins and alkaloids appear to augment the measured activities. Acknowledgemnet: The authors acknowledge Mr. A.Ozioko of BDCP, Nsukka, Enugu State Nigeria for providing the plant material. References. 1. Omeje, E.O. et al (2008) Asian Journal of Medicinal Plants. 2. Osadebe, P.O. et al (2008) Journal of Pharmacology and Phytochemistry- Accepted. 3. Osadebe, P.O., Ukwueze, S. (2004), Bio. Research 2(1):18 – 23. 4. Osadebe, P.O. et al. (2006) Journal of Pharmaceutical and Allied Sciences 3(1):263 – 268.

PA79 Anti-inflammatory properties of hexanic fraction of *Agave sisalana* in pleurisy model

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Carrageenin (CAR), when injected into the pleural cavity, causes several injuries and local inflammation attracting the neutrophil and mononuclear cells to this inflammation. Some plants have secondary metabolites with anti-inflammatory activity, such as steroidals sapogenins present in *Agave* genus. The anti-inflammatory activity of the Hexanic fraction of *Agave sisalana* (FHAS) was evaluated in pleurisy model. Male Unib:WH rats where separated and treated in groups FHAS 10 mg/kg, FHAS 25 mg/kg, PEG-40% and Dexamethasone (DEX) 2,0 mg/kg. All the groups received an injection of 0,2 ml λ-CAR 1% into the pleural cavity 1 hour later. After four hours the animals were sacrificed and their pleural cavities were washed with PBS 5 ml (heparinized). Pleural exudates were used to count cells in Neubauer chamber- the results are shown below. Table 1: values of celular infiltrate (mean and s.d)

Groups	Total account		Neutrophils		Mononuclear's	
	Number of cels (106)	% inhibiton	Number of cels (106)	% inhibiton	Number of cels (106)	% inhibiton
PEG	3,8 ± 0,87	------	2,86 ± 0,74	------	1,18 ± 0,29	----
DEX	0,9 ± 0,13*	76	0,29 ± 0,07*	90	0,61 ± 0,14*	48
FHAS 10	2,25 ± 0,48*	41	1,29 ± 0,30*	55	0,98 ± 0,19	17
FHAS 25	1,89 ± 0,48*	50	1,08 ± 0,31*	62	0,78 ± 0,16*	34

In conclusion, FHAS showed significant results for cellular infiltration on pleurisy model, which suggests new studies in its mechanisms of action.

PA80 Analgesic study of hexanic fraction of *Agave sisalana*

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The therapeutic use of medicinal plants is widely used throughout the world. It is an ancient tradition in several cultures. The objective of these tests is to analyze the analgesic property the Hexanic fraction in *Agave sisalana* (FHAS) with doses of 5, 10, 25 and 50 mg/kg. The analgesic investigations were carried out in two types of noxious stimuli: chemically (acetic acid-induced writhing) using a positive control, (indomethacin) and thermal (hot plate and tail flick tests) with morphine and sufentanil control, respectively, and Polietileneglycol 40% (PEG) as vehicle. FHAS decreased the acetic acid model writhings in doses of 5, 10 and 25 mg/kg (22, 54 e 48%, respectively) in comparison with PEG and the standard drug Indomethacin (45%). It showed an increasing latency time in tail flick tests in doses of 10 mg/kg (47,4% in 90 minutes), 25 mg/kg (61,5% in 60 minutes) and 50 mg/kg (66,2 in 45 minutes). Hot Plate showed increasing latency time in 10 (5,91 ± 0,89 s), 25 (6,58 ± 0,68 s) and 50 (7,49 ± 1,14) doses 120 minutes. These results showed that FHAS had central and peripheral acting effects, but they do not involve an opioid receptor. In conclusion, FHAS has analgesic activity. *Acknowledgements: CAPES, FAPESP*

PA81 Effect of methanolic extract of *Barleria lupulina* Lindl. in adjuvant induced arthritis in rats

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Barleria lupulina Lindl. has been traditionally used in rheumatoid arthritis but no scientific data has been published supporting the claimed ethnomedical use [1, 2]. Thus the present study was designed to illustrate the beneficial outcome of the methanolic extract of *Barleria lupulina* Lindl. in adjuvant induced arthritis in rat model with respect to the changes in pathological lesion and extra-articular manifestation. Female Sprague Dawley rats of body weight 150 – 250 g were used for the study. The animals were divided into six groups of 6 animals each viz. Normal control, arthritic control, Standard drug treated (Indomethacin), MEBL (Methanolic extract of *Barleria lupulina* Lindl) 300 and 600 mg/Kg. Arthritis was induced in all the groups (except normal control group) by the injection of 0.1 ml of Freund's Complete Adjuvant (Sigma) in the subplantar region of the left hind paw [3]. Arthritis developed 14 days after the adjuvant injection and then treatment was continued for another 14 days according to the treatment protocol. On 28th day, animals were sacrificed and antioxidant status and myeloperoxidase activity changes in control and experimental animal were analyzed. MEBL at the both doses significantly regulated the inflammation in the arthritic joints by reducing the paw volume and myeloperoxidase activity and by increasing the antioxidant levels. The effect might be attributed to the combined effect of phytoconstituents like flavonoids and glycosides present in the extract. *Acknowledgements: All India Council for Technical Education, New Delhi, India for scholarship to the presenting author* **References:** [1] Suba, V. et al. (2005) Phytother. Res. 19:695 – 969. [2] Wanikiat, P. et al. (2008) J. Ethnopharmacol. 116:234 – 244. [3] Bendele, A.M. (2001) J. Musculoskel. Neuron. Interact. 1:377 – 385.

PA82

New caulindoles from *Raputia simulans* Kallunki

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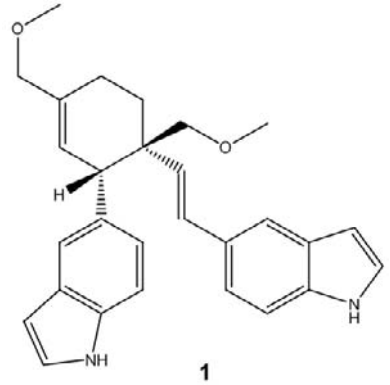
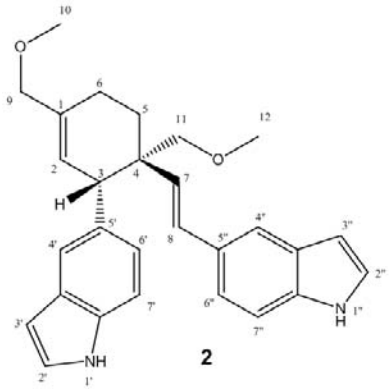
Genus *Raputia* Aubl. comprises 10 species of neotropical Rutaceae in the subtribe Cuspariinae. *R. simulans* Kallunki occurs in the upper Amazon basin of Brazil, Colombia and Peru [1]. Due to the rarity of the genus and the complicated taxonomy amongst similar genera (*Raputia* and *Neoraputia*), no actual phytochemical study of the genus *Raputia* has been reported to date. In continuation of our previous studies concerning the phytochemical investigation of the dichloromethane root extract of *Raputia simulans* Kallunki [2], we report herein the isolation and characterization of new caulindole-type bisindole alkaloids (**1** – **3**). The isolation procedure was performed using Counter Current Chromatography techniques while the structure determination was based on 1D and 2D NMR experiments. The caulindoles is a relatively new class of natural products and the only representatives reported so far were isolated from an Anonnaceae species [3].



1



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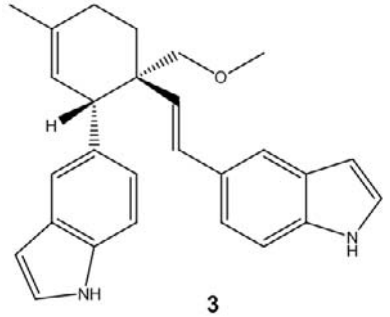

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References: [1] Kallunki, J. (1994) Brittonia 46:279 – 295. [2] Vougogiannopoulou, K. et al. (2008) 7th Joint Meeting of AFERP, ASP, GA, PSE & SIF, Abstracts. Planta Med 74:1080. [3] Makangara, J. et al. (2003) Phytochemistry 65:227 – 232.

PA83

New polyketides from the algicolous fungus *Phaeosphaeria spartinae*

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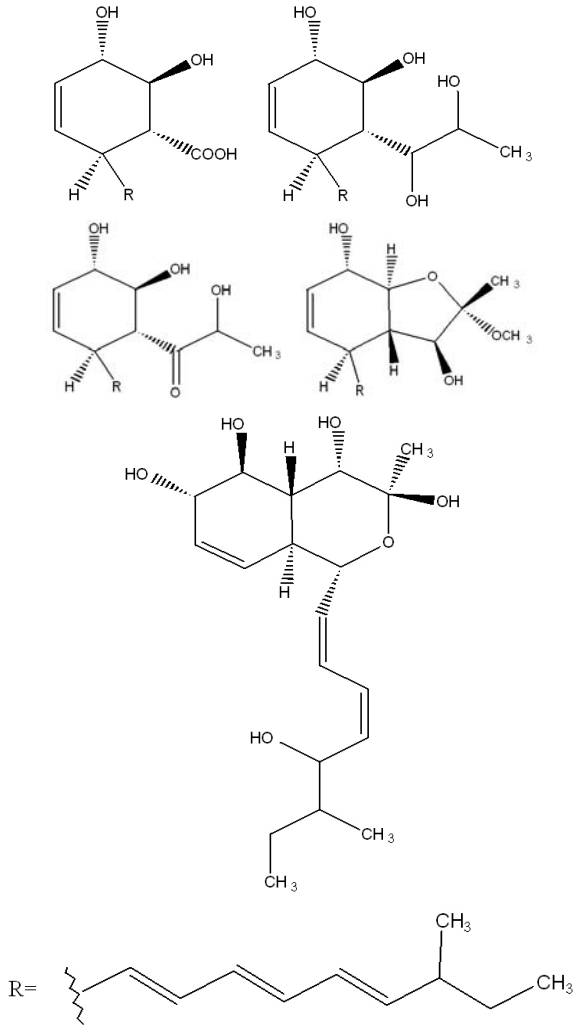
Marine algae are associated with fungal endophytes, which are a source of pharmacologically active natural products. The algicolous fungus *Phaeosphaeria spartinae* originated from the alga *Ceramium* sp. which was collected at the German coast (Büsum, North Sea). Its ethyl acetate extract showed high inhibitory activity toward acetylcholinesterase and papain. Further investigation of the crude extract provided new hydroxylated and unsaturated polyketides. The structures of the compounds were established on the basis of spectroscopic studies. Their biological activities are under investigation.



Acknowledgement: Financial support came from the Egyptian Government and from BMBF (project No. 03F0415A)

PA84

Myricetin from *Plinia cauliflora* (Mart.) Kausel and activity against *Candida albicans*

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The aim of this work was to obtain a pure compound from *P. cauliflora* leaves extract and to evaluate its anti-*Candida* activity. The leaves extract was obtained with 70% ethanol by percolation. After partition with ethyl acetate and water, the ethyl acetate fraction was chromatographed through a Sephadex LH-20 gel column eluted with methanol. Fractions (8 mL each) were collected and analyzed by TLC (silicagel plate, 025 μm

thickness) eluted with chloroform:methanol:*n*-propanol:water (5:6:1:4; v/v; organic phase) and sprayed with sulphuric anisaldehyde. Spots with similar Rf and colour were assembled The isolated compound was identified by its spectral data (^{1}H-NMR, ^{13}C-NMR, HMQC, COSY, HMBC, 1D-TOCSY) and compared to those reported in the literature [1,2]. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) was performed according NCCLS M27-A2 [3]. The identified fraction sample was diluted to 500 µg/mL and the *Candida albicans* (ATCC 64548) final inoculum was 2.5 – 5.0 x 10^3 cells/mL. The spectroscopic data of the isolated compound were in complete agreement with the flavonoid myricetin-3-*O*-β-allopyranoside. This compound showed MIC and MFC of 250 µg/mL and 500 µg/mL, respectively. This was the first flavonol isolated from the leaves of *P. cauliflora* and it was possible to determine for the first time its good activity against the pathogen *C. albicans*. *Support: PADC-UNESP and FAPESP* **References:** [1] Agrawal, P.K. (1989) Carbon 13 NMR of Flavonoids, Elsevier, Amsterdam. [2] Harborne, J.B., Willians, C.A. (2000) Phytochemistry 55: 481 – 504. [3] NCCLS. National Committee For Clinical Laboratory Standards (2002). Approved standard M27-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, PA.

PA85 Pinobatol – a novel spirodienone sesquineolignan

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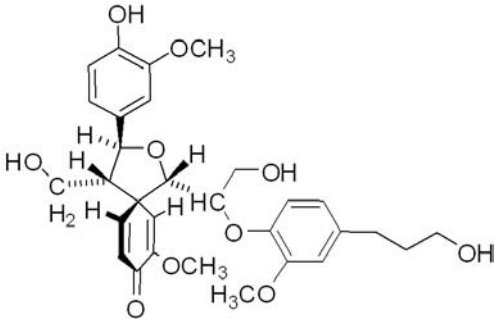
This poster reports the structural elucidation of a novel sesquineolignan with a spirodienone structure (Figure), which we have named as pinobatol [1]. Pinobatol has been isolated from the bioactive fraction from pine bark extract. The structure of it was identified by MS and NMR experiments. The assignment of all ^{1}H and ^{13}C NMR signals was achieved by the combination of techniques DQF-COSY, CH_2-edited HSQC, HMBC, NOESY and selective 1D-TOCSY. Spiro lignan structures are rare and only very few sesquineolignans with spiro skeleton have been described in literature. Interestingly, the spirodienone structure has been proposed as an intermediate formed by β-1-cross-coupling mechanism in the lignin (bio)synthesis. However, the monomeric structure has not been previously found.



References: [1] Sinkkonen, J. et al. (2007) Angew. Chem. Int. Ed. 46:4148 – 4150.

PA86 Phenolic compounds and antioxidative properties of buckwheat grain, hull and flours

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The cultivation of pseudocereal buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) has gained raising attention, due to many positive physiological effects. Buckwheat grain contains large amount of proteins, starch, vitamins and does not contain gluten, which classify buckwheat products as functional ones. Also buckwheat grain is reach in phenolic compounds [1,2]. Distribution of phenolic compounds and antioxidant activity of ethanolic extracts of buckwheat whole grain (1), dehulled grain (2), hull (3) and two types of flours (wholegrain (4) and light (5)) were investigated. For the purpose of extracts antioxidant properties investigation iron (III) reduction and DPPH assays were applied. Total phenol and flavonoid contents of each extract were also determined. The extracts were analysed by HPLC as well and showed to contain significant quantities of plant phenolics (Table). Hull extract contained 14.3% of phenolics, which is almost ten times more then in other extracts. Furthermore, hull extract exhibited the best antioxidant properties in both tests applied (IC_{50}= 0.37 mg/ml). Significant correlation was obtained for the results of these tests (0.97, p< 0.05). These results strongly correlate with the total phenol and flavonoid contents in the samples, as well. Regarding the obtained results hulls could be potentially used as a source of natural antioxidants.

Extract	Flavonoids and phenolcarbonic acids content (%)												
	Procatechin	p-hydoxibenzoic acid	chlorogenic acid	vanilic acid	caffeic acid	siringic acid	p-coumarin	sinapic acid	vitexin	rutin	hyperoside	isoquercitrin	quercetin
1	0.06	0.16	0.17	-	-	0.02	0.01	-	0.07	0.27	0.07	0.02	0.03
2	0.03	0.03	0.19	-	-	0.03	0.01	-	-	0.12	0.02	-	-
3	-	-	0.10	0.13	-	0.04	0.08	-	0.55	0.67	0.65	0.15	0.15
4	0.02	0.01	0.10	-	-	-	0.01	-	-	0.14	0.01	0.01	-
5	-	0.02	0.09	-	0.01	-	0.01	0.04	-	0.07	0.01	0.01	-

Acknowledgements: The authors wish to thank Serbian Ministry of Science for financial support project number TR 20068 **References:** [1] Kim, S.J. et al. (2009) Food. Chem. 110:814 – 820. [2] Gawlik-Dziki, U. et al. (2007) Food Sci. Technol. 42:137 – 143.

PA87 Investigation of volatiles, lipoidal matter and biological activity of the aerial parts of *Dichrostachys cinerea* L.

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GC/MS analysis of the volatile constituents obtained by steam distillation of the aerial parts of *D. cinerea* [1] revealed the identification of seventy compounds representing 86.13% of the total volatiles [2]. α-Pinene (26.47%), nonanal (5.11%) and 8,11,14-eicosatrienoic acid (4.90%) were the major compounds. Lipoidal matter was fractionated into unsaponifiable and fatty acids fractions [3]. GS/MS analysis of both fractions separately, revealed the identification of thirty four compounds representing 93.67% of the total unsaponifiable fraction with Isophytol (25.43%), butylated hydroxyl toluene (15.08%), octadecene (8.57%), and hexdecene (8.51%) as major compounds, while, thirty one compounds representing 92.08% of the total fatty acids fraction were identified, with methyl 9, 12, 15-octadecatrienoate (25.55%) and methyl-12-hydroxy-9-octadecenoate (22.43%) as major compounds. Unsaturated fatty acids constituted 53.63% while the saturated ones constituted 38.45%. Analgesic, antipyretic and anticonvulsant activity tests of two dose levels [50 &100 mg/kg body weight (b.wt.) oral doses, n = 6 rats] of the total ethanol extract and successive extracts (petroleum ether, chloroform, ethyl acetate & methanol) of the plant, after one and two hours were investigated. Novalgine (50 mg/kg b.wt.), paracetamol (20 mg/kg b.wt.) and carbamazepin (100 mg/kg b.wt.) were used as reference drugs, respectively. The obtained data were statistically analyzed using the Student's "t" test. Results with p < 0.01 were considered statistically significant. The highest activities were found to be exhibited by 100 mg of total ethanol extract (80.87% potency), 100 mg of methanol extract (121.76% potency) and 100 mg of ethyl acetate extract (74.15% potency) after two hours for the three activities respectively, in comparison with the corresponding reference drugs (100% activity). **References:** 1. Macleod, A.J., Cave, S.J. (1975) J. Sci. Food Agric. 26:351 – 358. 2. Adams, R. (1995) Allured publishing Corporation, Carol Stream Illinois, USA. 3. Johnson, A.R., Davenport, J.B. (1971) Division of John Wiely and Sons, Inc. New York.

Topic B: Conservation and biodiversity issues

PB1 Bulked AFLP analysis for assessing genetic diversity in *Echinacea* species

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Echinacea is an allogamous genus, thus its cultivars or populations are heterogeneous. Using amplified fragment length polymorphism (AFLP) to estimate the genetic diversity of *Echinacea* may be limited by the large number of individual plants that need to be processed. In the present study, effectiveness of several bulkings (10, 15, 20, 25 and 30 individuals) with 20, 36 and 55 primer pairs was assessed using AFLP in determining genetic diversity of eight *Echinacea* species/varieties/cultivars. The results indicated that the use of bulked DNA-based AFLP analysis is capable of detecting genetic diversity among *Echinacea* species/varieties/cultivars. The assessments showed that a bulk of 15 individuals could detect AFLP variations at most genomic sites. Additionally, 20 primer pairs could generate sufficient polymorphic fragments to achieve high resolving power of AFLP for the tested *Echinacea* genus. 1. Chen, C.L. et al. (2008) Exp. Agric. 44:497 – 507. 2. Kim, D.H. et al. (2004) Genome 47:102 – 111.

PB2 Allelopathic potential of phenolic constituents from *Polygonum cuspidatum* Sieb. & Zucc (Polygonaceae)

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Polygonum cuspidatum Sieb. & Zucc (Polygonaceae), originating from China and used in traditional Chinese medicine, has gained much notoriety in Europe and North America as a pernicious weed by reducing the diversity of plant species and significantly altering natural habitats [1]. Analysis of different crude extracts of *P. cuspidatum* from China and Switzerland by HPLC-DAD-ESI/MS revealed that the main phytochemical differences between the original and invasive varieties reside in the last one. Stilbene glycosides (piceatannol glucoside, resveratroloside) predominate, and proanthocyanidins (catechin, epicatechin, flavanol tetramer, flavonol gallates) are also obviously present. The novel weapon hypothesis states that some invasive weed species owe part of their success as invaders to allelopathy mediated by some allelochemicals [2]. With the seedling growth test model [3] of *Lepidium sativum*, the allelopathy of extracts and pure compounds isolated from MeOH extracts were studied. The MeOH extract of roots and DCM extract of leaves of both invasive and native varieties showed significant allelopathic activity (62.3%, 93.7% respectively for the invasive variety; 70.5, 92.0% respectively for the native variety). Among 17 compounds including anthraquinones, procyanidins, stilbenes, flavonoids, phenylpropanoids and aromatic acid, rhein, stilbenes, procyanidin monomers demonstrated potential activity. Especially, rhein and resveratrol with IC_{50} 0.04, 0.41 mM respectively, were much stronger than the known allelochemical (-)-catechin (IC_{50} 3.80 mM). The concentrations of these two compounds in root exudates and field soil, and their effect on the surrounding plant species are necessary to be further studied to support the novel weapon hypothesis which may contribute to the aggressiveness of *P. cuspidatum*. **References:** [1] The Nature Conservancy of Vermont (1998) Invasive exotic fact sheet: Japanese knotweed. Montpelier USA. [2] Inderjit et al. (2006) Trends Plant. Sci. 11:574 – 580. [3] Inoue, M. et al. (1992) J. Chem. Ecol. 18:1833 – 1840.

PB3 Metabonomic study of transgenic and non-transgenic sugarcane leaves based on NMR profile

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Metabolomics represents an holistic approach complementary to genomics and proteomics for studying a complex biological system's response to chemical, physical and genetic variations [1]. The aim of this work was to establish metabolic fingerprints of sugarcane (*Saccharum officinarum*) to identify differences between leaves of two varieties of transgenic sugarcane modified with proteinase inhibitors Bowman-Birk (BB) and Kunitz (K) from soybean and their respective controls. Principal Component Analyses (PCA) and ANOVA-test were required to recognize and evaluate the significance of possible discriminating metabolites. 42 samples of sugarcane leaves were analyzed through ^{1}H NMR (25 °C, Bruker AV-400 spectrometer, proton frequency of 400.13 MHz). The samples were submitted on 2 ways of extraction: direct extraction to general analyses and undirected extraction to precipitate sugars and concentrate polyphenols compounds which are often affected by genetic transformation [2, 4]. Some compounds were identified by 1D (^{1}H and J-Resolved) and 2D NMR experiments (COSY ^{1}H-^{1}H and HMBC ^{1}H-^{13}C) as isomers of 3' and 5'-chlorogenic acid, syringic acid, glucose, sucrose, threonine, alanine, aspartic acid, proline, fumaric acid, succinic acid, choline, glycine, asparagine and some unidentified polyphenols. PCA scores analyses from total bucket files exhibit no significant difference between the most transgenic plants and controls in both kind of extractions for BB and K varieties. According to the procedure followed the transgenic plants and wild type apparently have the same phenotype. Therefore, these results indicate that these transgenic varieties of sugarcane should not represent health risk for humans. The improved resistance of the sugarcane transgenic for the proteinase inhibitor genes is due to these proteins as no significant changes were observed in the metabolome [5]. *Acknowledgements: FAPESP, CAPES, CNPq.* **References:** [1] Lindon, J.C. et al. (2001) Prog. Nucl. Mag. Reson. Spectrosc. 39:1 – 40. [2] Kim, H.K. et al. (2006) Biotechnology in Agriculture and Forestry, v. 57 Plant Metabolomics. Springer. Berlin. [3] Choi, Y.H. et al. (2004) Plant Physiol. 135:2398 – 2410. [4] Choi, H-K. et al. (2004) Phytochemistry 65:857 – 864. [5] Falco, M.C. and Silva-Filho, M.C. (2003) Plant Physiol. Bioch. 41:761 – 766.

PB4 Evaluation of the essential oil composition of fruits of three endemic species of *Tornabenea* from Cape Verde Islands

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Tornabenea Parl. ex Webb is an endemic Cape Verdean genus of Apiaceae subfamily Apioideae tribe Laserpitieae [1]. The number of species in *Tornabenea* is still a matter of controversy [2]. Being fruit characters regarded as crucial in Apiaceae taxonomy and given the identification difficulties within the members of this genus, the aim of this study was to characterize the essential oil profile of the fruits of three *Tornabenea* species, to ascertain whether volatile components could serve as chemical markers and help in the species delimitation. The essential oils of *T. annua*, *T. insularis* and *T. tenuissima* herbarium and *in vivo* fruits, collected in five Islands from Cape Verde archipelago, and from plants grown in Portugal, were isolated by hydrodistillation and analysed by GC and GC-MS. The yellowish oils were obtained in variable average yields, lower in herbarium samples [0.05% (v/w)] and higher from *in vivo* samples [1.3% (v/w)]. Whereas *T. annua* fruits oils were all dominated by myristicin (92 – 100%), most of the *T. insularis* fruits oils were elemicin rich (82 – 90%). No clear information could be obtained for *T.*

tenuissima fruits oils. Cluster analysis confirmed these chemical differences. Although in minute amounts, most of the herbarium samples provided a good support for *Tornabenea* species discrimination, if they were separated according to their individual provenance. *Acknowledgements: Partially funded by a grant from European Social Fund 211 015 (PRODEP III – 3.2).* *References: [1]* Heywood, V.H. (1971) Bot. J. Linn. Soc. 64 (Suppl. 1):31 – 41. [2] Grosso, C. et al. (2008) Plant Biosyst. 142:87 – 93.

PB5 Volatile characterization and molecular polymorphism evaluation among Azorean *Laurus azorica*

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A combined analysis of *Laurus azorica* (Seub.) Franco volatile oils, RAPDs and ISSRs data, was performed to evaluate the relationship between both data sets. Volatiles from individual samples were isolated by distillation-extraction and analyzed by GC and GC-MS, as in [1]. DNA fingerprinting was performed using 51 RAPD primers and 25 ISSR primers, according to [2, 3]. NTSYS software [in 1] was used for DNA and volatile oils data cluster analysis. The oils consisted mainly of α-pinene (4 – 48%), 1,8-cineole (4 – 36%) and β-pinene (3 – 23%), in accordance with previous studies in populations [1]. Cluster analysis of chemical data showed a high correlation among all samples (S_{corr}= 0.86), with exception of three individuals: one from S. Miguel (S_{corr}= 0.63) and two from Graciosa (S_{corr}= 0.44). The smaller correlation of the latter samples was due to the higher relative amounts of 1,8-cineole (24 – 36%). β-Caryophyllene (6 – 12%), β-elemene (0.3 – 18%) and linalool (6 – 8%) were also among the main oil components, suggesting a volatile composition similar to that of *L. nobilis*. The selected RAPDs and ISSRs primers generated a total of 566 bands representing 95% of polymorphism between individual samples. Cluster analysis of molecular data showed a moderate correlation (S_{corr}= 0.51). Plants were grouped mainly according to their geographical location, providing no straight correlation between molecular and chemical clusters. Although molecular data was useful in assessing genetic diversity in *L. azorica*, different molecular tools and additional chemical profiling should be performed, in particular with Graciosa and S. Miguel accessions. *Acknowledgements: Partially funded by FCT under research contract PTDC/AGR-AAM/70136/2006. The authors thank the Lisbon Botanical Garden for gently providing the L. nobilis specimen.* **References:** [1] Pedro, L.G. et al. (2001) Phytochemistry 57:245 – 250. [2] Trindade, H. et al. (2008) Biochem. System. Ecol. 36:790 – 797. [3] Mendes, M.D. et al. (2009) Biochem. System. Ecol. 37:98 – 105.

PB6 Variation in *Angelica archangelica* root essential oils

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NordGen – Nordic Genetic Resource Center – is an institution for conservation and sustainable use of plants, farm animals and forest trees. Researchers from NordGen collected *Angelica archangelica* L. samples from Greenland and Faroe Islands and the plants were cultivated in Alnarp, south Sweden. There are nine samples from individual plants of the same accession (Greenland) and 16 samples from different accessions (Greenland and Faroe Islands). Root samples were collected in autumn 2008, dried and cut and sent to University of Helsinki for analysis. The essential oils were isolated by hydrodistillation, dried over anhydrous sodium sulphate and stored at +6°C. They were analysed by GC-MS using a polar Stabilwax column. The oil composition was very complex; 60 – 70% of the oil comprised monoterpene hydrocarbons, mainly α-pinene and α-phellandrene, 7 – 20% macrocyclic lactones, 13-tridecanolide, 15-pentadecanolide (muskalactone) and 17-heptadecanolide, and the rest consisted of oxygenated mono- and sesquiterpenes plus sesquiterpene hydrocarbons. Several chemotypes could be distinguished. The macrolides are important for the musk odour of angelica and their amount has been reported to increase during storage [1]. **References:** [1] Nivinskiené, O. et al. (2003) Chemija (Vilnius) 14:52 – 56.

PB7 Population structure and gene flow among wild populations of the *Saussurea involucrate* based on chloroplast DNA sequences

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Saussurea involucrate (Kar. et Kir.) Sch.-Bip. (Compositae) has been used as a traditional Chinese medicine [1]. To assess the population structure and gene flow among the extant populations, we sequenced *psb*A-*trn*H (442 bp) and *rps*16-*trn*Q (1116 bp) of the Chloroplast DNA sequence for 62 samples collected from its current three large populations (Bogedafeng, Heshuo, Tianchi). A total of 17 unique haplotypes were defined based on 23 polymorphic sites. Phylogenetic analyses suggested the Chloroplastic DNA sequence haplotypes were split into two well divergent clades. Interestingly, the two distinct haplotype clades were found to coexist in Tianchi area. The nested clade analysis revealed a significant phylogeographic structure among the *S. involucrate* populations (total cladogram: χ^2=32.75; P < 0.001), which was inferred to result from past fragmentation followed by range expansion. The population expansion was supported by the analysis of mismatch distribution and the tests of neutrality. In the end, we suggest, actions should be taken to conserve populations like Tianchi, in which a high level of population genetic diversity was observed. *Acknowledgements*: *This research was supported by the Natural Science Foundation of China (NSFC 30770153).* **References:** [1] The state Pharmacopoeia Commission of the PRC. (2005) Pharmacopoeia of the People's Republic of China. Chemical Industry Press. Beijing.

PB8 Differential expression of microsatellites in leaves and rhizomes of Turmeric (*Curcuma longa* Linn.)

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Curcuma longa Linn. commonly known as turmeric or Indian saffron belongs to the family Zingiberaceae. Turmeric is a perennial herb with simple and large leaves. Tubers, rhizomes and essential oil of turmeric have great importance in medicine and food [1]. Although essential oil contents of turmeric have been extensively studied, genome analysis of this plant falls behind the other crop species. A total of 12,593 rhizome and young leaf expressed sequence tags (ESTs) were analyzed using two bioinformatic programs to identify microsatellites [2, 3] and a statistical approach [4] was used to investigate whether microsatellite densities between rhizome and young leaves differed. Results indicated that the level of microsatellite densities in leaf and rhizome ESTs were not statistically different (P = 0.05). On the other hand, densities of tri-nucleotides and tetra-nucleotide microsatellites were statistically different between the two types of tissues (P ≤ 0.001). Tri-nucleotide microsatellites were statistically higher in leaf ESTs while they were lower in rhizome ESTs. Microsatellite density differences between the two tissues may indicate that genes containing tri- and tetra-nucleotide repeats have specific functions in these tissues. Microsatellites showing differences between tissues could also be used in tissue fingerprinting studies. Also our initial studies indicated that there are microsatellite density differences between *Curcuma* species. In the present study we also identified a total of 22 new set of microsatellite primer pairs. These genic (EST-based) microsatellite primer pairs could be used in genetic studies in turmeric improvement. *Acknowledgements: This research is supported by the Scientific Research Projects Administration Unit of Akdeniz University.* **References:** [1] Jain, S. et al. (2007) Phcog Rev. 1:119 – 128. [2] Ince, A.G. et al. (2008) Plant Cell Tiss. Org. 94:281 – 290. [3] Bilgen, M. et al. (2004) Bioinformatics 20:3379 – 3386. [4] Lawson, M.J. and Zhang, L. (2008) Gene 407:54 – 62.

PB9 Development of microsatellite primer pairs for *Cynara cardunculus* var. *scolymus* (L.) Fiori

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Globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] is a diploid (2n = 2x = 34) out-crossing species, originating in the Mediterranean Basin. It is poly-annual crop and mostly cultivated for its edible immature flower heads. Most commercial production of globe artichoke is based on vegetative propagation of selected clones. Differentiation of distinct clones under cultivation is difficult to determine with accuracy based on morphological observations. On the other hand molecular analyses using DNA fingerprinting techniques such as DNA markers have number of advantages. Among the DNA fingerprinting techniques, the multilocus AFLP (amplified fragment length polymorphism) and the single-locus microsatellite (simple sequence repeats) markers have been used in many crops species for identification of closely related plant species and genetic mapping. AFLP technique in comparison to microsatellites has several disadvantages. Microsatellites, on the other hand, produce robust and reliable markers in every organism studied so far. However the number of microsatellite primer pairs flanking the microsatellites is limited in globe artichoke. Expressed sequence tags (ESTs) have been used to obtain microsatellite primer pairs in many organisms [1]. In the present study utilizing globe artichoke ESTs we obtained 50 microsatellite primer pairs. EST-microsatellites were identified using Exact-Tandem Repeat Analysis program [2] and primer pairs flanking these microsatellites were designed using Primer3 software [3]. *Acknowledgements: This research is supported by the Scientific Research Projects Administration Unit of Akdeniz University.* **References:** [1] Ince, A.G. et al. (2008) Plant Cell. Tiss. Org. 94:281 – 290. [2] Karaca, M. et al. (2005) J. Genet. 84:49 – 54. [3] Rozen, S., Skaletsky, H.J. (2000) Methods in Molecular Biology, vol. 132, Bioinformatics Methods and Protocols, Humana Press, Totowa, USA.

PB10 Essential oil profile of *Thymus jankae* Celak. from Bosnia

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Among the aromatic plants belonging to the Lamiaceae family, the genus *Thymus* is noteworthy for the numerous wild species and cultivated plants. It is well known for different medicinal purposes such as antispasmodic and antimicrobial activity. A widespread chemical polymorphism is an important characteristic of this genus [1]. The qualitative composition and relative proportions of the volatiles are widely influenced by the genotype, the ontogeny, and the environmental conditions [2,3]. Volatile profile of odorous parts of *Thymus jankae* Celak., collected from natural habitat, was analyzed by capillary GC-MS. This work presents the first investigation of hydrodistilled essential oil and headspace composition of this species from Bosnia and Herzegovina. Forty-eight components were identified in both samples, representing 96.4% and 96.2% in total, for hydrodistilled essential oil and headspace, respectively. The major compounds in essential oil belong to the oxygenated monoterpenes (57.5%), with linalyl acetate (28.7%) and linalool (14.4%). Headspace sample also showed richness in linalyl acetate (52.4%), but second the most abundant compound was *alpha*-pinene (14.5%), a monoterpene hydrocarbon. Investigated essential oil and headspace from Bosnian population of *T. jankae* significantly differs from volatile profile of *T. serpyllum* from the same region [2]. To the best of our knowledge, there is no published data on medicinal properties of *T. jankae*. This issue will be subjected to our future research. **References:** [1] Stahl-Biskup, E., Sáez, F. (2002) Thyme: The Genus *Thymus*, CRC Press. [2] Ćavar, S. et al. (2009) Nat. Prod. Comm. 4:415 – 420. [3] Karuza-Stojaković, L. et al. (1989) Arh. Farm. 39:105 – 111.

PB11 Testing candidate plant barcode regions in the *Dendrobium* species

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There are 74 species and 2 varieties of *Dendrobium* found in China and more than half of them are used as *Herba Dendrobii* in China and other Asian countries. Because of its high market demand, *Herba Dendrobii* has a relatively high market price compared to other medicinal plants. Moreover, medicinal *Dendrobium* is listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This has led to substantial adulterations with other *Dendrobium* species or other orchid species. *Dendrobium* is difficult to identify and is an ideal model group to test DNA barcoding technique [1]. In our previous study, the candidate DNA barcoding sequence, *psbA-trnH* intergenic spacer region, could be used as a barcode to distinguish various *Dendrobium* species and to differentiate *Dendrobium* species from other adulterating species [2]. In this study, more samples were used to test the utility of six coding (*matK, rpoC1, rpoB, rbcL, accD, ycf5*) and two non-coding (*atpF-atpH, psbK-psbI*) chloroplast markers as potential plant barcoding regions. The results showed that six of the regions we tested were slightly variant across species (*rpoB, rpoC1, accD, rbcL, ycf5, atpF-atpH*), and *psbK-psbI* had significant variation and show promise for barcoding in *Dendrobium* species. *Acknowledgements: This research was supported by the International Cooperation Program of Science and Technology (No.2007DFA30990) and the Special Founding for Healthy Field (No. 200802043).* **References:** [1] Lahaye, R. et al. (2008) Proc. Natl. Acad. Sci. USA 105:2923 – 2928. [2] Yao, H. et al. (2009) Planta Med. 75: DOI: 10.1055/s-0029 – 1185385.

PB12 Contribution to the comparative studies of natural populations of *Veronica* from the Romanian Eastern Carpathians

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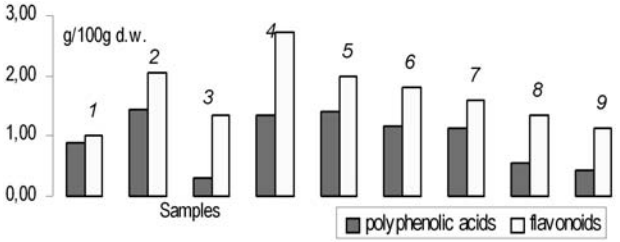
In the study regarding the chemical composition of 5 *Veronica* species, originating from habitats with reduced anthropization from the area surrounding the Moldavian Subcarpathians, we achieved a general phytochemical analysis to identify the big groups of active principles. By means of TLC and HPLC we analyzed the polyphenolic components, spectrophotometrically dosing the flavonoids, iridoids and polyphenolic acids.



Total polyphenolic acids (caffeic acid) and flavonoids (rutoside) *V. urticifolia* (1; 2), *V. austriaca* (3), *V. officinalis* (4; 5), *V. chamaedrys* (6; 7), *V. beccabunga* (8; 9)

Caffeic acid equivalents (mg/g d.w.)

Samples	No. of derivates	Cumulative quantity
1	3	10.58
2	2	41.72
3	-	-
4	2	42.10
5	6	33.05
6	3	23.52
7	2	26.14
8	2	8.47
9	2	8.80

With the *Veronica* populations, we noticed the qualitative and quantitative variability of the polyphenolic acids and flavonoids, dependent on the analyzed species and the original habitat. The HPLC analysis confirmed the presence in some extracts of the derivates of apigenine, luteoline as well as caffeic acid [1]. Similarly, we noticed the existence of an inter- and intra-specific variability of the iridoids and polyholosides. *Acknowledgements: The work is sustained in the PNCDI-2 program financed by the Romanian Government – National R&D Agency* **References:** [1] Blaschek, W. et al. (2006) Hagers Handbuch der Drogen und Arzneistoffe, (Hager ROM), Springer Verlag, Berlin, Heidelberg, New York.

PB13 Chemical variability of some natural populations of *Ajuga sp.* from the north-eastern part of Romania

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Seven natural populations of *Ajuga reptans* L. and eight of *A. genevensis* L., harvested in June, 2008, from four counties situated in the north-eastern part of Romania, were investigated to determine their content of flavonoids, polyphenolic acids and iridoids in order to appreciate the inter- and intraspecific chemical variability. Using the TLC qualitaive investigation technique, we resorted to spectrophotometry for the quantitative determination and completed with the HPLC analysis for a better appreciation of the spectrum similarities and differencies from the polyphenolic compounds group. The study showed the existence of an intraspecific variability for the populations belonging to the same genus, which is, probably, linked to the pedoclimatic offer of the original location. In the same time, we noticed that the flavonoidic fraction is constituted, in the case of both species, of aglycones, a fact already known by literature [1], exception being a single sample of *A. genevensis,* in which we identified a reduced quantity of luteolin-7-glucoside. Likewise, there exists an interspecific variability, especially at the level of polyphenolic acids, for *A. reptans* characteristic being the rosmarinic acid, while for *A. genevensis* it is the chlorogenic acid. In this respect, in the non-volatile monoterpens, the officinal species presents three components, while for *A. genevensis* their number is of two. *Acknowledgements: The work is sustained in the PNCDI-2 program financed by the Romanian Government – National R&D Agency* **References:** [1] Nikolova, M., Asenov, A. (2006) Nat. Prod. Res. 20:103 – 106.

PB14 Chemodiversity and conservation of *Santalum insulare* of French Polynesia

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Overexploited for its fragrant heartwood during the 19th century, the Polynesian sandalwood (*Santalum insulare*) is now an endangered tree scattered among the islands of Eastern Polynesia where several botanical varieties are recognized [1]. In order to sustainably manage this natural resource, chemodiversity approach was carried out to highlight restauration and conservation programm monitoring. So, sesquiterpenoid composition of heartwood extracts and leaf-flavonoid composition of samples from all its distribution area were analyzed. Multivariate statistical methods of the obtained data were performed to establish the diversity patterns. Regarding the essential oil quality from sesquiterpenoid diversity, two main chemotypes appeared: santalol chemotype as the major one and a (Z)-nuciferol chemotype restricted to few stands in Marquesas islands [2]. Investigations on leaf-flavonoid diversity put in evidence a remarkable coherence between chemotaxonomy and botanical taxonomy. Sandalwood varieties of each archipelago were clearly segregated for their flavonoid profiles, confirming in that the major role of flavonoids in chemotaxonomy but also the noticeable infra-specific biodiversity of the Polynesian sandalwood [3]. This observed diversity is subjected to conservation programm actions in French Polynesia. Thus, several stands from (Z)-nuciferol chemotype were enclosed against feral cattle and goats whereas the santalol chemotype were mutliplied and distributed to the local inhabitants by the forest service. Moreover, on islands from each of the flavonoid profiles, local sandalwood seed orchards were implemented firstly for conservation purposes and secondly for replantation by the government but also by the inhabitants. **References:** [1] Fosberg, R.F. et al. (1985) Candollea 40:459 – 470. [2] Butaud, J.F. et al. (2003)J. Essent. Oil Res. 15:323 – 326. [3] Butaud, J.F. et al. (2006) Nat. Prod. Comm. 1:969 – 972.

PB15 Genus *Hydrangea*: diversity of pigments and phenolic compounds

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The most important collection of *Hydrangea* in Europe is located in Angers (France). It consists of over 700 germplasm accessions distributed in 13 species. Originating from Asia and America, they were introduced in Europe in the 18th century for their ornamental interest but medicinal properties may also be found in this genus since extracts from *H. macrophylla* are already described as exhibiting anti-diabetic [1], lipid lowering and anti-oxidative [2], anti-allergic [3] and antimalarial activities [4]. Management of the collection requires botanical, genetic and biochemical studies allowing good, reliable characterization of species, subspecies and varieties. In this context, the biochemical characterization of the inflorescences was undertaken to evaluate the intra and interspecific diversities of pigments and other phenolic compounds. Inflorescences are generally white, except for three species: *H. macrophylla*, *H. involucrata* and *H .aspera* which exhibit rose or blue flowers. Among them only *H. macrophylla* was previously studied for sepal color variation [5]. In this study, 80 accessions were analyzed by means of HPLC/DAD, LC-MS/MS and NMR experiments: 46 *H. macrophylla*, 13 *H. aspera*, 6 *H. involucrata*, 5 *H. paniculata*, 3 *H. quercifolia*, 2 *H. arborescens*, 2 *H. anomala*, 2 *H. heteromala*, 1 *H. scandens*, 1 *H. seemannii* and 1 *H. integrifolia*. About 50 phenolic derivatives – essentially phenolic acids and flavonols (quercetin and kaempferol) – and 20 anthocyanins could be identified. The contents of pigments and other phenolic compounds appeared as very diverse both qualitatively and quantitatively and some compounds could be identified as chemospecific. On this basis, a statistical study using Principal Component Analysis allowed a clear distinction between both species and subspecies. Besides, different biological evaluations of crude extracts and secondary metabolites isolated from *Hydrangea* sp will also be discussed. *Acknowledgements: This research is founded by the Region »Pays de la Loire «* **References:** [1] Matsuda, et al. (2007) 2nd Symposium on Pharmaceutical Food Science OCT 18 – 19, Shizuoka, JAPAN. [2] Kim, H.K. et al. (2009) Biol. Pharm. Bull. 32:153 – 156. [3] Kurume, A. et al. (2008) Chem. Pharm. Bull. 56:1264 – 1269. [4] Ishih, A. et al. (2007)J. Nat. Med. 61:213 – 216. [5] Yoshida, K. et al. (2008) Phytochemistry 69:3159 – 3165.

PB16 Status, utilization and diversity of medicinal plants of pachmarhi biosphere reserve, india

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Pachmarhi Biosphere Reserve situated in Satpura Ranges of Madhya Pradesh is rightly known as Satpura Ki Rani (Queen of Satpura), being reservoir of biodiversity in Central India. The Reserve encompasses forest eco-region and is an important transition zone between the forest of western and eastern India. The dominant species are Teak (*Tectona grandis*) and Sal (*Shorea robusta*). It is home to a large number of rare and endemic species of algae, bryophytes, fern, gymnosperms, Orchids and angiosperms including a treasure-trove of medicinal plants. To understand the value of biodiversity, medicinal plants utilization and conservation in the area, phytosociological, systematic and ethno-medico-botanical studies have been carried out with the help of tribal and local people. About 265 species of wild medicinal plants have been identified including 109 tree species, 51 shrubs species, 78 species are herbs, 27 species are climbers and three species of grasses. Of these 265 species 29 species are highly threatened in which 14 species are endangered, 11 species vulnerable and 04 species are at a low risk. With the increasing harnessing of medicinal plants due to commercialization and globalization of herbal wealth, their availability in nature is subsequently declining. Therefore, in the study an attempt has been made to elucidate the

number of medicinal plants to leverage immediate attention required for their conservation and propagation in the region.

PB17 Infraspecific chemical taxa of *Achillea distans* Waldst. Et Kit. from the Rodnei Mountains (Eastern Carpathians) – Romania

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Achillea distans Waldst et Kit (Asteraceae) is an alpino-carpatho-balkan type species that vegetates on the upper limit of mountain forests and in subalpine shrubs. According to the length of ligulate florets and to their color, 2 subspecies are recognized: *Achillea distans* ssp. *distans* (I), with white flowers and 2 mm length of ligulae and *Achillea distans* ssp. *alpina* (Rochel) Soo (II), with pink flowers and 3 mm length of ligulae. [1,2,3]. Both subspecies were harvested from the Rodnei Mts., in the north of the Eastern Carpathians (Romania), near Iezer Lake, at 1700 – 1750 m altitude, in August 2006 and 2007, in the blossom period. The extraction and quantification of the essential oil from dried inflorescences was made in a Neo-Clevenger apparatus, and was analyzed by TLC and GS-MS. The Wiley Library was used as reference database [4]. The content of the essential oil was 0.40 ml/100 g dried material (I) and 0.25 ml/100 g (II). Both oils are colorless and the EP test for prochamazulenes and the TLC assay for azulenes were negative. By GC-MS analyses, 18 compounds were separated in (I), the most important being α-thujone (33.31%), β-thujone (25.52%), sabinene (15.60%), and 36 compounds in (II), among them being eucalyptol (20.97%), sabinene (6.37%), camphor (4.94%), but thujones were missing. We consider that the 2 subspecies are significantly different concerning the chemical composition of the essential oils and they may be considered as infraspecific chemical taxa or chemovarieties of *Achillea distans.* **References:** [1] Anon (1964) Flora RPR, Ed. Academiei, Bucuresti. [2] Tutin, T. et al. (1976) Flora Europaea vol. IV, Cambridge Univ. Press, Cambridge. [3] Coldea, G. (1990) Muntii Rodnei – studiu geobotanic, Ed. Academiei Romane, Bucuresti. [4] Oprean, R. et al. (2001) J. Pharm. Biomed. Anal. 24:1163 – 1168.

PB18 PCR based minisatellites are useful in *Origanum*, *Thymus*, *Sideritis* and *Salvia* genetic studies

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The Mediterranean Basin of Turkey houses many plant taxa. Among these taxa *Origanum*, *Thymus*, *Salvia* and *Sideritis* have attracted researchers and commercial producers in the region due to medicinal and aromatic properties of these plant species. In general, identification of taxon or species of medicinal and aromatic plants in a genus can basically be accomplished using three main approaches such as conventional taxonomic studies based on morphological and anatomical characteristics of individuals, chemo-typing studies based on chemical constituent differences between individuals and DNA-based genotyping studies based on nucleic acid sequence variations between individual genotypes. Species or taxon identification based on morphological characteristics in *Thymus* is very difficult. Morphological characterization of *Origanum*, *Salvia* and *Sideritis* has problems in some taxon. Random amplified polymorphic DNA (RAPD) offer several advantages in species or taxon identification; however, recent studies showed that the RAPD technique has several limitation. In our previous studies [1,2,3] we found that primers flanking the minisatellite regions using polymerase chain reactions (PCRs) could be used in plant genetic studies. Minisatellites are tandemly repeated DNA with a longer motif, up to several dozen base pairs in length in comparison to microsatellites which consist of short repeat motifs. In the present study we report a total of 22 minisatellite flanking primers which generate reproducible polymerase chain reaction amplified products in touch-down PCR amplification profile. These primers are valuable in taxon identification and genetic relationship studies in *Origanum*, *Thymus*, *Salvia* and *Sideritis*. *Acknowledgements: This research is supported by the Scientific Research Projects Administration Unit of Akdeniz University.* **References:** [1] Karaca, M., Ince, A.G. (2008) J. Genet. 87:83 – 86. [2] Karaca, M. et al. (2008). J. Sci. Food Agric. 88:2508 – 2516. [3] Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. 56:211 – 221.

PB19 Identification of genotypes with high essential oil contents in *Origanum*, *Thymus* and *Sideritis*

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A large number of medicinal and aromatic plant species naturally grown in the Mediterranean Basin of Turkey contain secondary metabolites such as alkaloids, flavanoids, phenols, polysaccharides, terpenes, and quinones that are used in the food, pharmaceutical, cosmetic, and pesticide industries. Many of these plant species are undergoing domestication and cultivar development. Unfortunately some of these plant species are among the most endangered species which need to be protected to ensure their sustainable use in the region. In the present study several species in *Origanum*, *Thymus* and *Sideritis* were collected from several locations in the Mediterranean Basin of Turkey, and essential oil contents and DNA fingerprinting analyses of each genotype collected from a location and other locations were studied. Preliminary studies indicated that there exist great variations in essential oil and genetic content of plant genotypes of each genus collected within and between the locations. Some of the genotypes within a location showed extremely higher essential oil contents while the other genotypes possess limited contents of essential oil. Using simple sequence repeat (SSR), minisatellite, chloroplast and mitochondrial DNA markers we obtained species specific DNA markers for some species. Upon completion of this study we will be able to define genotypes and locations from *Origanum*, *Thymus* and *Sideritis* with higher essential oil contents and define DNA marker specific to species in each the three genera [1,2]. Since we recorded positions of the each genotype using a global positioning system, we can recollect those genotypes containing superior essential oil contents and use them in cultivar development studies. *Acknowledgements: This research is supported by the Scientific Research Projects Administration Unit of Akdeniz University.* **References:** [1] Karaca, M. et al. (2008) J. Sci. Food. Agric. 88: 2508 – 2516. [2] Ince, A.G. et al. (2009) Genet. Resour. Crop. Ev. 56: 211 – 221.

PB20 Medicinal plants from Brazilian Cerrado as potential antiulcer drugs

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Global expansion of consumption of alcohol and non-steroidal anti-inflammatory drugs (NSAID) and inappropriate diets have contributed to growing ulcer etiopathology. Thus, peptic ulcer is considered a disease of modern times, related to increasingly frequent addictions and stressful lifestyle. Treatment with natural products presents promise of a cure. Central Brazil is one of the major biogeographic regions of the world and also the most threatened. Many of these plants are used as natural medicines by people living in the Cerrado area to treat several diseases. An ethnopharmacological inventory made in the Cerrado of central Brazil showed a high number of medicinal plants utilized to treat gastric pain and gastritis. As a part of a State Collaborative Program denominated BIOTA-Fapesp, our project aims to investigate 20 medicinal species that potentially act to prevent gastric injuries. This research is based on ethnopharmacological investigation, followed by the chemical and pharmacological investigation of medicinal plants. The antiulcerogenic mechanisms were determined through the effect of the isolated substances (or enriched fractions) on specific receptors, enzymes and substances produced in response to the gastric lesion, such as the expression of Epidermal Growth Factor, COX-2 and angiogenesis. Simultaneously, the antioxidant activity of extracts/substance was evaluated, specifically those related to the mechanisms of antiulcerogenic activity. Additionally, assays for the detection of mucus, prostaglandins, gastrin and antimicrobial action against *Helicobacter pylori* were also evaluated. Our project has shown that the apparent incompatibility between chemical and pharmacological studies of a plant species can be resolved with the strong determination to deal rationally with the problem – the

search for phytomedicine with efficacy and safety of use. *Acknowledgements: Biota/FAPESP and CNPq*

PB21 Authentication of plants in *Astragalus* by DNA Barcoding technique

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Astragalus possesses important medicinal efficacy in Fabaceae, with a large number of medicinal plants and poisonous plants. However, it is arduous to identify some of the species in this genus because of morphological similarity and frequent variation. In this study, The DNA barcode, a short DNA sequence originating from the genome, was firstly investigated for the plants in *Astragalus*. We compared sequences of six potential barcodes, four coding (*trnH-psbA, rpoC1, rbcL, matK*) chloroplast regions and two noncoding (*ITS, ITS2*) nuclear ribosomal DNA among 319 different species of *Astragalus*. The results were as follows: 1. The amplification efficiency for six candidate DNA barcodes decreased successively, *rpoC1 > trnH-psbA = ITS2> matK> ITS> rbcL*. 2. The intraspecific variation and interspecific variation of six promising markers showed that *trnH-psbA, ITS2* and *ITS* were the more discriminatory regions. While other three plastid regions were of lower divergences. 3. In this study different species of *Astragalus* can be differentiated effectively by comparing the DNA barcoding regions. Overall, our findings indicated that DNA barcoding is an efficient and powerful taxonomic tool in *Astragalus*. *Acknowledgements: The International Cooperation Program of Science and Technology (No. 2007DFA30990), the Special Founding for Healthy Field (No. 200802043).* **References:** [1] Gregory, T.R. (2005) Nature 434:1067. [2] Kress, W.J., Erickson, D.L. (2007) PloS One 2:508. [3] Kress, W.J., Erickson, D.L. (2008) Proc. Natl. Acad. Sci. USA 105:2761 – 2762. [4] Frézal, L. and Leblois, R. (2008) Infect. Genet. Evol. 8:727 – 736. [5] Lahaye, R. et al. (2007) Proc. Natl. Acad. Sci. USA 105:2923 – 2928. [6] Hebert, P.D.N. et al. (2003) Proc. R. Soc. Lond. B 270:313 – 321. [7] Song Jingyuan, et al. (2008) Planta Med. 74:1112. [8] Yao, H. et al. (2008) Planta Med. 75, DOI: 10.1055/s-0029 – 1185385. [9] Han, J. et al. (2009) Planta Med. 75, DOI: 10.1055/s-2009 – 1216417. [10] Luo, K. et al. (2009) Planta Med. 75, DOI: 10.1055/s-2009 – 1216448. [11] Pang, X.H. et al. (2009) Planta Med. 75, DOI: 10.1055/s-2009 – 1216450. [12] Gao, T. et al. (2009) Planta Med. 75, DOI: 10.1055/s-2009 – 1216451.

PB22 Comparative essential oil composition of fennel (*Foeniculum vulgare* Mill.) fruits collected during different three years

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Fennel (*Foeniculum vulgare* Mill., Apiaceae) is a well-known aromatic and medicinal plant [1]. Essential oils obtained by hydrodistillation from fresh ripe fruits of fennel collected during three different years (2001 – 2003) from experimental cultures were investigated to evaluate their chemical profile related to climatic conditions. The constituents of the essential oils have been characterized using gas chromatography and mass spectroscopy analysis (GC-MS) [2]. The yield of the essential oil ranged from 2% in 2003 to 12.6% in 2002 and 11% in 2001, respectively. The main compounds in all fennel volatile oils were: t-anethole, estragole, fenchone and limonene. A discrete variation in the aromatic fraction content from 80.50% (2003) to 83.81% (2001) and 85.81% (2002) was observed. On the other hand, we noticed a significant increase of the monoterpenes level from 12.55% (2002), 13.96% (2001) to 19.29% (2003). The essential oil of fennel fruits showed a characteristic chemical profile from year to year. The content and its monoterpenes components were the most susceptible features of fennel essential oil to be affected by climatic conditions. **References:** [1] Parejo, I. et al. (2004) J. Agr. Food Chem. 52:1890 – 1897. [2] Wang, C. et al. (2003) Zhongguo Zhong Yao Za Zhi 28:240.

PB23 Ethnobotanical use of wild and cultivated plants in traditional medicine of Middle Bosnia and Herzegovina

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In the years 2006 – 2009 a research was carried out concerning ethnobotanical use of wild and cultivated plants on the territory of middle Bosnia and Herzegovina (W. Balkan Peninsula; SE Europe), a region characterized by great plant diversity with about 3.572 different species of vascular plants [1]. This area is less frequently studied from the perspective of plant usage in traditional medicine. The purpose of this study was the collection of any information about usage of wild and cultivated plants in human therapy, especially verbally delivered prescriptions. To all used plants corresponding material for investigations and documentation was collected and deposited in the herbarium of the Department of Pharmacognosy, Vienna. For the further analyses and comparisons, it was necessary to insert all collected data in the so called "VOLKSMED" data base of Austrian prescriptions [2]. In total, 27 different places were visited, 33 different persons questioned, approx. 140 different wild and cultivated species and 548 different preparations for the use in human therapy were recorded. Among those wild plants, different species of the genera *Hypericum*, *Thymus*, *Achillea*, *Teucrium*, *Mentha and Urtica* were particularly highly recommended by the majority of the informants as being beneficial for all ailments. The most frequent indications were urinary-genital ailments (18,8%), gastrointestinal tract disorders (17,3%), cardio-vascular (15,9%) and respiratory tract problems (15,7%). Not so frequent were indications like disorders of nervous system (7,8%), skin ailments (6,4%) and rheumatism (6%). **References:** [1] Foreign Trade Chamber of Bosnia and Herzegovina (2006) Medicinal and Aromatic Plants, Mushrooms, Wild Forests Products. MAG Plus. Sarajevo. [2] Saukel, J. et al. (2006) Pflanzen in der österreichischen Volksmedizin. Die "VOLKSMED-DATENBANK". Vortrag bei der 19. Wissenschaftlichen Tagung der Österreichischen Pharmazeutischen Gesellschaft. Innsbruck.

PB24 Aqueous portion of *Byrsonima intermedia* A. Juss (Malpighiaceae): indication of gastroprotective and healing action of a medicinal plant from Brazilian Cerrado

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Byrsonima intermedia is a plant used in folk medicine as an antiulcer and healing agent. In the present work an aqueous portion (AcoAq) obtained from leaves of this species was investigated for its ability to prevent and heal gastric ulcer *in vivo*. The preventive and healing actions of the AcoAq were evaluated in experimental models in male Wistar rats (n = 10) that simulated the disease in human gastric mucosa. The treatment significantly decreased the severity of gastric damage formation induced by absolute ethanol. AcoAq (100 mg/kg, p.o.) presented effective gastroprotection (41.0 ± 9.6 mm) reducing ulcerative lesions when compared to control (147.0 ± 5.7 mm). This gastroprotective action was completely reversed by the nitric oxide inhibitor (99.86 ± 10.5 mm) or sulfhydryl blocker (166.1 ± 24.7 mm). AcoAq also showed effective healing action in chronic gastric disease after 14 days (100 mg/kg, p.o.) by morphometric analysis and immunohistochemical evaluation (PCNA-cell proliferation), COX2 (cyclooxygenase-2), SOD (superoxide dismutase) and CXCR4 (angiogenesis). Morphometric analysis demonstrated increase in epithelial height of regenerated mucosa (μm) of AcoAq (958.1 ± 34) when compared to the control group (741.4 ± 16). Results of immunohistochemical analyses from PCNA presented intense cell proliferation and also increased expression of COX2, SOD and CXCR4 after 14 consecutive days of AcoAq treatment. The intense cellular proliferation by PCNA confirms the results of the morphometric analysis. Expression of COX2 and SOD and intense angiogenesis are involved in cellular healing of *B. intermedia*, indicating a cicatrizing effect of this medicinal species in addition to activation of nitric oxide and sulfhydryl compounds that exert great influence on the protection against these severe harmful agents. *Acknowledgements: Biota/FAPESP and CNPq*

PB25 Antinociceptive activity of methanolic extract from *Rhamnidium elaeocarpum* barks

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We evaluated the antinociceptive action of a methanolic extract from *R. elaeocarpum* (MeOH) and its mechanisms of action in rodent experimental models. The antinociceptive effect was evaluated by formalin method where male Swiss mice (n = 5 – 8) received by oral route saline, piroxicam (30 mg/kg) or ME (250 mg/kg). After 1 hour all animals received 20 µL of formalin solution 2.5% in PBS at their hinder right paw. After injection of formalin, mice were observed during 5 min (neurogenic phase) and between 15 – 30 min after infection (inflammatory phase). The time spent of licking the injected paw was recorded with a chronometer and considered as indicative of nociception index. The evaluation of involvement of nitric oxide or serotonin in the antinociceptive mechanism of ME, mice were pre-treated with L-argenine (500 mg/kg, i.p, 30 min before ME administration) or PCPA-p-chlorophenylalanine (100 mg/kg i.p, once a day for 4 consecutive days), respectively. The statistical significance of differences between groups was detected by ANOVA followed by Dunnett test ($p < 0.05$). The group of animals that received MeOH showed significative reductions in time of reaction during the inflammatory phase comparing to animals treated with vehicle. The antinociceptive action of extract did not reverse by L-argenine (NO precursor). But MeOH antinociceptive property was significantly reversed by PCPA (an inhibitor of serotonin synthesis).Thus, methanolic extract from *Rhamnidium elaeocarpum* exert antinociceptive action by serotonergic system. *Acknowledgements: Biota/FAPESP, CNPq, FAPESP proc. Nô¤ 07/57377 – 8* **Reference:** [1] Hunskaar, S., Hole, K. (1987) Pain 30:103 – 104.

PB26 The influence of the nutritional space upon the raw material and volatile oil yields in *Valeriana officinalis* L., under the ecological conditions of Cluj-Napoca, Romania

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Our research determined the optimum nutritional space for *Valeriana officinalis* L. (the Magurele 100 cultivar), as an ecological crop created through seedling transplants in the second decade of April, using the following densities: V_1 = 200 thousand of plants/ha, V_2 = 133 thousand of plants/ha, V_3 = 100 thousand of plants/ha, V_4 = 80 thousand of plants/ha, V_5 = 67 thousand of plants/ha. Some of our results are presented below: The number of harvested plants/m^2 was lower than the number of the transplanted ones (differences of 4 – 8%); the decrease was minimal with lower densities. The raw material yield (roots and rhizomes) in *Valeriana* is much influenced by the number of plants per surface unit. The planting variants V_2 and V_3 proved to be the optimal ones, with three-year mean yields of 1.7 – 1.8 t/ha of dry roots and rhizomes. The three-year mean yield of volatile oil/ha was of 10.8 – 15.5 l/ha, with the highest values for V_2 and V_3. Considering the number of plants in V_2 and V_3, any increase or decrease of it causes a significant cut down in the raw material and volatile oil yields. The economic calculations (groß profit, profitableness rate, production unitary cost) revealed superior values when the planting was done with densities between 100 – 133 thousand plants/ha (with 50 cm spacing between rows and 15 – 20 cm between plants in a row).

PB27 Assessment of somaclonal variation in purple coneflower (*Echinacea purpurea* (L.) Moench) by RAPD (Random Amplification of Polymorphic DNA) fingerprinting analyses

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In order to induce somaclonal variation in purple coneflower (*Echinacea purpurea* (L.) Moench), the selected material from five elite plants was cultured in vitro. Optimum callus formation was observed on Murashige and Skoogs' (MS) medium supplemented with 10 mg/l of 2,4-D. The best shoot regeneration was achieved upon transferring the callus to MS medium containing 2.5 mg/l BAP and 0.5 mg/l IAA. Complete plantlets were obtained upon transfer of the regenerated shoot to MS medium containing 1 mg/l IBA. A number of 13 plants regenerated from callus were successfully transferred to the greenhouse, following previously standardized hardening procedures. DNA was extracted from the parental plants and from the callus regenerated plants. RAPD (Random Amplification of Polymorphic DNA) analyses were carried out to detect somaclonal variation. Two, out of 13 regenerated plants exhibited somaclonal variation. These four somaclones were different from the parental plants by at least one polymorphic amplification product. The two somaclones had common origin (the same elite plant) but they displayed non-maternal bands for two different primers, OPB 09 and OPX 03. The conclusion that can be drawn from here is that the somaclones are genotypically, and maybe even phenotypically different. The remaining regenerants were genetically stable as compared to the elite donor plants. RAPD markers were an efficient tool for the early detection of somaclonal variants in purple coneflower tissue culture.

PB28 Evaluation of Biodiversity among some of the *Salvia* L. species in Iran

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Salvia L. genus is one of the most important medicinal plants of Lamiaceae [1]. In this context, the purpose of the present study was to display the biodiversity among six species as *S. Reuterana* Boiss., *S. macrosiphon* Boiss., *S. Moorcroftiana* Wall. ex Benth., *S. Sharifii* Rech. f. ex Esfand., *S. multicaulis* Vahl., *S. hydrangea* Dc., and 62 accessions of *Salvia* collected from natural habitats of Iran using 46 quantitative morphological characters as vegetative and reproductive, and molecular markers as Amplified Fragment Length Polymorphism.

Coefficient of Variation in morphological data	C.V. max. and min. in morphological Data	Genetic Variation	Genetic Distance (Nei) in species	Genetic Similarity (Jaccard) in species
Between species = 130.1	Max = 135, the length of hair in petiole, *S. Reu.*	Between species = 63.3	D max = 0.3, *S. Shar., S. mult.*	Sj max = 0.75, *S. Reu., S. mac.*
Between accessions = 85.35	Min = 45.6, length of calyx, *S. hyd.*	Between accessions = 46.47	D min = 0.024, *S. Reu., S. mac*	Sj min = 0.05, *S. hyd.*, S. Moor.

Regarding our molecular data, a total 12 primers used generated 440 bands of which 80% percent (PIC = 0.80) were polymorphic in *Salvia* species. The genetic variations of the species were higher than the accessions, confirming the morphological analysis [2]. In addition, high phenotypic and genotypic diversity might have arisen due to adaptation, segregation and recombination. Concerning the variability detected between *Salvia* taxa in Iran it can be concluded that there would be a high gene flow and diversity among *Salvia* species. **References:** [1] Walker, J.B. et al. (2004) Am. J. Bot. 91:115 – 1125. [2] Reals, A. et al. (2004) Bot. J. Linn. Soc.145:353 – 371.

PB29 Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria

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Diabetics' population in Nigeria is about 10 million and about half of this number is in Lagos State because of its very cosmopolitan nature [1]. An ethnobotanical survey was conducted into the herbal antidiabetic reme-

dies in the traditional pharmacopiea in five districts of Lagos State, Nigeria by means of semi-structured questionnaire and oral interview. 100 respondents from the predominantly Yoruba tribe mostly males (76%) were knowledgeable in traditional treatment of diabetes. About half of the respondents with 20 – 30 years experience in treating diabetes used mainly herbs (96%), and diagnostic methods included polyuria, polyphagia, polydypsia, and attraction of ants to urine as diagnostic methods. 92% of diabetic patients were usually out-patients aged 21 – 60 years. Diabetes trado-specialists (80%) rarely referred their patients but usually treated referred cases (96%). Treatment was usually with liquid formulations on a weekly (39%) or monthly (23%) basis, and lasted for 12 and 16 weeks (39%) with minimal side effects. A total of forty nine different plant species belonging to 48 genera in 33 families were used in formulating the fifty recipes documented, each containing at least three plant s. The principal antidiabetic plants included *Vernonia amygdalina, Bidens pilosa*, *Carica papaya*, *Citrus aurantiifolia*, *Ocimum gratissimum*, *Momordica charantia* and *Aframomum melegueta.* Of these, the antidiabetic activity of all except *A. melegueta* has been investigated [2], and promising results recorded. **References:** [1] Ogbera, A.O. et al. (2005) Int. J. Endocrinol. Metab. 4:165 – 173. [2] Marles, R.J., Farnsworth, N., (1996) Prot. J. Bot. Med. 1:85 – 135.

PB30 Traditional antifever phytotherapies in Sagamu and Remo North districts of Ogun State, Nigeria

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Febrile illnesses are common ailments in various parts of the world which has benefited from orthodox medicine and herbs. Since no documentation is available on herbal therapy of such ailments in south western Nigeria, we therefore conducted an ethnobotanical survey in both an urban settlement in Sagamu Local Government Area (LGA) and a rural settlement in the Remo North LGA of Ogun State in south western Nigeria, implicated in the treatment of various types of fever. Methodology involved administration of semi-structured questionnaire to traditional medical practitioners, her sellers, herbalists and villagers in the LGAs, as well as oral interviews using trained interviewers. Seventy respondents mostly aged 31 – 50 covered in this survey were drawn from among the herbalists (20), herb sellers (15), traditional medical and practitioners (35) that are mostly educated. Four types of fever including malaria, yellow fever, typhoid and cold were identified, with malaria and yellow fever being very common. Majority of the respondents were quite knowledgeable in the aetiology, symptoms, and seasonality of all fevers except cold. 116 antifever herbal recipes documented were administered as oral decoction or infusion for both curative and preventive purposes. Malaria and yellow fever were treated by almost equal number (35 – 39) of recipes. Treatment is usually devoid of known side effects. *Cymbopogon citratus, Citrus aurantifolia, Enantia chlorantha, Carica papaya, Morinda lucida* and *Lawsonia inermis* were frequently included in antifever herbal recipes for malaria, typhoid and yellow fevers. Survey has therefore lent credence to various herbs used for the prevention and treatment of febrile illnesses in south western Nigeria.

PB31 HPLC-DAD fingerprinting and chemical characteristics of unifloral Croatian honeys

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Monofloral honeys are products connected with defined botanic species and geographical areas. Croatia, due to its peculiar climate and specific botanical species, produces several unifloral honeys. The aim of this work was to develop a direct and accurate HPLC-DAD method to study the non volatile components of honeys in order to use the chromatographic profile as a marker of the monofloral origin of the Croatian honeys. Moreover, CIE L*C*h* (lightness, chroma, hue) chromatic coordinates, total phenols, diastase activity and 5-(hydroxymethyl) furfural (HMF) were determined. The antioxidant and antiradical activities of honeys were also evaluated with FRAP and DPPH tests, respectively [1]. Honey samples of *Paliurus spina-christi*, *Salvia officinalis*, *Mentha* spp., and *Satureja* spp. honeys selected for this investigation were obtained from professional beekeepers. The melissopalynological and sensorial characteristics were evaluated as the first step to assess the botanical origin of the monofloral honeys. HPLC-DAD fingerprinting was performed with an HPLC Varian system ProStar using a Licrocart Purosher Star RP-18e column (250 x 4.0 mm 5 µm) and samples did not need any purification step. Absorbance was recorded in the range 200 – 600 nm and chromatograms were acquired at 280, 313 and 360 nm. Chromatograms obtained for the studied Croatian monofloral honeys showed strong differences according to the botanical origin, allowing to easily discriminating the studied honeys. Also L*C*h* chromatic coordinates proved to be useful to easily discriminate the studied honeys. *Mentha* spp. honeys showed the highest total polyphenols amount (702.1 ± 26.6 mg/kg), antioxidant (6.47 ± 0.59 mmol Fe^{2+}/kg) and antiradical (1.81 ± 0.38 mmol TEAC/kg) activities. HMF highest value was 15.1 ± 0.7 mg/kg, while the lowest diastase activity was 11.9 ± 1.1: those values respected the legal limit fixed by the EC [2] and indicate a proper way of production and storage. *Acknowledgements: This paper was supported by UKF grant 25/08, PIP, API-HERBA, KONCEPT MEDIA and GODAX-PRO.* **References:** [1] Tuberoso, C.I.G. et al. (2009) J. Agric. Food Chem. (*in press*). [2] Official J. Eur. Communities CD 2001/110/EC L 10/47 – 52.

PB32 Headspace volatile profiles of willow (*Salix* spp.) nectar and honeydew honeys: identification of chemical biomarkers

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Honey is an excellent nutritional food with health benefits. It has been used for the treatment of flue and common cold, healing of wounds and burns, as anti-microbial agent as well as the source of antioxidants [1,2]. Consumer preference, and hence the price of the honey, mainly depends on its botanical origin and organoleptic characteristics. Willow (*Salix* spp.) nectar and honeydew honey samples from Croatia are for the first time at the focus of this research and were characterized according to the National and EU regulations [3]. The assessment of honey botanical origin (besides melissopalynological analysis) now days is oriented toward finding marker compounds. Aroma profile is one of the most typical authenticity feature of the honey and therefore volatile component analyses of the samples were performed by means of headspace solid-phase microextraction (HS-SPME) followed by gas chromatography and mass spectrometry (GC, GC-MS). PDMS/DVB fiber coating was used and > 50 compounds were identified. Willow nectar honey contained 3-methylbutanoic and 3-methylpentanoic acids that may be considered as biomarkers as well as relatively high percentage of phenylacetonitrile and β-damascenone. Potential biomarkers of willow honeydew honey were 3-methylbutanoic and 2-methylbutanoic acids while found methyl salicylate was specific marker. Ubiquitous honey volatiles were also identified in all the samples such as hotrienol, benzaldehyde, *cis*- and *trans*-linalool oxides, lilac aldehydes and others. *Acknowledgements: UKF grant 25/08, PIP, API-HERBA, KONCEPT MEDIA and GODAX-PRO.* **References:** [1] Cuevas-Glory, L. et al. (2007) Food Chem. 103:1032 – 1043. [2] Al-Mamary, M. et al. (2002) Nutr. Res. 22:1041 – 1047. [3] Official J. Eur. Communities CD 2001/110/EC L 10/47 – 52.

PB33 Anti-inflammatory activity of the methanol extract of *Kaempferia galanga* Linn. in experimental animals

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Kaempferia galanga Linn. (Zingiberaceae) has been reported for the treatment of various disorders in folk medicine including muscle pain and rheumatism. The analgesic activity of this plant extract has been reported but its anti-inflammatory effect is not investigated [1]. Thus,

the aim of this study is to assess anti-inflammatory activity of the methanol extract of *Kaempferia galanga* in rats.The *in vivo* models used for evaluation of anti-inflammatory activity in rats were carrageenan-induced hind paw edema and cotton pellet-induced granuloma. The results showed that only the methanol extract of *Kaempferia galanga* at doses of 100 and 200 mg/kg demonstrated anti-inflammatory activity. This activity seemed to be dose- and time-dependent. The anti-inflammatory activity of the extract was markedly observed at the dose of 200 mg/kg with its inhibition was observed at the 2nd h by 42.68%, however, the inhibition of inflammation was efficiently maintained for the duration of the experiment (5 h). This activity seemed to be dose- and time-dependent, but less potent than aspirin (100 mg/kg). In summary, the results demonstrated that the methanol extract of *Kaempferia galanga* markedly exhibits the anti-inflammatory activity which supports the local people use of this plant in the treatment of many inflammatory conditions. **References:** [1] Ridtitid, W. et al. (2008) J. Ethnopharmacol. 118:225 – 230.

PB34 HPLC analysis of flavonoids of *Astragalus gossypinus* (Fabaceae), as a medicinal plant in the West of Iran

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Most of *Astragalus* genus use for the production of the economically important gum, traganth. The roots of several *Astragalus* species present a very old and well- known drug in traditional medicine for its usage in the treatment of nephritis, diabetes, leukemia, uterine cancer and as an antiperspirant diuretic and tonic [1]. The purpose of this investigation was the study of the flavonic patterns in 29 plant populations in the west of Iran. Flavonoids were extracted from air-dried leaves, under reflux twice with a Me OH- H2O (7:3) mixture. Pooled extracts of each plant were concentrated at a reduced pressure and the final extract was taken up in a small volume of 80% MeOH [2]. The flavonoids were separated by HPLC using (Auto sampler 360, pump 322, and Diode array detector) and ultra base C- 18 column (5 µm, 4.6 mm/250 mm) [3]. Cluster and discriminate analysis by SPSS (Statistical Package for the Social Sciences) and MVSP (Multivariate Statistical package) with Ward and UPGMA (Unweighted Pair Group Method With Arithmetic Mean) methods showed 7 chemotypes of *Astragalus gossypinus* in different populations from west of Iran. These chemotypes determined on the base of different quality and quantity of four standards quercetin, flavon, rutin and catechin with other derivatives. A discriminate analysis showed the chemotaxonomic value of the 7 chemical polymorphisms. **References:** [1] Ozipek, M., Calis, I. (2003) Journal of the faculty of pharmacy: 23(2):85 – 94. [2] Semmar, N. et al. (2005) Biochem. Syst. Ecol. 33:187 – 200. [3] Grayer, R.J. et al. (2004) Biochem. Syst. Ecol. 32:901 – 913.

PB35 Root constituents of *Taraxacum udum*

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Plants of the genus *Taraxacum* (Asteraceae) have long been used as medicinal herbs. Various studies of *Taraxacum* extracts and their constituents have demonstrated anti-inflammatory, antinociceptive, antioxidative and anticarcinogenic activities, among others. These diverse effects have mainly been attributed to the presence of phenolic compounds and sesquiterpene lactones, including taraxinic acid and its derivatives [1]. In continuation of our chemical studies of plants from the genus *Taraxacum* [2,3], we have investigated roots of hitherto not studied *Taraxacum udum* Jord., a species of the section Palustria endangered in Poland. The dried plant material was extracted with ethanol, and the extract, after sequential fractionation on silica gel followed by semipreparative HPLC, gave a total of five known sesquiterpene lactones and five known phenolic compounds. The sesquiterpene lactones were identified as the germacranolides taraxinic acid and its 11β,13-dihydro-derivative, their β-glucopyranosyl esters, and the guaianolide macrocliniside A. The phenolics were identified as syringin, dihydrosyringin, methyl *p*-hydroxyphenyl acetate, dihydrodehydrodiconiferyl alcohol 9-O-β-glucopyranoside, and syringaresinol-4'-O-β-glucopyranoside, the latter being reported from *Taraxacum* species for the first time. In addition, a new natural product was isolated and characterized as taraxinic acid 6-acetyl-β-glucopyranosyl ester on the basis of spectroscopic data. Esters of taraxinic acids with glucose appeared to be major constituents and their content in the roots (0.14% dry wt.) was about ten times that found in roots of other *Taraxacum* species investigated so far. **References:** [1] Schutz, K. et al. (2006) J. Ethnopharmacol. 107:313 – 323. [2] Michalska, K., Kisiel, W. (2003) Planta Med. 69:181 – 183. [3] Kisiel, W., Michalska, K. (2005) Fitoterapia 76:520 – 524.

PB36 Using DNA barcoding to authentication of Cistanches and its fakements

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DNA barcoding has been designed as a system to facilitate species identification and recognition. One of the challenges in barcoding, however, is to discrimination of closely related species. The dried succulent stems of Cistanches (*Cistanche deserticola* and *Cistanche tubulosa*.) are one of the most widely used traditional Chinese medicines. However, it is often confused and substituted with the roots of *Orobanche pycnostachya* var. *pycnostachya*, *Boschniakia rossica*, *Cistanche salsa*, and *Cistanche sinensis*. The results showed that the region of *psbA-trnH* had significant variation and showed promise for barcoding in cistanches. Additionally, the genetic distance of *psbA-trnH* sequences was found to be significantly different from those of other species, with percentages of variation ranging from 0.050 to 1.238%. In contrast, the intraspecific variation among cistanches species studied ranged from 0 to 0.033%. The sequence difference between the *psbA-trnH* sequences of cistanches species and one *Orobanche pycnostachya* var. *pycnostachya* ranged from 0.381 to 1.308%. The monophyletic branches of the phylogenetic tree reveal that the *psbA-trnH* intergenic region is suitable for discrimination between these species. **References:** [1] Miller, S.E. (2007) P. Natl. Acad. Sci. USA. 104:4775 – 4776. [2] Lahaye, R. et al. (2008) P. Natl. Acad. Sci. USA. 105: 2923 – 2928.

PB37 Towards biomolecular aided agriculture: metabolic fingerprint of wild and cultivated Sicilian medicinal plants belonging to the folk tradition

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For chemotaxonomic and agro-technological purposes, extracts of wild thyme (*Thymus vulgaris* L., 30 samples), oregano (*Origanum vulgare* L., 61 samples) and rosemary (*Rosmarinus officinalis* L., 57 samples) collected in different areas of the Sicilian region, as well as cultivated sage (*Salvia officinalis* L., 7 samples), were screened for their phenolic compound content. All these plants belong to Sicilian culinary tradition and folk remedies. In order to have as many details as possible on the composition of these plants and their variation, extracts of different polarity (lipidic and alcoholic) for each cultivar were obtained and analysed. Several groups of selected molecules, typical of the different species and a part of their secondary metabolism (chemotaxonomical markers), were chosen, containing flavonoids, terpenoids, and organic acids as more representative chemical classes. The markers were firstly identified in the extracts through exhaustive analyses using the LC/UV-DAD/MS technique and then processed with high-throughput HPLC to obtain qualitative and quantitative compositional data. These data, together with the yield of extractions from the vegetable material, provided enough information to build up an analytical matrix from which the best extract in terms of presence and percentage of polyphenols could be selected. These results together, with the agronomical features (climatic conditions, quality of soil, etc.), helped in the identification of the best plant population and the most favourable area for every species to be cultivated.

PB38 Conservation status, present threats and their causes, grieving the medicinal plant species of the genus *Sempervivum* in natural occurrence sites in the Romanian S-E Carpatians

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Romanian medicinal plants of the genus *Sempervivum* s.l. (*S. marmoreum* and *S. heuffelii*), characteristic carpato-balcanic perennial monocarpic Crassulaceae, distributed throughout the Romanian S-E Carpatian Mountains, inhabit mainly arid, rocky habitats [1,2]. They are an enjoyed food ingredient in some Romanian regions [3]. Traditionally planted on tile-roofs, they are still highly prized ornamental plants. We report hereby on the conservation status, current threats and their direct/indirect causes, grieving the medicinal plant species of the genus *Sempervivum* s.l. in their natural sites in the Romanian S-E Carpatians. Data were gathered by direct personal observation throughout the investigated area during the past 20 years, complemented by literature search and interviews with local workers and sheep herders -when a semi-structured questionnaire was used- assessing the occurrence, uses, abundance, threats and conservation measures envisaged/applied for the *Sempervivum* spp. at any given location. *Major threats identified*: Illegal harvest for decorative and medicinal uses, grazing by both domestic and wild herbivores, especially goats; Habitat destruction for residential development and for stone exploitation. Population decreased in 7sites studied by more than 60%. *Direct and indirect causes*:-Weak and poorly enforced laws – any harvest within National Parks and Natural Reserves is illegal, elsewhere individual non-commercial harvest for personal use is not prohibited -Ignorance and no interest amongst locals for conservation in general and for *Sempervivum* in particular -Proliferation of "traditionalist" and naturistic healers with no taxonomic knowledge nor interest for conservation, who have depleted many accessible sites. **References:** [1] Barca, V., Niculae, M. (2005) Contrib. Bot. Cluj. XL:28 – 39. [2] Barca, V., Niculae, M. (2006) Contrib. Bot. Cluj. XLI:2 23 – 33. [3] Ravarut, M. (1953) Flora RPR, Crassulaceae, Edit. Acad RPR, Bucharest.

PB39 Structural characteristics of *Chrysanthemum morifolium* Ramat. (Romica cultivar) regenerated in vitro

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The micropropagation of *Chrysanthemum morifolium* Ramat. (Romica cultivar), belonging to the collection of "Anastasie Fătu" Botanical Garden from Iasi (Romania) was achieved through tissue culture technique and involved callus induction followed by shoot multiplication, rooting and establishment of plantlets in soil [1]. The purpose of this study was to determine the range of variation in certain structural characters of the vegetative organs of *in vitro* regenerated plants at *Chrysanthemum morifolium* Ramat. (Romica cultivar). The material subjected to the comparative anatomical analyses was represented by vegetative organs of the parent plant (PP) and regenerated plant (RP), on mature stage [2]. The density of glandular and non-glandular hair (mm^{-2}) on both leaf surfaces was statistical analysed using "t" test at 0.05 confidence level. Despite the great opportunity of genetic variation in callus cultures, the regenerated plants did not differ in their structural appearance from the normal plants [3]. **References:** [1] Vântu, S. (2006) An. şt. Univ. "Al. I. Cuza" Iaşi, s. II a (Biol. veget.) 51:71 – 77. [2] Toma, C. et al. (1985) An şt. Univ. "Al. I. Cuza" Iaşi, s. II a (Biol.) 31:45 – 48. [3] Bandyopadhyay, T. et al. (2004) Plant Cell Tiss. Org. Cult. 78:113 – 121.

PB40 Yield components and oil content of safflower in Eastern Algeria

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Safflower (*Carthamus tinctorius* L.) is a member of the family Asteraceae, cultivated mainly for its seed, which is used as edible oil and as birdseed. Traditionally, the crop was grown for its flowers, used for coloring, flavoring foods, making dyes (carthamidin and carthamin), and in medicine. Since safflower is a drought tolerant crop, the objective of this research was the investigation of the seed yield and oil content of safflower under semi-arid conditions in eastern Algeria. The results showed that SYPRUS variety gave the highest seeds number per plant (800.17) and yield of seeds (420.53 g/m^2). While OT-455 variety gave the highest weight of one hundred seeds (4.44 g). Considering the yield of the fixed oil (% of seeds), GILA variety produced the highest percentage (38.47%). The research revealed that the most suitable safflower variety, under semi-arid conditions of eastern Algeria was SYPRUS variety which's providing from ICARDA (International Center for Agricultural Research in Dry Areas, Syria). Analyses of variance (ANOVA) showed highly significant differences among the varieties for yield components and oil content. Correlation coefficients between variables (5 traits) are calculated, and the cluster analysis of observations (varieties) is also used to clarify the clustering pattern of genotypes tested.

PB41 HPLC analysis of flavonoids of *Astragalus gossypinus* (Fabaceae), as a medicinal plant in the West of Iran

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Most of *Astragalus* genus are used for the production of the economically important gum, traganth. The roots of several *Astragalus* species present a very old and well- known drug in traditional medicine for its usage in the treatment of nephritis, diabetes, leukemia, uterine cancer and as an antiperspirant diuretic and tonic [1]. The purpose of this investigation was the study of the flavonic patterns in 29 plant populations in the west of Iran. Flavonoids were extracted from air-dried leaves, under reflux twice with a MeOH-H_2O (7:3) mixture. Pooled extracts of each plant were concentrated at a reduced pressure and the final extract was taken up in a small volume of 80% MeOH [2]. The flavonoids were separated by HPLC using (Auto sampler 360, pump 322, Diode array detector) and an ultra base C-18 column (5 µm, 4.6 mm/250 mm) [3]. Cluster and discriminate analysis by SPSS (Statistical Package for the Social Sciences) and MVSP (Multivariate Statistical package) with Ward and UPGMA (Unweighted Pair Group Method With Arithmetic Mean) methods showed 7 chemotypes of *Astragalus gossypinus* in different populations from west of Iran. These chemotypes determined on the base of different quality and quantity of four standards quercetin, flavon, rutin and catechin with other derivatives. A discriminate analysis showed the chemotaxonomic value of the 7 chemical polymorphisms. **References:** [1] Ozipek, M. and Calis, I. (2003) Journal of the Faculty of Pharmacy 23:85 – 94. [2] Semmar, N. et al. (2005) Biochem. Syst. Ecol. 33:187 – 200. [3] Grayer, R.J. et al. (2004) Biochem. Syst. Ecol. 32:901 – 913.

PB42 *Ex situ* conservation of *Plumbago indica* using biotechnology

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Present report dwells on *in vitro* conservation of *Plumbago indica*, roots of which are used as medicine. A naphthoquinone compound, 'plumbagin', synthesized in roots, acts in low doses, as gastro stimulant and appetizer and as deterrent against syphilis and leprosy. The plant has been categorized as rare and therefore its conservation is of national importance. In the first phase of the work, an effective protocol of *in vitro* regeneration has been standardized. Culture of nodal explants in Murashige and Skoog's medium supplemented with 2×10^{-3} gl^{-1} BAP resulted in sprouting of buds into shoots at nodal axils and induction of new shoot buds at high frequency. Shoots in presence of 1×10^{-3} g l^{-1} putrescine developed roots. In the second phase of experiment, shoot tips of these *in vitro* plants were used for conservation. The study revealed that (1) synthetic seeds, made up of small shoot tips, coated with sodium alginate-polymerized MS medium could be preserved in absence of light at 22 ± 2 °C for a period of 4 months and (2) shoot tip cultures in MS medium containing 3 percent mannitol and 2×10^{-3} g l^{-1} BAP were maintained by 'reduced growth storage technique' over a per-

iod of one year without periodic transfer. Growth recovery was possible in both forms of germplasm when those were brought back to normal culture condition. Conserved plants as checked by PCR based genetic marker were all genetically stable. The finding of our study ensures feasibility of *in vitro* conservation of this plant.

PB43 Introduction of 2 Topodemes, 2 Pedodemes and 1 Basodeme of *Artemisia scoparia* as a medicinal plant from west of Iran

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Artemisia L. (Asteraceae) is the largest in Tribe *Anthemideae* and one of the largest in the family [1]. *Artemisia* species are reported as an herbal medicine for treatment of diabetes, high blood pressure, anti-migraine, anti-fungal, antihelmintic, anti-bacterial, digestive, lipolytic, mucolytic, vermifuge [2]. *Artemisia scoparia* Waldst & Kit is an important source of chemicals of immense medicinal and pharmaceutical importance which are effective as immunosuppresants, hepatoprotective, anti-spasmodic, hypotensive and anti-inflammatory agents [3]. This study was carried out to determine *Artemisia scoparia* intraspecific diversity by D.S.S (Determination of Special Station) method [4], in the west of Iran, from 2006 to 2008. At first, in this method by using the sources (different flora and some books) were determined the distribution localities of *Artemisia scoparia*. Then with referring to the determined localities, on the base of present of individual species under study and by using minimal area method, the special stations were determined and necessary data (Floristic-Ecologic) were collected from each one of the special stations. Data analyzes were carried out by Anaphyto software with A.F.C and C.A.H methods and MVSP software with C.C.A method. The results of this analysis showed the existence of intraspecific diversity for this plant in the west of Iran. MVSP software with UPGMA cluster analysis, P.C.A and P.C.O methods were used for determination of kind and level of intraspecific diversity. Between obtained results of this study, we present 2 Topodemes, 2 Pedodemes and 1 Basodeme for *Artemisia scoparia* as a medicinal plant from west of Iran. It is necessary to mention that these 2 Topodemes, 2 Pedodemes and 1 Basodeme from view point of chemical components are different. **References:** [1] Watson, L.E. et al. (2002), BMC Evolutionary Biology 2:17. [2] Nezhadali, A. et al. (2008), E-Journal of chemistry 5:557 – 561. [3] Sing, D. et al. (2006), Phcog Mag. 2: [4] Atri, M. (2007) First Botanical Systematic in Iran Sep: 6.

PB44 Chemical composition and antibacterial activity of essential oils from different populations of *Artemisia incana* (Asteraceae) from Iran

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Artemisia L. is the largest genus of the Anthemideae-Asteraceae and comprises more than 500 taxa growing worldwide [1]. The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity [2]. Many essential oils possess antibacterial activity to both Gram negative and Gram positive bacteria [3]. The essential oils of six populations of air-dried *Artemisia incana* obtained by hydrodistillation and were analyzed by gas chromatography-mass spectrometry (GC-MS). Results showed that the different studied populations have different essential oil components, from view point of quality and quantity. The antibacterial effects of these 6 populations were studied against 8 bacterial strains (4 Gram positive and 4 Gram-negative bacteria). The obtained results showed strong antibacterial activity of their essential oils. The highest zones of inhibition were exhibited by essential oil of different populations of *A. incana*, ranging from 9 to 30 mm. In spite of high antibacterial effects of essential oils of *A. incana* components, antibacterial ability of different populations of this taxon was very different. It is necessary to mention that each one of these 6 populations occur in particular ecological conditions and therefore have the particular components. **References:** [1] Kubitzki, K. (2007). The families and genera of vascular plants, Springer-Verlag, Berlin. [2] Celikel, N., Kavas G. (2008) Czech J. Food Sci. 26:174 – 181. [3] Oyedemi, S.O. et al (2008) Afr. J. Biotechnol. 7:4140 – 4146.

PB45 Some models of sustainable use of medical plants in Dinaric Alpes (SE Europe)

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Many medicinal plants appeared on "red lists of endangered flora". In order to preserve natural gene-pool of medicinal plants, it is necessary to set models that would result in sustainable models for their use [1]. Sustainability implies such exploitation of biomass from natural habitats that would respect biological and ecological characteristics of given plant species and if possible market requirements. This approach includes determination of variables that could be used as basis for developments of mathematical – statistical models in order to reach "function of sustainability". Sustainability function implies resultant between two key variables – production of biomass of given medical plant and amount of biomass that is used or is exploited in natural populations. Graphic model of that function is very diversified and it largely depends on plant species, used part of plant, vegetation season, ecological conditions in which given plant is developed, form of picking, total anthropogenic pressure – cutter, pasture, wood cutting, global changes. This ecological – statistical approach is applied to several species of medicinal plants that are intensively exploited from western Balkan [2,3]. In Mediterranean belt, that is *Salvia officinalis*, in sub – Mediterranean belt it is *Helichrysum italicum*, *Satureja montana* and *Satureja subspicata*, in sub alpine belt those are species *Gentiana lutea* and *Arctostaphylos uva-ursi*, and in belt of deciduous forests, that is species *Atropa belladonna* and *Origanum vulgare*. Researches show that gradient of sustainable use is different in each belts. In Mediterranean and sub – Mediterranean belt it is about 50 – 60%, in mountain belt it is 30 – 40%, while in belt of forests it is up to 70% (per km^2). That means it is necessary to leave about 50 – 60% of units of sage in free habitats, approximately same number of units of genus *Satureja*, about 70% of units of gentian, or about 30% of biomass of species *Atropa and Origanum* in order to reach effect of sustainability. **References:** [1] Redzic, S. (2006) Proc 1st IFOAM Intern Conf Organic Wild Production, 117 – 141. [2] Redzic, S.S. (2007) Collegium Antropol. 31:869 – 890. [3] Redzic, S.J. (2006) Ecol. Food Nutr. 45:189 – 232.

PB46 Analysis of secondary metabolites in selected *Penstemon* species – preliminary chemotaxonomic investigations

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Species from the *Penstemon* genus (Scrophulariaceae family) are perennial, herbaceous plants with decorative flowers, often used in horticulture. They come from North and Central America. Genus *Penstemon* Sch. includes about 250 species and plenty of horticultural varieties and forms. Their characteristic feature is the presence of iridoid compounds, widely described in numerous papers. There are also other groups of compounds present in Genus *Penstemon* which practically have not been examined yet. Therefore, an attempt to analyze flavonoids, phenolics, as well as iridoids in the herbs of selected *Penstemon* species has been undertaken. At first, the spectrophotometric analysis of total phenolics, flavonoids and iridoids in the selected herbs of *Penstemon* species has been made. All these experiments showed huge differences between the examined species. The next step of our investigations was qualitative chromatographic analysis of iridoids and other polar compounds in the examined plant materials. With this end in view, we have prepared purified extracts containing an iridoid fraction. The obtained fractions from 10 *Penstemon* species or varieties were examined using the TLC method in the system: SiO_2 plate/acetone:chloroform:water (16:4:1) + visualization with the vanillin reagent. We compared the results with those obtained by the HPLC method in reversed – phase system. Chromatographic analyses showed huge qualitative and quantitative differences between the investigated samples, which can lead to more general conclusions: the *Penstemon* genus demonstrates chemical differences even between morphologically similar species; the applied analytical

method can be utilized in chemotaxonomic investigations of *Penstemon* species on a large scale.

PB47 Ethnopharmedicinal survey of medicinal herbs used by rural and tribal community in Betul district of Madhya Pradesh, India

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The study of local knowledge about traditional herbal medicine is becoming increasingly important in defining strategies and actions for conservation of medicinal plants. This study therefore considered worthwhile to collect information from local rural and tribal population living in Betul district of Madhya Pradesh (India) concerning the use of medicinal plants; identify the most important species used; determine the relative importance of the species surveyed and calculate the informant consensus factor (ICF) in relation to medicinal plant use. Data collection relied predominantly on qualitative tools to record the interviewee's personal information and topics related to the medicinal use of specific plants. The present study revealed that 119 plant species grown in the study region are in use by rural and tribal community in traditional medicine for the treatment of various diseases. Most of the locals interviewed dealt with well-known safe medicinal plants such as *Allium sativum, Acacia arabica*, *Emblica offficinalis*, *Momordica charantia* and *Ocimum sanctum* with use value of 0.62, 0.54, 0.52, 0.51 and 0.50 respectively. Dental, inflammation-pain and female problems scored the highest ICF of 0.85, 0.78 and 0.77, respectively. The literature from different Indian traditional systems of medicine evidenced some concordance with the solicited plant uses mentioned by the rural and tribal informants. *Acknowledgements:* CDACR, Bhopal, India for financial assistance.

Topic C: Plants and aging of the population

PC1 Antioxidant profiling of new chemical entities from synthetic and natural origin

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Antioxidant compounds have become essential to prevent diseases partly induced by oxidative stress, such as cancer or neurodegenerative diseases (e.g. Alzheimer, Parkinson). To further understand and characterize their antioxidant properties, the radical scavenging activity of a large set of reference antioxidants and synthetic compounds was tested against three different radicals by four 96-well microplate assays. The antioxidant activities were ranked by cluster analysis in order to define the antioxidant profile of each compound. The first assay was realised with a protein, the alkaline phosphatase (ALP) hydrolyzing the 4-methylumbelliferyl phosphate (MUP) to a fluorescent substrate, the 4-methylumbelliferone (MU). The marker of oxidative damages was monitored by decrease of ALP's catalytic activity induced by peroxyl radicals generated by the 2,2'-azobis-(2-methlpropionamidine) dihydrochloride (AAPH). The second assay, based on the oxygen radical absorbance capacity (ORAC) was still carried out with peroxyl radicals, generated by AAPH. The marker of oxidative damages was monitored by the fluorescence decrease of fluorescein. The two last assays were spectrophotometric, the effectiveness of scavenging activity being monitored by, respectively, the absorbance decrease at 755 nm for 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\cdot-}$) and at 515 nm for 2,2-diphenylpicrylhydrazyl radical ($DPPH^{\cdot}$). From the cluster analysis, several antioxidant groups have been constituted and the similarity of the antioxidant profile of each group compared with the antioxidant profile of reference compounds (ascorbic acid, caffeic acid, chlorogenic acid, gallic acid, glutathione, mangiferin, mannitol, melatonin, quercetin, resveratrol, trolox, uric acid). Thus for new chemical entities from synthetic or natural origin, the position in the antioxidant space with respect to the one of reference compounds can be established.

PC2 Optimization of field cultivation of *Baptisia tinctoria* (L.) R. Br. by fertilizer, mulch and mycorrhiza treatments

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Baptisiae tinctoriae radix (L.) R. Br. is used as phytomedicinal compound in immunostimulating Esberitox®. Since 1992 field cultivation has lead to guaranteed quality of the drug regarding purity, hygiene and stability of compounds. To increase yield, different trials concerning mulching, fertilization and application of mycorrhiza were carried out to enhance survival of plants as well as fresh weight of roots. Mulching with tea and oak leaves is known to increase survival of plants due to their content of tannins. Mineral fertilization should lead to higher yield of fresh weight and was carried out in two different concentrations. Application of mycorrhiza to establish a beneficial symbiosis is known to cause a better adaptation of plants to stresses like nutrient deficiency, drought and pests and diseases. The trial was carried out in three successive plantations for the 3-year cycle. All treatments were carried out each year at the beginning of the vegetation period. Fresh weight and survival rates were investigated every year at the end of the season and yield calculated by multiplication of fresh weight and survival rates. Results show that application of tea and oak leaves show positive effects but with a decrease over the years. Fertilization leads to negative yields especially when doubled. Only mycorrhiza application leads to a stable increase over the 3-year cultivation. We conclude that all applications except mycorrhiza show a decreasing effect over the years when applied every year. Further trials will be carried out to investigate whether an increase could be achieved if a treatment would be applied only in the first year or if a combination of treatments would lead to better results.

PC3 Phenolic constituents of *Crithmum maritimum* and their radical scavenging activity

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Crithmum maritimum L. (Apiaceae) is a perennial aromatic plant of the European littoral, traditionally used as a tonic and spice herb [1]. The aim of the present study was to measure radical scavenging activity (RSA) of *C. maritimum* extracts obtained with different polarity solvents and then to isolate by bioguided fractionation the antioxidative compounds, using three different free radicals: 2,2-diphenyl-1-picryl-1-picrylhydrazyl radical ($DPPH^{\cdot}$), hydroxyl ($HO^{\cdot}$) and superoxide anion ($O_2^{\cdot-}$). Thirteen phenolic compounds were purified and identified using HPLC-UV combined with TLC and spectroscopic methods (NMR, MS). In addition to 5-*O*-caffeoylquinic acid (**1**), rutin (**2**) and hyperoside (**3**) also described in this species[2], ten additional compounds were isolated and identified for the first time in aerial parts of this halophyte: 4-*O*-caffeoylquinic acid (**4**), 4,5-*O*-dicaffeoylquinic acid (**5**), 3,5-*O*-dicaffeoylquinic acid (**6**), 3,4-*O*-dicaffeoylquinic acid (**7**), caffeic acid (**8**), isoquercitrin (**9**), diosmetin (**10**), quercitrin (**11**), quercetin (**12**) and luteolin (**13**). All these isolated polyphenols exhibited potent RSA against $DPPH^{\cdot}$ radical ($2\,\mu M < IC_{50} < 6\,\mu M$), $O_2^{\cdot-}$ radical ($1\,\mu M < IC_{50} < 10\,\mu M$) and $HO^{\cdot}$ radical ($0.6\,\mu M < IC_{50} < 1\,\mu M$), for the most actives. **References:** [1] Özcan, M. et al. (2001) Nahrung 45:353 – 356. [2] Katsouri, E. et al. J. Ess. Oil Res. 13:303 – 308.

PC4 Anti-inflammatory compounds from *Crithmum maritimum*

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Crithmum maritimum L. (Sea Fennel) is a halophyte perennial herb of the European littoral, traditionally used for its antibacterial, vermifuge, antispasmodic and tonic properties [1]. We investigated in vitro the anti-inflammatory activity of extracts from the aerial parts of *C. maritimum* using peripheral blood mononuclear cells (PBMC) of healthy individuals. The dichloromethane extract at doses of 50 and 10 µg.mL^{-1} exhibited a dose-dependent anti-inflammatory activity on LPS-induced production of TNF-α by PBMC, using an Enzyme-Linked Immunosorbent Assay. Thus, in addition to four compounds already described in the sea fennel [2,3] like dillapiole (**1**), scopoletin (**2**), scoparone (**3**) and falcarindiol (**4**), we isolated six other substances which are identified for the first time in the genus *Crithmum*: vomifoliol (**5**), ω-hydroxyisodillapiole (**6**), ferulaldehyde (**7**), iso-scopoletin (**8**) and diosmetin (**9**). Furthermore, a new structure was characterized: (2*E*)-2-ethylidene-3-[(10*E*,12*R*)-12-hydroxybut-10-en-10-yl]-4,4-dimethylcyclopentanone (**10**). Among them, three metabolites, (**2** – **4**) showed potent anti-inflammatory activities with IC_{50} of 9.15 µM, 19 µM and 37 µM respectively, on our experimental model. **References:** [1] Özcan, M. et al. (2001) Nahrung 45:353 – 356. [2] Cunsolo, F. et al. (1993) J. Nat. Prod. 56:1598 – 600. [3] Katsouri, E. et al. (2001) J. Ess. Oil Res. 13:303 – 308.

PC5 Proliferation enhancing effect of rice extracts on neuronal PC12 cells

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Rice is well-known as source of vitamin E, beta-glucan and gamma-oryzanol which may be useful for the treatment of Alzheimer's disease [1,2,3]. Two varieties of rice, white rice and red rice, were extracted by different methods to obtain 9 extracts as followed: S1 and S2-lyophilized rinse-water from red and white rice, S3-lyophilized water extract from white rice, S4 and S5- alcoholic extract from white and red rice, S6 and S7- cold-express extract from white and red rice, S8 and S9 – supercritical fluid extract from white and red rice. All extracts were tested proliferation activity on neuronal cell (PC12) by MTT assay at dosage of 50 and 100 µg/ml [4]. The results showed that all extracts had no cytotoxic activity against PC12. At dose of 50 µg/ml the S1, S2, S3 and S4 extracts exhibited high proliferative effect with significant level at $p < 0.05$ by 4 independence experiments. The S1 extract showed the highest proliferative effect at dose of 100 µg/ml by 3 independence experiments. These results were shown below and they were concluded that water extracts can enhance growth of neuronal cells more than the ethanolic extracts of both types of rice. Table: Percentage of PC12 cell growth (mean ± SEM) by MTT assay after treated with rice extracts exposure time 48 hours by independent experiment (N = 4)

dose	S1	S2	S3	S4
50 µg/ml	119.21 ± 0.027***	119.42 ± 0.031)**	120.05 ± 0.031***	112.17 ± 0.035*
100 µg/ml	128.37 ± 0.016)*	120.89 ± 0.042)	117.61 ± 0.027	118.73 ± 0.018
Significant difference with control ***p < 0.001 **p < 0.01 *p < 0.05				

Acknowledgements: Thai National Research Council for financial support.
References: [1] Yu, S. (2007) J. Agric. Food Chem. 55:7308 – 7313. [2] Kayali, H. et al. (2005) Neurosurg. Rev. 28:298 – 302. [3] Ogawa, Y. et al. (2008) Free Radic. Res. 42:674 – 87. [4] Kolla, N. (2005) Rev. Psychiatr. Neurosci. 30:196 – 201.

PC6 Identification of natural compounds that promote proteasome activation and confer lifespan extension

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The proteasome is the major cellular proteolytic machinery responsible for the degradation of both normal and damaged proteins shown to be down-regulated during senescence. On the contrary, its activation confers lifespan extension and maintenance of the young morphology for longer in human primary fibroblasts. Furthermore, it represents one of the main secondary antioxidant mechanisms. In this study, extracts derived from plants of the Greek flora (such as *Punica granatum, Rosa damascena, Quercus* sp., *Hedera helix, Origanum dictamnus, Liquiritieae glabra, Myrtus communis)* were studied in order to identify natural compounds that promote proteasome activation. We have identified 3 compounds (flavonol and triterpenic type) that promote the following characteristics to cultures of HFL-1 primary human fibroblasts: a) proteasome activation up to 2-folds accompanied by increased amounts of functional proteasome, b) increased resistance and survival to oxidative challenges, c) decreased intracellular oxidative load and levels of reactive oxygen species (ROS) and, d) as a natural consequence of the above mentioned characteristics, delay of the appearance of the senescent morphology and lifespan extension. Moreover, when these compounds were supplemented to senescent fibroblasts, a rejuvenating effect was observed. Besides, since tyrosinase, a known proteasome substrate, plays a pivotal role in the melanin pigment biosynthetic pathway which is mainly responible for the age spots, the whitening properties of these compounds were tested and revealed. These data demonstrate the beneficial effect of natural compounds in human fibroblasts undergoing replicative senescence, while, this study provides new insights towards enhancement of cellular antioxidant mechanisms by natural compounds.

PC7 Evaluation of *in vitro* antioxidant activity of selected Peruvian medicinal plants

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The increasing evidence that oxidative stress is involved in several serious inflammatory and degenerative human diseases has escalated interest in the research of antioxidant activity of naturally occurring molecules in food and biological systems [1]. It has been proved that a great number of aromatic, spicy and medicinal plants contain chemical compounds exhibiting antioxidant activity [2]. In this study we investigated the antioxidant properties of 18 Peruvian medicinal plants selected by their traditional medicinal uses in Coronel Portillo Province of Ucayali Department, Peru. The *in vitro* antioxidant capacity of medicinal plants was evaluated by the oxygen radical absorbance capacity (ORAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assays. Total phenolic compounds and total flavonoids were also determined for all plant species studied, in order to evaluate their contribution to antioxidant activity. Considering results of ORAC and DPPH tests, *Calycophyllum spruceanum* (8438 µmol TE/g extract, EC_{50} 5.34 µg/ml), *Caesalpinia spinosa* (7362 µmol TE/g extract, EC_{50} 3.50 µg/ml) and *Naucleopsis glabra* (8310 µmol TE/g extract, EC_{50} 5.45 µg/ml) performed the strongest antioxidant properties from all species tested. The total phenolic content has a significant correlation with the antioxidant activity of investigated plant extracts; nevertheless flavonoids seem not to be the main group of metabolites responsible for the effect. The results of

our study indicate that *Calycophyllum spruceanum, Caesalpinia spinosa* and *Naucleopsis glabra* are the most perspective species for further phytochemical research focused on determination of compounds responsible for their antioxidative properties. *Acknowledgements: This research was supported by Czech University of Life Sciences Prague (CIGA 20085001) and MSM 6046070901.* **References:** [1] MacDonald-Wicks, L.K. et al. (2006) J. Sci. Food Agric. 86:2046 – 2056. [2] Miliauskas, G. et al. (2004) Food Chem. 85:231 – 237.

PC8 Saffron extract and trans-crocetin inhibit the glutamatergic synaptic transmission on rat cortical neurones

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Crocus sativus L. is a small perennial plant from the Iridaceae family. The stigmata, commonly known as saffron, were used over centuries as a spice and dye but also as a medicinal plant. It has been shown that an ethanolic (80 vol.-%) saffron extract and trans-crocetin, a carotinoid from saffron, interact with the phencyclidine binding site of the NMDA receptor. The aim of the present study was to examine the influence of the ethanolic saffron extract CSE and trans-crocetin on the glutamatergic synaptic transmission in rat cortical brain slices. Postsynaptic potentials (PSPs) were elicited by electrical field stimulation in pyramidal cells of the cingulate cortex and recorded using intracellular placed microelectrodes. PSPs are induced by glutamate released from presynaptic terminals which activates postsynaptic NMDA and non-NMDA receptors. Additionally, glutamate induces a membrane depolarisation when applied directly to the brain slices. CSE (10 – 100 µg/ml) decreased the glutamate-induced membrane depolarisation and inhibited the evoked PSPs. In further experiments, the non-NMDA component of the PSPs was separated by application of the NMDA receptor antagonist APV (10 µM) and the NMDA component by application of the non-NMDA receptor antagonist CNQX (10 µM). CSE (100 µg/ml) decreased the isolated non-NMDA and NMDA component of the PSPs. CSE decreased the kainate (1 µM) induced membrane depolarisation, whereas the AMPA (1 µM) induced membrane depolarisation was not affected. Our results indicate that CSE has an antagonistic effect on NMDA and kainate receptors. AMPA receptors seem to be not involved. Trans-crocetin (1 – 50 µM) investigated under the same conditions as CSE induced inhibitory effects on the membrane depolarisation and evoked PSPs comparable to CSE. The inhibition of the glutamatergic synaptic transmission in rat cingulate cortex by CSE and trans-crocetin represents a possible new pathway by which *Crocus sativus* L. acts within the CNS.

PC9 Adaptogenic and nootropic activities of aqueous extract of *Carum carvi* Linn. (Caraway) fruit: An experimental study in Wistar rats

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Stress has been involved in the etio-pathogenesis of a diverse variety of diseases, varying from cognitive dysfunction, diabetes, hypertension and aging [1].In the present study, the aqueous extract of *Carum carvi* (CA) was evaluated for adaptogenic and nootropic activities in rats. Furthermore, the extract was studied for *in vitro* antioxidant potential to correlate its antistress activity. For the evaluation of antistress activity in both normal and stress induced rats, Urinary vanillylmandelic acid (VMA) [2] and ascorbic acid (AA) were selected as non-invasive biomarkers. Daily administration of CA at doses of 100, 200 and 300 mg/kg body weight one hour prior to induction of stress inhibited the stress induced urinary biochemical changes in a dose dependent manner. Nootropic activity was evaluated by conditioned avoidance response (CAR) using Cook's pole climbing apparatus in rats [3]. The cognition, as determined by the acquisition, retention and retrieval was observed to be significant and dose dependent. Further more the extract was also studied for their *in vitro* lipidperoxidation inhibition (antioxidant) [4] activity in brain and liver homogenates and compared to known antioxidant ascorbic acid. The present study provides scientific support for the antistress (adaptogenic), antioxidant and nootropic activities of *Carum carvi* extract and substantiate its traditional use as a culinary spice in foods is beneficial and scientific in combating stress induced disorders. **References:** [1] Chrousos, G.P., Gold, P.W. (1992) J. Am. Med. Assoc. 267:1244 – 1252. [2] Pisano, J.J. et al. (1962) Clin. Chem. Acta 7:277 – 284. [3] Cook, L., Weidley, E. (1957) Ann. NY. Acad. Sci. 66:740 – 752. [4] Ohkawa, H. et al. (1979) Anal. Biochem. 95:51 – 358.

PC10 Discovering the mechanisms of plant longevity to fight human aging and age related diseases

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Aging is one of the most complex and challenging problems in biology. Therefore, many models from yeast to mammals are widely used to discover the mechanisms of human aging in order to postpone it and prevent age related diseases. According to the evolutionary theory of aging [1], natural selection only affected early life traits like fitness and reproduction, due to predation and accidents that prevent the becoming old of animals in the wild. But it likely failed to prevent late life phenotypes like aging and even contributed or caused them by antagonistic pleiotropy. In contrast to animals, plants, especially trees, live in a protected environment and are not subject to predation. This should have allowed natural selection to prevent late life's deleterious effects in at least some plants. The presence of very long living tree species like *Pinus longaeva* that lives up to 5000 years [2] and 9550 years old spruce which is the oldest living tree [3], suggest that natural selection has found a way to prevent aging. In this study, the present literature on plant longevity and senescence was scrutinized and a working frame including leaf senescence [4] and seed longevity [5] was described for further studies. The wide range health benefits of pycnogenol [6], a bark extract of pine that is also a long living tree, gives the hope to discover other phytochemicals ensuring plant longevity from especially oldest living plants that have the potential to be weapons in our war against aging and age associated diseases. **References:** [1] Ljubuncic, P., Reznick, A.Z. (2009) Gerontology 55:205 – 216. [2] Lanner, R.M. and Connor, K.F. (2001) Exp. Gerontol. 36:675 – 685. [3] Umeå University (2008, April 16). World's Oldest Living Tree – 9550 years old – Discovered In Sweden. ScienceDaily. Retrieved [4] Lim, P.O. et al. (2007) Annu. Rev. Plant Biol. 58:115 – 136. [5] Rajjou, L., Debeaujon, I. (2008) C. R. Biol. 331:796 – 805. [6] Rohdewald, P. (2002) Int. J. Clin. Pharmacol. Ther. 40:158 – 168.

PC11 Inhibitory effect of *Lavandula viridis* methanol extract on acetylcholinesterase and butyrylcholinesterase enzymes

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The brain is an organ particularly vulnerable to oxidative stress, and the hypothesis that this process is involved in neurodegenerative events, neuronal cell death and progression of Alzheimer's disease (AD) has emerged [1]. Thus, the interest in naturally-occurring antioxidants, which can be used to protect human beings from oxidative stress damage, has increased [2]. In addition, the continuing search for novel anticholinesterases from plants as therapeutic agents for central nervous system disorders is based on the need for agents targeted to the brain areas affected, with reduced toxicity and side-effects. The aim of this work was to evaluate the antioxidant potential and, the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory capacity of methanol extracts from *Lavandula viridis* L' Hér. (Lamiaceae), an aromatic species endemic to the south western Iberian Peninsula. The tested extract showed a strong antioxidant potential in all the three assays conducted, Folin-Ciocalteau (F-C), trolox equivalent antioxidant capacity (TEAC) and oxygen radical antioxidant capacity (ORAC). The inhibition of AChE and BChE was assessed by using a 96-well microplate reader based on *in vitro* Ellman's method [3]. Results showed that the *L. viridis* extract displayed remarkable inhibitory activity of both enzymes, although slightly more active against AChE (IC_{50}= 244.90 ± 13.44 µg ml^{-1}) than BChE (IC_{50}= 285.28 ± 15.97 µg ml^{-1}). These results are in accordance with previous results obtained by our group for *L. viridis* essential oil [4]. The present work showed the simultaneous antioxidant and cholinesterase inhibitory potential of *L. viridis* extract, both relevant for the treatment of AD. *S. Gonçalves acknowledges a grant from Portuguese Science and Technology Foundation (FCT, Grant SFRH/BPD/31534/2006).*

References: [1] Moreira, P.I. et al. (2005) Drug News Perspect 18:13 – 19. [2] Scalbert, A. et al. (2005) Crit. Rev. Food Sci. Nutr. 45:287 – 306. [3] Ellman, G.L. et al. (1961) Biochem. Pharmacol. 7:88 – 95. [4] Gonçalves, S., Romano, A. (2008) Planta Med. 74:1200.

PC12 *Juglans nigra* green husks as a source of bioactive phytochemicals

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Juglans spp. (Juglandaceae) have been used in folk medicine for thousands of years to treat a wide range health disorders. Recently, a correlation between phenolic contents and antioxidant activity was established for *J. regia* L. leaves [1]. The present work evaluates the antioxidant properties of *J. regia* L. and *J. nigra* L. husks and assesses the biological and chemical potential of the *Juglans nigra* L., which is economically less valued. Green husks from both species were extracted with 70% aqueous ethanol using an Ultra-Turrax homogeniser. Total phenols were evaluated by Folin-Ciocalteu method. Antioxidant assessment was carried out with the DPPH method and the assay based on the superoxide-driven reduction of NBT by photochemically reduced riboflavin. Phenolic profiles were established by HPLC-PDA-ESI/tandem MS, in negative ion mode. A higher phenolic content was verified for *J. nigra* (83.1 ± 0.16 mg of gallic acid equivalents/g dry plant) relatively to *J. regia* (15.7 ± 0.03 mg/g). In addition, reactivity for DPPH and superoxide anion was also higher for *J. nigra* extract (Table 1). Different phenolic profiles were observed and significant quantities of ellagic acid and its derivatives were detected in *J. nigra* extract. Table 1: Free radicals scavenging activity of the *Juglans* spp. green husk extracts.

70% Aqueous ethanol extracts	DPPH[a]	Superoxide anion[a]
Juglans nigra L.	11.0224 ± 0.0309	5.5270 ± 0.3702
Juglans regia L.	54.1366 ± 0.0281	8.1987 ± 0.5814

[a] EC_{50} expressed in µg dry extract/mL in the reaction mixtures. Each value is the mean ± SD of three replicates.

The importance of the ellagic acid as antioxidant, preventing oxidative DNA damage [2], suggests the *J. nigra* green husks as a potential source of bioactive compounds involved in the degenerative diseases prevention. *Acknowledgements: FCT and POCTI/FEDER for financial support and LEM/UC integrated in RNEM of Portugal for the HPLC/MS analyses.* **References:** [1] Kulisic-Bilusic, T. et al. (2008) Food Technol. Biotechnol. 46:368 – 375. [2] Aiyer, H.S. et al. (2008) J. Mol. Sci. 9:327 – 341.

PC13 Inhibition of LPS-induced nuclear factor NF-kB activation by *Cymbopogon citratus* leaves in macrophages: a strategy to develop new anti-inflammatory drugs

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Inflammation is the cause of a large number of diseases like cancer, rheumatoid arthritis, diabesity, psoriasis, multiple sclerosis and cardiovascular diseases. Actually, the lack of responsiveness to the current anti-inflammatory drugs, their side effects, delivery problems and cost of manufacture, reinforced the development of new and safe anti-inflammatory agents. The nuclear factor (NF)-kB transcriptional system regulates the expression of many genes involved in inflammatory response [1]. Therefore, inhibition of NF-kB activation is now widely recognized as a valid strategy to combat diseases with a strong inflammatory component. Natural occurring products have been providing an important source of many pharmaceutical drugs currently available. Previously, significant antioxidant properties were verified for a lipid- and essential oil-free infusion from *Cymbopogon citratus* (Gramineae) leaves [2]. In this study was analyzed the inhibitory potential of that extract on lipopolysaccharide (LPS)-induced NF-kB activation in a murine macrophage cell line, Raw264.7. Our results demonstrated, by western blot analysis using specific antibodies, that *C. citratus* extract inhibited LPS-mediated IkB kinase (Ikk) phosphorylation, inhibitory kB (IkB) degradation and consequently prevented p65 protein translocation into the nucleus. The present data support both the use of *C. citratus* as source of new anti-inflammatory drugs as well as its traditional use for inflammation treatment. *Acknowledgements: FCT and POCTI/FEDER for financial support. Research supported by a FCT PhD fellowship (SFRH/BD/46281/2008)* **References:** [1] Cruz, M.T. et al. (2001) Nitric Oxide 5:53 – 61. [2] Figueirinha, A. et al. (2008) Food Chem. 110:718 – 728.

PC14 Acetylcholinesterase inhibitory activity of selected plants used in TCM to improve cognitive function

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As people are becoming older, mental degeneration in the form of Alzheimer's disease, Parkinson's disease and different types of dementia is a major public health concern. Alzheimer's disease is the most common form of dementia diagnosed after the age of 60 worldwide. It is a chronic and progressive process and a multifaceted neurodegenerative disorder affecting different brain areas. Currently, acetylcholinesterase (AChE) inhibitors are the main class of drugs prescribed for symptomatic treatment of Alzheimer's disease. However, these only slow down the disease progression. A cure for Alzheimer's disease has yet to be found. Plants from all over the world are being investigated intensively for compounds with AChE inhibiting activity. In the context of a recent study, 31 plants used in Traditional Chinese Medicine for the improvement of memory and cognition in old age were tested for their acetylcholinesterase inhibitory properties (*in vitro*) using a modified version of the colorimetric method of Ellman [1]. The final product was detected photometrically at 412 nm. The plant material was extracted with water in the traditional Chinese way. Significant inhibition of the enzyme expressed by the IC 50 values was observed for the aqueous extracts from *Rhiz. Coptidis* (huáng lián; IC 50 = 4.68 µg/mL), *Rad. Angelicae sinensis* (dāng guī; IC 50 = 0.13 µg/mL) *Rad. Paeoniae alba* (bái sháo; IC 50 = 0.59 µg/mL), and *Fr. Viticis* (màn jīng zǐ; IC 50 = 0.53 µg/mL). These results substantiate the traditional use of the investigated plant parts for improvement of cognition. **Reference:** [1] Ellman, G.L. et al. (1961) Biochem. Pharmacol 7:88 – 95.

PC15 Purification and antioxidative activities of a water-soluble polysaccharide isolated from *Cordyceps gunnii* (*berk.*) Berk. mycelium

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Cordyceps sinensis is a well known tonic food or invigorant with broad-spectrum medicinal properties that is widely used in China [1,2]. *Cordyceps gunnii (berk.)* Berk is also widely known as the Chinese rare caterpillar fungus and has similar pharmacological activities to *C. sinensis*. The water-soluble polysaccharide CPS 50-I was extracted from the mycelia of *C. gunnii* and further purified by DEAE-Sephadex A-25 and Sephadex G-75. Its characteristics were determined by chemical analysis, gas chromatography, HPSEC and IR spectroscopy. The results show that CPS 50 – 1 is a white powder containing 94.57% carbohydrate and four kinds of monosaccharides including xylose, mannose, glucose and galactose with a molar ratio of 0.13: 0.89:0.54: 1. CPS 50-I has a molecular weight of ~9874 Da and $[\alpha]_D^{20}$ =+85 (c 0.5, H_2O). The protective effect of

CPS50-I was investigated against oxidation resistance in D-galactose (D-gal)-induced aging mice. The results showed that CPS50-I had an obvious protective effect against D-gal-induced aging in mice. *Acknowledgements: Financial support from Natural Science Foundation of Tianjin City (09JCZDJC21800, 08ZCGHHZ00800, 09JCYBJC15800).* **Reference:** [1] Hou, A.I. et al. (2008) Chem. Res. Chinese U. 24:584 – 587. 2. Wang, B.J. et al. (2003) Chem. Res. Chinese U. 19:34 – 37.

PC16 Influence of aging on gastric ulcer healing activity of the essential oil from *Citrus aurantium*

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Aging causes drastic gastrointestinal functional changes in gastric mucosa. Defensive factors have been reported to show an age-related decrease in humans and other animals. The NSAIDs commonly used in elderly persons triple the risk of gastrointestinal complications such as gastric ulcer. Previous studies have reported gastroprotective effects of essential oil from *Citrus aurantium* (EO) to protect the gastric mucosa against injuries caused by different necrotizing agents. However, the confirmation of gastroprotective action of EO does not imply that this same preparation also presents a healing effect on injured gastric mucosa in animals at different ages. So this study aimed to evaluate the healing action of EO from *C. aurantium* in chronic ulcers induced by acetic acid in 8- and 48-week-old rats. The EO healing action was evaluated in acetic-acid-induced gastric ulcer in male Wistar rats (n = 10). We analyzed the effective healing action on chronic gastric ulcer after 14 days (OE = 250 mg/kg, p.o.) by evaluating morphometry and immunohistochemistry (PCNA-cell proliferation), COX2 (cyclooxygenase-2), and VEGF (vascular endothelial growth factor). Macroscopic analysis showed that 8- and 48-week-old rats presented healing proportions of 44% and 99%, respectively in relation to the control group. The morphometric analysis demonstrated that both groups increased the epithelial height of regenerated mucosa (μm) when compared to the control group. Results of immunohistochemical analyses from PCNA presented more intense cell proliferation in 8-week-old rats treated with EO than 48-week-old rats. The 8-week-old rats also increased COX2 expression after 14 consecutive days of EO treatment. But the 48-week-old rats expressed VEGF more intensely than 8-week-old rats treated with EO. Elevated VEGF expression in 48-week-old rats and more intense cell proliferation and COX2 expression in 8-week-old rats treated with essential oil from *Citrus aurantium* were the factors that exerted great influence on protection against the severe harmful agents. *Acknowledgements: CNPq, FAPESP*

PC17 Butyrylcholinesterase inhibitors from *Angelica archangelica* L. roots

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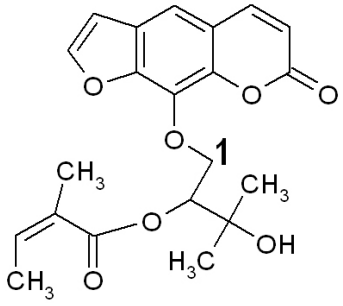
Acetylcholinesterase (AChE) inhibitors are widely used as a drug for the symptomatic treatment of Alzheimer's disease (AD). BuChE appears to be a very important new therapeutic target. Especially as the activity of BuChE increases with the higher stage of the AD, while the activity of AChE decreases [1]. Herbal extracts seem to be a significant source of new potential AChE and butyrylcholinesterase (BuChE) inhibitors, like galantamine isolated from the bulbs of daffodils. Recent data have shown that *Angelica* sp. and coumarins are able to inhibit the activity of acetylcholinesterase, but their potency was not very strong. On the other hand, there is no much information about the effects of those groups of compounds on butyrylcholinesterase activity. In the present study we reported the identification and isolation of inhibitors from extracts obtained from roots of *Angelica archangelica* on two cholinesterase AChE and BuChE. Our results confirm the weak effect of *Angelica* roots on AChE activity. The BuChE inhibition was much more pronounced and achieved the rate higher than 50% at the concentration of 100 μg/ml for hexane extract. The HPLC-DAD profile of hexane extract showed the presence of considerable amount of xanthotoxin, bergapten, imperatorin, isoimperatorin and osthol. Between identified compounds only imperatorin have demonstrated inhibition of BuChE with IC_{50}= 14.3 μM. The TLC bioautography guided fractionation and spectroscopic analysis led to the isolation and identification of compound **1** (2-methyl-2-butenoic acid-2-hydroxy-2-methyl-1-[[(7-oxo-7 H-furo[3,2-g][1]benzopyran-9yl)oxy]methyl]propyl ester) which showed significant BuChE inhibition activity with IC_{50}= 7.5 μM. Only C8-substituted furanocoumarins were BuChE inhibitors.



Reference: [1] Greigt, N.H. et al. (2005) P. Natl. Acad. Sci. USA 102:172130 – 172138.

PC18 Influence of winemaking conditions on phenolic content and antioxidant potential of red wines

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Moderate consumption of red wine is linked to the reduced mortality from cardiovascular disease. These health benefits of red wine have been attributed to its phenolic compounds [1]. The object of this study was to investigate the effect of temperature in winemaking technologies on phenolics in wine in order to increase their content. Wines from three different cultivars were used in experiment. Musts were subjected to different treatments: Experiment (**1**) 60 °C for 1 hour, Experiment (**2**) 80 °C for 5 min., Control (**C**)-no treatment. Total phenolic content was determined according to the Folin-Ciocalteu method. Radical-scavenging capacity (RSC) was measured by evaluating the quenching of the stable DPPH and calculation of mean scavenging concentration (RSC_{50}).

Cabernet Sauvignon	*C*	*1*	*2*
RSC_{50} (μl/ml)	1,22	0,68	0,97
TPC (mg/l GAE)	911,55	1410,39	1098,97
Prokupac	*C*	*1*	*2*
RSC_{50}(μl/ml)	1,81	0,79	1,25
TPC (mg/l GAE)	564,43	1159,37	870,57
Burgundy	*C*	*1*	*2*
RSC_{50}(μl/ml)	1,79	1,26	0,58
TPC (mg/l GAE)	731,06	1038,37	1196,66

Results showed that thermic treatment increased phenolic content and radical scavenging properties of all analyzed samples. Obviously, higher temperature increased extraction of phenolic compounds from grape skins. Also, samples treated at 80 °C mainly showed smaller phenolic content than those treated at 60 °C, due to the decomposition of phenolic compounds with increase of temperature. 1. Cimino, F. et al. (2007) Food Chem. 103:75 – 81.

PC19 Antioxidant flavonoids from bark of *Elaeagnus angustifolia*

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Elaeagnus angustifolia L. (Elaeagnaceae), a plant distributed widely from the northern regions of Asia to the Himalayas and Europe, has been extensively used in traditional medicine to treat ulcer, relax muscle, ease fever, kill pains and cure inflammation [1,2]. However, to date, little has been reported on the chemical compositions of *E. angustifolia*. In this study, 95% EtOH extraction of the title plant bark led to the isolation of different classes of flavonoids, including 4 flavan-3-ols, [(+)-catechin (**1**), (-)-epicatechin (**2**), (+)-gallocatechin (**3**) and (-)-epigallocatechin (**4**)], 2 flavonols [kaempferol (**5**) and quercetin (**6**)], as well as a flavone [luteolin (**7**)], and their structures were elucidated on the basis of phy-

sicochemical and spectroscopical evidence (NMR and MS) [3]. Among the flavonoids **1**, **2**, **3**, **5** and **7** were isolated from *E. angustifolia* for the first time. The antioxidant activities of the flavonoids were evaluated by DPPH free radical-scavenging assay. Results suggested that compounds **1**, **2**, **3**, **4**, **5**, **6** and **7** showed significant antioxidant potential (DPPH IC_{50} values of 5.32, 5.42, 6.81, 5.37, 5.41, 5.30 and 6.68 μM, respectively) compared with α-tocopherol and BHT (DPPH IC_{50} values of 6.86 and 6.91 μM, respectively), which were used as controls. *Acknowledgements: Financial support from Natural Science Foundation of Tianjin City (09JCYBJC15800, 09JCZDJC21800) and China Postdoctoral Science Foundation is gratefully acknowledged.* **Reference:** [1] Hosseinzadeh, H. et al. (2003) J. Ethnopharmacol. 84:275 – 278. 2. Ahmadiani, A. et al. (2000) J. Ethnopharmacol. 72:287 – 292. 3. Agrawal, P.K. (1989) Carbon-13 NMR of Flavonoids. Elsevier, New York.

PC20 Investigation of South African Amaryllidaceae for inhibitors of acetylcholinesterase

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South Africa has a rich diversity of plant species which contain various classes of bioactive compounds [1]. It is known that the Amaryllidaceae provide a number of alkaloids and other compounds [2,3] which are inhibitors of the enzyme acetylcholinesterase and thus may have some application in management of Alzheimer's disease (AD). They furnish, for example, the benzazepine alkaloid galanthamine, which is a competitive and reversible inhibitor of cholinesterase and is currently administered in many countries to patients with AD. The fresh bulbs of a series of Amaryllidaceae belonging to the genera *Crinum*, *Nerine*, *Strumaria* and *Ammocharis* were extracted with 90% ethanol and tested for the inhibition of acetylcholinesterase in a rapid TLC benchtop bioassay [4]. The extracts showed varying degrees of inhibition in the bioassay, with activities being attributed to polar and apolar constituents and both alkaloidal and non-alkaloidal components. Several species contained galanthamine. The same species of plant (*Nerine laticoma*, for example), collected in different localities, showed a wide variation in content of active natural products. Some modifications were also made to the operation procedure of the original enzyme bioassay [4]. **References:** 1. Mulholland, D.A. and Drewes, S.E. (2004) Phytochemistry 65:769 – 782. 2. Kissling, J. et al. (2005) Phytother. Res. 19:984 – 987. 3. Elgorashi, E.E. et al. (2006) S. Afr. J. Bot. 72:224 – 231. 4. Marston, A. et al. (2002) Phytochem. Anal. 13:51 – 54.

Topic D: Natural products and neglected diseases

PD1 Tehranolide, A sesquiterpene lactone with an endoperoxide group that probably has the same effect as the antimalarial agent Artemisinin

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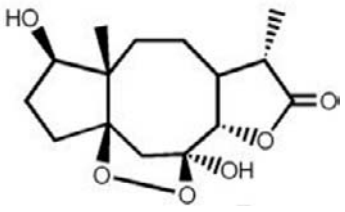
The malaria situation is aggravated by the appearance of strains of *Plasmodium falciparum* resistant to antimalarial drugs as well as by the resistance of vector *Anopheles* mosquitoes to DDT and other insecticides. Nearly 300 – 500 million people are infected by malaria and the incidence of this disease is dramatically increasing, since many strains of *Plasmodium falciparum*, the parasite responsible for the majority of fatal malaria infections, have become resistant to chloroquine and other traditional antimalarial drugs. Fortunately, these strains are still susceptible to the artemisinin derivatives. All of the artemisinin compounds contain stable endoperoxide bridges. The extract of the aerial parts of *A. diffusa* collected in the Province of Khorassan (Iran) afforded, in addition to several eudesmanolides, a new type of sesquiterpene lactone (Tehranolide) with an endoperoxide group. Previously, we reported antimalarial effect of extract of *A. diffusa* against *P. berghei*. Since the endoperoxide group is an essential requirement for the antimalarial activity of artemisinin, we have presumed the antimalarial properties of the crude extract are attributed to Tehranolide. We report here the *in vivo* laboratory evaluation of anti-malarial effects of Tehranolide, was done on *P. berghei* infected NMRI mice. The antimalarial effects of Tehranolide in 27 mg/ml concentration (high dose) were injected s.c. every day for 12 days after infection in malaria mice. Three groups of mice (n = 5) were investigated for antimalarial efficacy, the degree of parasitaemia, assessment of pathology including body weight, physiological activities, hepatomegaly and splenomegaly. Parasitaemia was measured every other day by counting Geimsa-stained blood smears which were taken from end tail cutting.



Tehranolide

References: Rustaiyan, A. et al. (2009) PHCOG MAG 4:1 – 7.

PD2 Aloes. Homonataloin and aloenin are important bioactive components for use in cosmetic and medicinal industries

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Aloes provide a fascinating subject for research from a chemical, biochemical, pharmaceutical, taxonomic, medical and economic point of view. The genus *Aloe* contains about 420 species [1]. The majority of these plant species are desert plants which inhabit in the Desert of South Africa. Some of these species are tall trees in size of 0.5 m or more, while the majority is shrubs 0.5 to 1.5 m tall. Some plants species are very small, measuring only a few cm [2]. *Aloe* plant contains many biological activities compounds, such as anthrones and anthraquinones, chromones, phenolic compounds, alkaloids, polysaccharides and other components. As a continuation of our investigation (1991 – 2009) me have studied the content and distribution of aloenin and homonataloin in the leaves from 67 *Aloe* species, originated from South Africa and introduced during the 25 the last years in the Botanical Garden in the Negev Desert of Israel. It was found that 28% of this *Aloe* species contain homonataloin and only 5% contain aloenin. *Aloe vera* (barbadensis) does not contain aloenin. Aloenin could be useful as cancer chemopreventive agent against tumor promotion [3]. Aloenin is a major constituent which has significantly promoted hair growth. Aloenin also has demonstrated recuperative effects on human skin [4]. Homonataloin, which possessed antimalarial activity, inhibited the chloroquine-resistant *Plasmodium falciparum* [5]. *Aloe* species containing aloenin and homonataloin (besides *Aloe vera* and *Aloe ferox*) are suitable as commercial sources of Aloes gel for use in the cosmetic and medicinal industries. **References:** [1] Reynolds, T. (2004) *Aloes*: The genus *Aloe*. CRC Press. [2] Van Wyk, B.E. et al. (1996) Guide to the *Aloes* of South Africa. Briza Publications, Pretoria. [3] Shimpo, K. et al. (2002) Phytother. Res.16:491 – 493. [4] Yamamoto, M. (1993) Jap. J. Tox. Env. Health 39:409 – 414. [5] Van Zyl, R. et al. (2002) S. Afr. J. Bot. 68:106 – 110.

PD3 Antioxidant property of a Thai traditional formula for longevity

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Antioxidant properties of a Thai traditional formula for longevity, which is composed of 6 herbs as follows: *Albizia procera*, *Diospyros rhodocalyx*, *Tinospora crispa*, *Cyperus rotundus*, *Streblus asper* and *Piper nigrum* were studied. Each herb including the formula was extracted by 95% ethanol and concentrated by using vacuum evaporator. The antioxidant properties were detected by DPPH method. Vitamin C and Trolox were used as

reference standard. The *in vitro* oxidative hemolysis of sheep red blood cells was used as a model to study the free radical-induced damage of biological membranes by AAPH. From DPPH method, *Albizia procera* extract possessed the most potent antioxidant properties (IC_{50} 44.34 μg/ml) while vitamin C and Trolox had IC_{50} at 17.47 μg/ml and 22.75 μg/ml, respectively. From AAPH hemolysis method, *Albizia procera*, the formula for longevity, *Cyperus rotundus*, *Diospyros rhodocalyx*, *Piper nigrum*, *Tinospora crispa* and *Streblus asper* extracts at the concentration of 5 mg/ml could prolong the time of 50% hemolysis from 78 minutes to 157, 142, 126,114, 108, 101, 100 minutes, respectively, while the time of 50% hemolysis of trolox at the concentration of 0.5 mg/ml was 160 minutes. The phytochemical screening tests showed the presence of phenolic compounds, tannins and flavonoids in the formula and *Cyperus rotundus*, *Albizia procera*; phenolic compounds and flavonoid in *Piper nigrum*, *Diospyros rhodocalyx* and *Streblus asper*. Phenolic compounds were found in *Tinospora crispa* extract.

PD4 Trypanocidal activity of pterogynidine and nitensidine E using two distinct *Trypanosoma cruzi* strains

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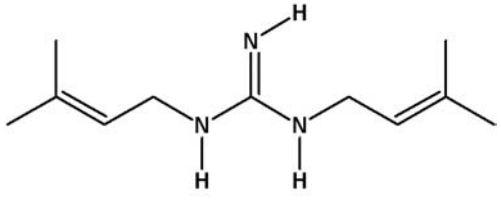
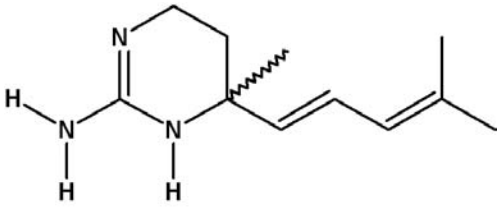
Pterogynidine (**1**) and Nitensidine E (**2**) were isolated from different parts of *Pterogyne nitens*, which is a native tree common in South America [1,2]. Recently, Regasini et al. [3] demonstrated that nitensidine E was the most active compound against different tumor cell lines, while pterogynidine was inactive. The structure of nitensidine E is the first report of natural occurrence of a cyclic monoterpene derivative on a guanidine moiety [3].



pterogynidine (1)



nitensidine E (2)

We described the trypanocidal activity of both compounds and IC_{50} values (μg/ml) were determined as follows: pterogynidine: 22.5 (Y strain) and < 0.07 (BOL strain); nitensidine E: 2.41 and 3.00, respectively, demonstrating a higher correlation between the number of prenyl units and the trypanocidal activity. *Acknowledgements: FAPESP (2004/07932 – 7; 2007/02076 – 3; 2008/06021 – 1); CAPES.* **References:** [1] Lorenzi, H. (1998) Plantarum. Nova Odessa, Brasil. [2] Bukart, A. (1952) Aemé Agency. Buenos Aires, Argentina. [3] Regasini, L.O. et al. (2009) J. Nat. Prod. 72:473 – 476.

PD5 *In vitro* shistosomicidal activity of (-)-6,6-dinitrohinokinin: a semi-synthetic lignan derivative obtained from (-)-hinokinin

Silva MLA[1], Rodrigues V[2], Albuquerque S[2], Bastos JK[2], Silva R[1], Pereira Júnior OS[3], Bianco TNC[1], Cunha WR[1], Santos FF[1], Donate PM[2], Magalhães LG[2], Pereira AC[1], Da Silva Filho AA[1]
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Schistosomiasis is one of the most significant neglected tropical diseases in the world, affecting more than 200 million people. Praziquantel (**PZQ**) is currently the only effective drug available for the treatment of this infection caused by *Schistosoma* species [1,2]. Therefore, the aim of this work was to evaluate the *in vitro* shistosomicidal activity of (-)-*6,6*-dinitrohinokin (**DiNI**), obtained by partial synthesis of (-)-hinokinin, a semi-synthetic derivative of (-)-cubebin [3]. Coupled adult worms of *S. mansoni* LE strain were recovered from mesenteric veins of the infected mice and cultured in 24-well plates at 37^0C in RPMI1640 media. Coupled adult worms were kept for 5 days and the viability, pairing, egg production, and egg development were monitored every 24 h by incubation in the presence of **DiNI** at concentrations of 31.5, 16.9 and 7.9 μM[1]. As negative control group it were used adult worms treated with 10% of DMSO. **DiNI** (31.5 and 16.9 μM) caused the death of all *S. mansoni* adult worms after 24 h of incubation. Also, **DiNI** (7.9 μM) induced a significant reduction in the motor activity after 24 h, as well as the death of all *S. mansoni* adult worms after 120 h of incubation. **DiNI**, at 31.5, 16.9 and 7.9 μM, significantly decreased the egg production by 47%, 86% and 90%, respectively, after 48 h of incubation. **DiNI** (at 31.5, 16.9 and 7.9 μM) was able to separate the adult worm pairs (into male and female) and to cause significant tegumental alterations in the worms after 120 h. This result shows the possibility of starting a promising work, with future possibilities for an effective drug to combat schistosomiasis, which has patent deposited in Brazil (**PI 0503951 – 7**), EUA (**US 11995,789**), Europe (**EP 06761026.1**) and Japan (**JP 2008 – 508031**). *Acknowledgements: FAPESP* (1998/14956 – 7, 2004/08784 – 1 and 2006/60132 – 4) *and CNPq for financial support.* **References:** [1] Magalhães, L.G. et al. (2009) Parasitol. Res. in press. [2] Smithers, T. (1965) Parasitol. 55:695 – 700. [3] Silva, R. et al. (2005) Bioorg. Med. Chem. Lett. 15:1033 – 1037.

PD6 Evaluation of the *in vitro* trypanocidal activity of plant extracts from the Brazilian Cerrado

Cunha WR[1], Dos Santos FM[1], Peixoto JA[1], Veneziani RCS[1], Crotti AEM[1], Silva MLA[1], Da Silva Filho AA[1], Turatti ICC[2], Bastos JK[2], Albuquerque S[2]
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Chagas' disease is endemic in Latin America and affects 16 – 18 million people, while other 100 million are exposed to the risk of infection. *Trypanosoma cruzi*, the etiological agent of this disease, causes a pathology which features depend on both the inherent characteristics of the host and the virulence of the parasite. In this study we report the screening of the *in vitro* trypanocidal activity of twenty extracts obtained from ten different plant species growing in the Brazilian Cerrado: *Aspidosperma macrocarpum* Mart. (Apocynaceae), *Aegiphila sellowiana* Cham. (Verbenaceae), *Byrsonima intermedia* Juss. (Malpighiaceae), *Cyperus rotundus* L. (Cyperaceae), *Leandra lacunosa* Cogn. (Melastomataceae), *Miconia ligustroides* (DC.) Naudin. (Melastomataceae), *Miconia sellowiana* Naudin. (Melastomataceae), *Myrcia variabilis* Mart. ex DC. (Myrtaceae), *Solanum lycocarpum* St. Hil. (Solanaceae) and *Tibouchina stenocarpa* Cogn. (Melastomataceae). The most active extracts were submitted to phytochemical analyses. The high-resolution gas chromatography analysis of the *n*-hexane extract of *T. stenocarpa* (IC_{50} = 23.6 μg/mL), the most active extract amongst all tested samples, allowed the identification of β-amyrin, α-amyrin, lupeol, friedelin, β-friedelanol, campesterol, stigmasterol, and β-sitosterol. Oleanolic and ursolic acids were isolated from the methylene chloride extract of *T. stenocarpa* (IC_{50} = 51.5 μg/mL), while ursolic acid was isolated from the methylene chloride extract of *M. variabilis* (IC_{50} = 38.4 μg/mL). Solasonine and solamargine were identified as major compounds by mass spectrometry analysis in the hydroalcoholic extract of the fruits of *S. lycocarpum* (IC_{50} = 57.1 μg/

mL). The results showed that the trypanocidal activity may be related to the major compounds identified in the crude active extracts. *Acknowledgements: FAPESP and CNPq for financial support*

PD7 Antimycobacterial activity of medicinal plants from Mozambique

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Lannea stuhlmanni, *Maytenus heterophylla*, *Maytenus senegalensis*, *Sarcostemma viminale* and *Tabernaemontana elegans* are medicinal plants used in Mozambique to alleviate symptoms and treat pulmonary diseases, including tuberculosis [1]. Hereby we present results from an ethnopharmacological study conducted in order to validate the traditional use of these species against mycobacteria strains, specifically drug-sensitive and drug-resistant ones. Therefore *in vitro* antimycobacterial activity of nine hydroethanol extracts from different plant parts were screened through a rapid radiometric method. All extracts were tested in triplicate and the minimum inhibitory concentrations (MIC) refers to the mean arithmetic value. Five extracts have shown activity against the drug-sensitive *Mycobacterium tuberculosis* ATCC 700457. *L. stuhlmanni* root, *M. senegalensis* leaf, *S. viminale* root, and *T. elegans* leaf and root extracts were the most active extracts, demonstrating MIC ranged from 150 – 175 μg/mL. *T. elegans* root extract was the most active. Concerning the drug-resistant strains (*M. tuberculosis* ATCC 35822 – isoniazide resistant; *M. tuberculosis* ATCC 35838 – rifampin resistant), *T. elegans* root extract, *T. elegans* leaf extract and *M. heterophylla* root extract have shown the lowest MIC values, ranging from 150 – 175 μg/mL. In order to localise the biological activity some active extracts were partitioned by liquid-liquid extraction. *M. senegalensis* ether fraction exhibited the most promising results against the drug-sensitive mycobacterium strain (MIC = 150 μg/mL). Prospective studies include the establishment of the chemical profile of the active extracts and fractions and identification of the active compounds. Results confirm the medicinal value of these plants. **References:** [1] Jansen, P., Mendes, O. (1991) Plantas Medicinais – Seu Uso Tradicional em Moçambique. Imprensa do Partido. Maputo.

PD8 Optimization of the medicinal mushroom *Ganoderma australe* biomass production using Response Surface Methodology

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Mushrooms or fruiting bodies of many Basidiomycetes used in traditional therapies presenting medicinal effects are commonly produced in solid-state fermentation, generally after 20 – 60 days of growth. Recently, a number of substances of mushroom origin have been isolated, identified and shown to have physiological activities, such as antitumor, immunomodulating, cardiovascular, antibacterial, antiviral, antiparasitic, hepatoprotective and antidiabetic activities. Submerged fermentation of the mycelial form of mushroom-producing fungi has received much attention as a promising alternative for efficient and faster production of the biomass of medicinal mushrooms and their active metabolites [1]. The aim of this work was to study the effect of the composition of the nutrient media on the growth of vegetative mycelium in submerged cultures of the Basidiomycetes *Ganoderma australe*, which is a species of pharmaceutical interest [2]. Initially 95 different carbon sources were screened with the Biolog MicroPlate Analysis and then 9 of them were tested in shake flasks cultures [3]. The effect of various organic and complex nitrogen sources on biomass production was also examined and response surface methodology based on central composite design was applied to explore the optimal medium composition. When the optimized culture medium was tested in a 20-L stirred tank bioreactor, using 1.37% (w/v) glucose and 3.0% (w/v) yeast extract, high yields of biomass (11.0 g L^{-1}) and productivity of 0.17 g L^{-1} h^{-1} were obtained. **References:** [1] Tang, Y.J. et al. (2007) Food Technol. Biotechnol. 45:221 – 229. [2] Rusell, R., Paterson, M. (2006) Phytochemistry 67:1985 – 2001. [3] Kubicek, C.P. et al. (2003) Fungal Genet. Biol. 38:310 – 319.

PD9 Antiprotozoal, antibacterial and antifungal activities of plants of the Lauraceae family collected in the Brazilian Amazon rainforest

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[1]Programa de Pós-graduação em Microbiologia, Universidade Estadual de Londrina; [2]Programa de Pós-graduação em Química, Universidade Federal do Amazonas; [3]Depto de Química, Universidade Federal do Amazonas; [4]Depto de Análises Clínicas, Universidade Estadual de Maringá, PR Brazil

Lauraceae is a world-wide distributed family of flowering plants which most are aromatic [1]. Due to this characteristic, several species are used for cosmetics and gastronomic purposes, but the medicinal property of the majority of the species against infections and diseases is not completely elucidated [2]. As the microbial resistance to usual drugs increases, the search for new chemotherapics, including diseases which the cure has not been reached yet or the available treatments present great toxicity to humans benn is needed. In this study, the crude extracts of 13 species and the essential oil of 10 species (*Aniba panurensis, Aniba rosaeodora, Dicypellium manauense, Endlicheria chalise, Licaria canella angustata, Licaria martiniana, Mezilaurus duckei, Mezilaurus itauba, Ocotea nigrescens, Ocotea splendens, Pleurothyrium vasquezii, Rhodostemonodaphne negrensis, Rhodostemonodaphne parvifolia, Sextonia rubra)* were evaluated against bacteria (*S. aureus, B. subtilis, P. aeruginosa, E. coli*), yeasts (*C. albicans, C. tropicalis, C. parapsilosis*), and protozoa (*T. cruzi, L. amazonensis*). The minimum inhibitory concentration (MIC) was determined for bacteria and yeasts in a 96-well plate after 24 and 48 h of incubation at 37 °C, respectively. Growth inhibition of 50% (IC_{50}) was determined for *T. cruzi* after 96 h at 28 °C and *L. amazonensis* after 72 h at 25 °C, both in 24-well plates. Among the plant extracts, 12% were able to inhibit the growth of *S. aureus* and *B. subtilis* (MIC 140 – 260 μg/mL), while more than 50% of the extracts resulted in IC_{50} less than or equal to 200 μg/mL for both protozoa. The majority of the essential oils showed similar activity to the extracts inhibiting the growth of the same bacteria (MIC 250 – 1000 μg/mL) and all of them inhibited *C. tropicalis*, mostly at concentrations below 62.5 μg/mL. About 80% of the oils showed IC_{50} below 50 μg/mL against *T. cruzi* and *L. amazonensis*. Then, Lauraceae species provided great results and studies might be continued. *Acknowledgements: CNPq, FINEP, PRONEX/Fundação Araucária, FAPEAM.* **References:** [1] Van Der Werff, H., Richter, H.G. (1996) Ann. Mo. Bot. Gard. 8:419 – 432. [2] Marques, C.A. (2001) Floresta e Ambiente 8:195 – 206.

PD10 Synthesis of the potent antimycobacterial agent evocarpin

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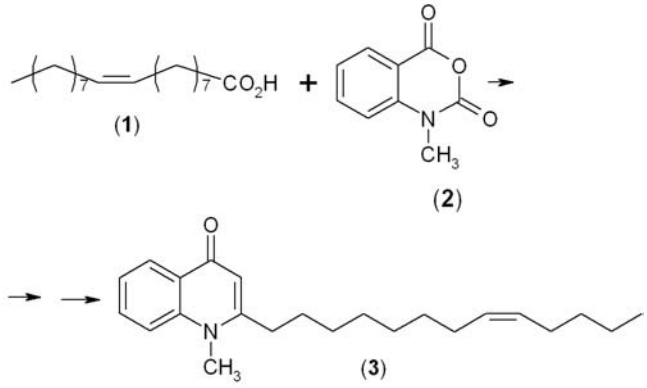
Evocarpin, originally isolated from the fruits of the Chinese medicinal plant *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae) displayed a potent antimycobacterial activity on fast growing strains of mycobacteria [1,2]. As part of our on going project, we have prepared evocarpin from the cheaply available unsaturated fatty acid, oleic acid (**1**). The synthesis was started by Wittig reaction of an aldehyde ester, which was readily prepared by ozonolysis of methyl oleate with pentyltriphenylphosphonium bromide which was obtained by the reaction between bromopentane and triphenylphosphine. The resulting unsaturated ester underwent alkaline hydrolysis to afford the corresponding unsaturated acid, and subsequent methylation of the acid with methyllithium afforded an unsaturated methyl ketone. Further treatment of this methyl ketone

with N-methylisatoic acid anhydrid (**2**) under strong basic conditions gave evocarpine and its *trans* isomer (2:1) [3], which was subjected to preparative HPLC to give evocarpin (**3**).



Acknoledgements: We are grateful for financial support by the Austrian Science Fund (FWF), Project No. P21152. **References:** [1] Adams, M. et al. (2005) Int. J. Antimicrob. Agent. 26:262 – 264. [2] Adams, M. et al. (2006) Patent. Int WO 2006/094327 A1. [3] Coppola, G.M. (1985) J. Heterocyclic Chem. 22:491 – 494.

PD11 *In vitro* anti-yeast effect of *Nigella sativa* seed quinones

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N. sativa L. seeds (Ranunculaceae), commonly known as black seed or black cumin, have been traditionally used as a curative remedy for numerous disorders as well as spice and food preservative. Several pharmacological activities of the seeds have been attributed to the quinone constituents of its volatile oil, particularly to thymoquinone (TQ). Thymohydroquinone (THQ) and dithymoquinone (DTQ). Although many of the biological effects, e.g. antibacterial, antiparasitic and anti-inflammatory have been widely investigated in the past [1], antifungal activity of the plant has been rarely assessed [2]. In this study we describe the *in vitro* inhibitory effect of *N. sativa* quinones on growth of human pathogenic and food spoilage yeasts, namely on *Candida albicans*, *Debaryomyces hansenii, Kluyveromyces marxianus, Pichia anomala, Saccharomyces cerevisiae,* and *Yarrowia lipolytica* using the broth microdilution method [3]. For the tests purposes TQ was purchased from Sigma-Aldrich (CZ), whereas DTQ and THQ were synthesized from TQ according to the previously described methods [4,5]. The results showed that TQ and THQ possessed inhibitory effect against all yeasts tested in this study at concentrations of 64 μg/ml and below, except THQ, which inhibited the growth of *C. albicans* strains with MICs value 256 μg/ml. The strongest antimicrobial effect showed TQ and THQ, inhibiting the growth of *Z. microellipsoides* with minimum inhibitory concentration 8 μg/ml. DTQ possessed no inhibitory activity. Regarding to our results, we suggest TQ and THQ as a perspective anti-yeast agents for possible applications in pharmaceutical or food industry. *Acknowledgements: This research was supported by Czech Science Foundation (Project No. 525/08/H060).* **References:** [1] Ghosheh, O.A. et al. (1999) J. Pharm. Biomed. Anal. 19:757 – 762. [2] Aljabre, S.H.M. et al. (2005) J. Ethnopharmacol. 101:116 – 119. [3] Jorgensen, J.H. et al. (1999). In: Murray P.R. (ed.) Manual of Clinical Microbiology. ASM Press. Washington, DC. [4] Smith, L.I. et al. (1944) J. Am. Chem. Soc. 66:1323. [5] El-Dakhakhny, M. (1963) Planta Med. 11:465.

PD12 Polysaccharides from *Sutherlandia frutescences* leaves have immunomodulating properties

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Sutherlandia frutescens, syn. *Lessertia frutescens*, is a plant of long traditional use in South Africa, and over the last years it has obtained great interest as a plant that has positive effects in relation to the treatment of HIV/AIDS. Patients having HIV/AIDS have often a low immune response. If this can be stimulated the wellbeing of the person will increase. As polysaccharides have been shown to have immunomodulating properties [1,2,3] it was of interest for us to study polysaccharides from the leaves of *Sutherlandia frutescens* and focus on their structure and effect in immunoassays related to the immune system. Traditionally, water extracts have been the choice of preparation of remedies used. For this reason we prepared water extracts, and from these purified polysaccharides both of the xylan type and of the pectin type were prepared. Determination of their monosaccharide compositions as well as their linkages showed that the xylan mainly was 1,4 linked, while the pectins contained typical rhamnogalacturonan type I regions as well as side-chains containing arabinogalactan type II structures. The polysaccharides had a marked effect in the complement system and did also show proliferation of B cells and maturation of dendritic cells, all effects indicating immunomodulating properties. **References:** [1] Paulsen, B.S., Barsett, H. (2005) Advances in Polymer Science, Bioactive pectic polysaccharides (Polysaccharides I) Springer Berlin/Heidelberg, Germany, pp69 – 101. [2] Inngjerdingen, K.T. et al. (2007) Glycobiology 17(12):1299 – 1310. [3] Inngjerdingen, M. (2008) Glycobiology 18(12):1074 – 1084.

PD13 A protocol for HPLC based activity profiling of plant and fungal extracts against tropical parasites

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Of the more than two hundred thousand secondary metabolites known so far, many have become rewarding leads in the development of new therapeutics. In the area of tropical parasitic diseases, important natural product leads have been quinine, artemisinin, and the avermectins [1,2]. HPLC based activity profiling is an effective strategy to speed up the discovery of new leads. It conveniently combines the superior separation power of HPLC micro scale compound separation with efficient high-throughput biological screening methods. Active substances can be structurally characterized by HPLC hyphenated methods (HPLC-PDA, -MS^n, -HR-MS, -NMR etc.), and if so desired, be effectively targeted. The need of handling large numbers of fractions is minimized [3]. We established a library of 640 plant and fungal extracts in 96-well format which was screened for antimalarial, antileishmanial and anti-trypanosomal activity. Active extracts were subjected to a newly developed medium throughput HPLC based activity profiling protocol, to identify the active compounds. 350 μg of an extract were injected onto an analytical column (SunFire RP-18, 3.5 um, 3 * 150 mm, Waters), and thirty five one- minute fractions were collected into 96 deep well microtiter plates. After parallel evaporation of the microfractions, suitable dilution schemes permitted parallel activity profiling for antimalarial, antileishmanial and anti-trypanosomal activity. The protocol was validated with extracts and positive controls such as *Artemisia annua* which were treated likewise. The activity was confined to one or few of the micro fractions, which could be chemically characterized online by HPLC-hyphenated methods. Examples of the use of this protocol for the identification of active constituents in a complex extract are shown. **References:** [1] Cragg, G.M., Newman, D.J. (2005) Pure Appl Chem 77:7 – 24. [2] Wilcox, M.L. et al. (2001) Trends Parasitol. 17:58 – 60. [3] Potterat, O. et al. (2006) Curr. Org. Chem. 10:899 – 920.

PD14 HPLC-profiling for antiplasmodial compounds – 3-methoxycarpachromene from *Pistacia atlantica*

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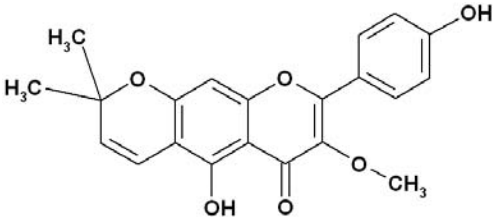
In the course of a medium throughput screen of 640 plant extracts for antimalarial activity [1] an ethyl acetate extract of *Pistacia atlantica* DC. (Anacardiaceae) was active. With analytical scale time-based HPLC separation and testing for antiplasmodial activity in combination with hyphenated methods (HPLC-PDA, -MS^n, HR-MS, off line microprobe NMR) the active substance was identified. After isolation, assignment of the ^1H and ^{13}C NMR resonances were carried out by extensive analysis of its NMR spectra and the spectra of its acetylated derivative. The new antiplasmodial flavonoid 3-methoxy carpachromene had an IC_{50} of 3.4 μM towards *Plasmodium falciparum* K1 strain was identified. In a cytotoxicity assay using rat skeletal myoblasts (L-6 cells) it had an IC_{50} of 21.9 μM. This compound is amongst the most potent antiplasmodial flavanoids reported so far and is chemotaxonomically quite unusual for the Anacardiaceae family.



References: [1] Kunert, O. et al. (2008) Phytochem. Lett. 1:171 – 174.

PD15 HPLC based activity profiling of *Ganoderma lucidum* extract for antiplasmodial activity and isolation of new active lanostane triterpenoids

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Ganoderma lucidum mushroom (Curtis) P. Karst (Ganodermataceae) is known as lingzhi in Chinese and reishi or mannentake in Japanese. It has been used medicinally for thousands of years and is apraised as one of the most powerful remedies in traditional Chinese medicine [1]. Over 200 substances from this source have been isolated and structurally identified, mostly polysaccharides and lanostane triterpenes. In a medium throughput screen of plant and fungal extracts for antiplasmodial activity [2] an ethyl acetate extract from lingzhi mushroom was active with a 79% inhibition at 4.9 μg/ml. With analytical scale time-based HPLC separation and testing of one-minute fractions for their antiplasmodial activity in combination with hyphenated methods (HPLC-PDA, -MS^n, HR-MS, off line microprobe NMR) the active substances were identified. The substances were isolated using normal phase medium pressure column chromatography and semi-preparative HPLC, and structure elucidation was achieved by extensive ^1H and ^{13}C NMR analysis. HPLC based activity profiling is an efficient tool to identify active minor compounds in complex extract matrices [3]. This way we could identify new active compounds from lingzhi mushroom despite the fact that it has already been studied extensively. This is the first report of antiplasmodial activity of this triterpenoid class. **References:** [1] Pattersen R.R. et al. (2006) Phytochemistry 67:1985 – 2001. [2] Kunert, O. et al. (2008) Phytochem. Lett. 1:171 – 174. [3] Potterat, O. et al. (2006) Curr. Org. Chem. 10:899 – 920.

PD16 Identification of the mechanism of action of eupomatenoid-5 isolated from *Piper regnellii* var. *pallescens* on *Trypanosoma cruzi*

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Parasitic protozoa cause several diseases, affecting hundreds of millions, particularly in underdeveloped countries [1]. Chagas disease is one of these clinical conditions triggered by infection with the protozoan *Trypanosoma cruzi*. The currently drugs available for treatment of this infection are unsatisfactory due to limited efficacy and toxic side effects, making the search for more specific pharmacological agents a priority [2]. In previous work, eupomatenoid-5 isolated from *Piper regnellii* var. *pallescens* showed activity against the proliferative stages of *T. cruzi* [3]. Here, we decided to investigate the mechanism of action of the eupomatenoid-5 on *T. cruzi*. The components of the trypanothione-dependent antioxidant system from *T. cruzi* have been pointed out as potential chemotherapeutic targets [2] by presenting differences with mammalian host. Thus, epimastigotes were pre-treated with eupomatenoid-5 and challenged with doses of H_2O_2 in IB medium. Then, the cells were resuspended in LIT medium and the growth index (GI) determined after 5 days. Control of the eupomatenoid-5 and H_2O_2 were also performed. For lipoperoxidation assay, the same treatment was performed and determined as the amount of thiobarbituric acid-reactive substances in terms of malondialdehyde [4]. The results showed a decrease of GI after pre-treatment and challenged with non-toxic dose (20mM) (35.4%) and sub-lethal dose of H_2O_2 (100mM) (17.9%), while the control eupomatenoid-5 GI was 61.5%. Additionally, the same treatment increased lipoperoxidation compared with the controls H_2O_2 and eupomatenoid-5. These results have indicated that eupomatenoid-5 can act in the detoxification system of *T. cruzi* making these cells more susceptive to H_2O_2. Studies in trypomastigote and amastigote intracellular forms are being performed. *Acknowledgements: This study was supported through grants from DECIT/SCTIE/MS and MCT by CNPq, FINEP, PRONEX/Fund. Araucária.* **References:** [1] Turrens, J.F. (2004) Mol. Aspects Med. 25:211 – 220. [2] Mielniczki-Pereira, A.A. et al. (2007) Acta Trop. 101:54 – 60. [3] Luize, P.S. et al. (2006) Biol. Pharm. Bull. 29:2126 – 2130. [4] Hernández, S.M. et al. (2006) Acta Trop. 98:94 – 102

PD17 The biological activity of piperovatine and piperlonguminine isolated from *Piper ovatum* Vahl on epimastigote form *of Trypanosoma cruzi*

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Trypanosoma cruzi is the etiological agent of Chagas disease, a debilitating disease that affects about 18 million people [1], causing the deaths of 45,000 patients annually [2]. The current treatment for this infection is very limited, and available drugs (Nifurtimox and Benznidazole) have many side effects [3]. Several studies of crude plant extracts have identified potential compounds to treat this disease. In this study was evaluated the effect of piperovatine and piperlonguminine isolated from *Piper ovatum* Vahl on epimastigote form of *T. cruzi*, in LIT medium during 5 days. Ultrastructural and morphological alterations were observed by electron microscopy. The piperovatine and piperlonguminine concentration which inhibit 50% of growth (IC_{50}) of epimastigotes were 11.5 μg/ml and 17.0 μg/ml, respectively. Epimastigotes treated with piperovatine were fixed with 2.5% glutaraldehyde. For transmission electron microscopy, cells were post-fixed in osmium tetroxide, dehydrated in acetone, and embedded in Epon. Ultrathin sections were observed in Zeiss 900 TEM. For scanning electron microscopy, parasites were placed on a specimen support with poly-L-lysine, dehydrated in ethanol, critical-point

dried in CO_2, coated with gold, and observed in a Shimadzu SS-550 SEM. The ultrastructural studies on the epimastigote showed alterations in the mitochondria and cytoplasmatic extraction, with multiple vacuoles. Observations by scanning electron microscopy revealed alterations in the shape and size of the epimastigotes. These findings add a new insight in the search for new antiprotozoal agents from natural sources. *Acknowledgements: This study was supported through grants from DECIT/SCTIE/MS and MCT by CNPq, FINEP, and CAPES.* **References:** [1] Salas, C. et al. (2008) Bioorgan. Med. Chem. 16:668 – 674. [2] WHO, Control of Chagas Disease (2002). [3] Menna-Barreto, R.F. et al. (2009) Micron 40:157 – 168.

PD18 Phytochemical study and biological evaluation of the stem of *Derris ferruginea* Bentham

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The genus *Derris* Loureiro belongs to the tribe Milletiae of the Leguminosae. It includes about fifteen species widely found in the tropical areas of Africa and Asia [1]. These plants have been traditionally used over centuries as fish poisoning, insecticide and pesticide, particularly due to their large production of rotenone [2]. Biological activities of *Derris* species are various: cytotoxic, antibacterial, antifungal, and antioxidant [3,4,5]. Though major secondary metabolites found in the genus are known to be flavonoids, including prenylated flavanones, and isoflavonoids such as rotenoids, very few phytochemical informations were available on *D. ferruginea* [6]. Different crude extracts were obtained from the stem (2 kg, Soxhlet apparatus). They were fractionated using successively MPLC, LC, FCPC® (Fast Centrifugal Partition Chromatography, Kromaton Angers), Sephadex LH-20® gel and finally purified on preparative HPLC. Chemical structures of the isolated compounds were elucidated using ^{1}H and ^{13}C NMR spectrum as well as Mass Spectrometry. Most of these compounds were identified as prenylated flavonoids (flavanones and isoflavonoids). Biological effects of these compounds will be reported here, particularly antimicrobial, antiparasitic activities and inhibition of the formation of AGEs (Advanced Glycation End Products involved in age- and diabetes-related chronic diseases). Refrences: 1. The Angiosperm Phylogeny Group (2003) Bot. J. Linn. Soc. 141:399 – 436. 2. Moretti, C. et al. (1982) J. Ethnopharmacol. 6:139 – 160. 3. Cheenpracha, S. et al. (2007) Can. J. Chem. 85:1019 – 1022. 4. Khan, M.R. et al. (2006) Fitoterapia 77:327 – 330. 5. Wangensteen, H. et al. (2006) Fitoterapia, 77:290 – 295. 6. Subba, N.Y. et al. (1946) Proc. Indian Acad. Sci. 24:344 – 348.

PD19 The in vitro antidiarrhoeal activity of *Holarrhena antidysenterica* (bark) wall extracts

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The in vitro antidiarrhoeal activity of ethanolic (by maceration) & aqueous (by soxhlet) extracts of the bark of *Holarrhena antidysenterica* and its comparison with that of standard drugs was evaluated. It showed significant activity against *Shigella boydii, Shigella soneii and Shigella flexneri* whereas the activity of both extracts was found to be moderate against *Shigella dysenteirae.* The minimum inhibitory concentration (by micro dilution) against the strains of *Shigella* was recorded between 250 to 500 µg/ml. Viable cell count method was also used to decipher bactericidal or bacteriostatic action of each extract. All the extracts were bactericidal within 2 h. The extract was found to be more effective than the standards at lower end of the concentrations tested (0.5 mg/ml and 1.0 mg/ml). The antimicrobial activity of the extract is comparable to standard antibiotic ciprofloxacin. The results support the efficacy of ethanolic and aqueous extracts of bark of *Holarrhena antidysenterica* as bacteriocidal and antidiarrhoeal agent. Our present work suggests that preclinical and clinical trials of both aqueous and alcoholic extracts of the stem bark of *Holarrhena antidysenterica* can be carried out against different enteric pathogens, causative agents of diarrhoea in population.

References: [1] Ahmad, I. et al. (1998) J. Ethnopharmacol. 62:183 – 93. [2] Ballal, M. et al. (2001) Indian J. Pharmacol. 33:392 – 393.

PD20 Constituents of *Baccharis dracunculifolia* DC (Asteraceae) with *in vitro* antileishmanial, antiplasmodial and cytotoxic activities

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Baccharis dracunculifolia D.C. (Asteraceae) is the most important plant source of the Brazilian green propolis. The aim of this work was to evaluate the antileishmanial and antiplasmodial activities of *B. dracunculifolia* and its isolated compounds. The leave rinse extract of *B. dracunculifolia* (BdE) showed *in vitro* antileishmanial activity against *Leishmania donovani*, displaying an IC_{50} value of 45 µg/mL, while green propolis hydroalcoholic extract (GPE) showed an IC_{50} value of 49 µg/mL. Among the isolated compounds, ursolic acid and hautriwaic acid lactone, showed the highest antileishmanial activities, displaying IC_{50} values of 3.7 µg/mL and 7.0 µg/mL, respectively. The pentacyclic triterpene Uvaol, as well as the flavonoids acacetin and ermanin showed IC_{50} values of 15.0 µg/mL, 18.0 µg/mL and 40.0 µg/mL, respectively. Regarding the antiplasmodial assay against *Plasmodium falciparum*, BdE and GPE showed similar IC_{50} values (about 20 µg/mL). Hautriwaic acid lactone displayed moderate antiplasmodial activity, with IC_{50} values of 0.8 µg/mL (D6 clone) and 2.2 µg/mL (W2 clone). In order to compare the effect on the parasites with toxicity with mammalian cells, the cytotoxic activity of the samples were evaluated against the Vero cells, showing that all evaluated compounds exhibited no cytotoxicity in the maximum dose tested. *Acknowledgements: To FAPESP for financial support (2006/60142 – 4) and FAPESP (2007/04175 – 9, CAPES* (PDEE/BEX 0387/04 – 5) *and CNPq* (119831/2007 – 4) *for fellowships.*

PD21 *In vitro* antischistosomal activities of phenylpropanoids and lignans against *Schistosoma mansoni* adult worms

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Phenylpropanoids (isoeugenol, α-asarone, coumaric acid, ferulic acid, caffeic acid, sinapic acid) and lignans **1** and **2** obtained by oxidative coupling of caffeic and sinapic acids, respectively, were evaluated *in vitro* against *Schistosoma mansoni* adult worms. Couple adult worms of *S. mansoni* LE strain were recovered from mesenteric veins of the infected mice and cultured in 24-well plates at 37°C in RPMI 1640 media. Samples were dissolved in 10% DMSO and diluted into the medium to give 10, 25, 50 and 100 µM. Coupled adult worms were kept for 5 days and the viability, pairing, egg production, and egg development were monitored every 24 h. As negative control (NCG) was used adult worms treated with 10% DMSO. Praziquantel (PZQ, 10 µM) was used as positive control. Regarding the viability, neither phenylpropanoids nor lignans caused the death of the *S. mansoni*. Lignans **1** and **2** (10, 25, 50 and 100 µM) reduced motor activity of the adult worms and significantly decreased daily egg production. Also, **1** and **2** (50 and 100 µM) was able to separate the adult worm pairs into male and female after 120 h of incubation. All tested phenylpropanoids were inactive against *S. mansoni* adult worms, while PZQ (10 µM) caused death of worms and tegumental alterations. *Acknowledgements: To FAPESP for financial support (2006/60142 – 4) and fellowships (2008/09273 – 1; 2008/01174 – 4).*

PD22 Bioguided fractionation of the antimalarial plant *Argemone mexicana*: isolation and quantification of active compounds from effective clinical batches

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Despite major scientific advances and considerable health related efforts, malaria remains one of the world's leading killers in endemic countries, with an estimated 250 million cases every year, giving rise to an estimated 880 000 deaths, mostly among African children. For various reasons the access to safe and effective medicines such as artemisinin-based combined therapies is a major issue for a large proportion of the patients, especially those living in rural areas who use traditional medicinal plants for their primary healthcare [1]. Based on the promising clinical results of an *Argemone mexicana* L. (Papaveraceae) traditional preparation used to treat malaria in southern Mali [2], a bioguided fractionation of the decoction prepared with a clinical batch of the plant was performed in order to identify its active ingredients. Fractions were obtained by a combination of liquid-solid extraction and liquid-liquid partitions. From the active fraction, three alkaloids were isolated by semi-preparative HPLC and tested against *P. falciparum in vitro*: allocryptopine, berberine and protopine. A QNMR method was developed to quantify these three alkaloids within a mixture. The ^{1}H NMR signal of the methylene dioxide group of each alkaloid was used for integration and anthracene was used as the internal standard. Allocryptopine was found to be the most concentrated alkaloid in the traditional decoction, with an antiplasmodial IC_{50} value of 1.46 µg/ml. Qualitative and quantitative results are critically discussed in regard of the clinical efficacy of this traditional preparation. The outcome of the quantitative NMR measurements are compared to results obtained using other analytical methods. **References:** [1] Bourdy, G. et al. (2008) Int. J. Parasitol. 38:33 – 41. [2] Willcox, M.L. et al. (2007) Trans. R. Soc. Trop. Med. Hyg. 101:1190 – 1198.

PD23 Rhinacanthin production by four hairy root lines of *Rhinacanthus nasutus* (L.) Kurz

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Rhinacanthus nasutus (L.) Kurz is a medicinal plant used in Southeast Asia for treatment of several skin diseases [1]. The plant extract possessed several pharmacological activities such as antifungal and antiviral activities [2]. The naphthoquinone compounds, namely rhinacanthins, are major chemical constituents in this plant. In this study, technique of hairy roots induction by *Agrobacterium rhizogenes* was used for increasing the rhinacanthin level. Four hairy root lines of *R. nasutus*, including HR11325, HR13332, HR13333 and HR15834, were induced on the leaf explants by *A. rhizogenes* strains ATCC 11325, 13332, 13333 and 15834, respectively. Transformation percentages were 55%, 25%, 70% and 60%, respectively. The fragments of *rol*B and *rol*C genes were observed in all hairy root lines using PCR technique indicating the successful integration of the T-DNA fragment of Ri plasmid of *A. rhizogenes* to the genome of the hairy roots. Rhinacanthin production from these hairy roots was determined using HPLC technique. HR11325 produced rhinacanthin-C as a major compound together with rhinacanthin-D, and -N with the yields of 2.163, 0.042 and 0.006% w/w, respectively. In contrast, HR13332, HR13333 and HR15384 contained only rhinacanthin-C and -D. The amount of rhinacanthin-C in these three hairy roots were 0.843, 0.824, and 1.148% w/w, while those of rhinacanthin-D were 0.039, 0.012 and 0.017% w/w, respectively. *Acknowledgements: National Research Council of Thailand (NRCT), Prince of Songkla University* **References:** [1] Farnswort, N.R. et al. (1992) Thai Medicinal Plants: Plant Recommended for Primary Health Care System. Prachachon. Bangkok. [2] Wu, T.S. et al. (1998) Chem. Pharm. Bull. 46:413 – 418.

PD24 The antimicrobial activity of medicinal plants to treat sexually transmitted infections (STI's)

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There are numerous ethnobotanical reports of plants used for the treatment of sexually transmitted infections, yet few studies have been undertaken to validate the use against pathogens infecting the urogenital tract. For this study, twenty plants were assessed for antimicrobial activity against STI pathogens i. e. *Trichomonas vaginalis, Candida albicans, Oligella ureolytica, Ureaplasma urealyticum, Neisseria gonorrhoeae* and *Gardnerella vaginalis*. Plant selection was based on the ethnobotanical literature [1,2,3]. Extracts were prepared by submerging the dried macerated plant material in a mixture of methanol and dichloromethane (1:1) for 24 h. Antimicrobial activity was assessed using the micro-well minimum inhibitory concentration assay with specific alterations to facilitate fastidious growth of pathogens [4]. *Tarchonanthus camphoratus* (leaf extract) showed the most significant broad spectrum activity with MIC values ranging between 0.5 – 0.7 mg/mL against five of the six pathogens tested. Other noteworthy activity was found for *Hypoxis latifolia* showing sensitivity towards *T. vaginalis* at 0.8 mg/mL. *Tarchonanthus camphoratus* (leaf extract) showed notable sensitivity when tested against *C. albicans* (0.5 mg/mL). The highest activity noted for *N. gonorrhoeae* was for *Hypericum aethiopicum* (root) at 0.3 mg/mL. *Polygala fruticosa* and the root extract of *Hypericum aethiopicum* showed highest sensitivities towards *G. vaginalis* at 0.2 mg/mL. Efficacy of the plant extracts against the pathogen *O. ureolytica* showed MIC values below ≤0.1 mg/ml for nine plant species. The highest activity noted against *U. urealyticum* was for *Psidium guajava* at 0.8 mg/mL. This *in vitro* evaluation validates the ethnobotanical use as an anti-infective to treat sexually transmitted diseases. **References:** [1] Hutchings, A. et al. (1996) Zulu Medicinal Plants – an Inventory. University of Natal Press. Pietermaritzburg, South Africa. [2] Watt, J.M., Breyer-Brandwijk, M.G. (1962) The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd Edition, Livingstone, London, UK. [3] Neuwinger, H.D.et al. (2000) African Traditional Medicine: A Dictionary of Plant Use and Applications. Medpharm. Stuttgart, Germany. [4] NCCLS (2003) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria. 6th Edition, USA.

PD25 Antibacterial Activity of Rhinacanthin Rich *Rhinacanthus nasutus* Extract

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Rhinacanthin rich *Rhinacanthus nasutus* (RRn) extract was prepared using the method described by Panichayupakaranant et al. [1]. In a recent study we have shown that the RRn extract possessed antifungal activity against *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum* with the potency was equal to that of rhinacanthin-C, the most antifungal active constituent of *R. nasutus* leaf extract [1]. In this study, antibacterial activity of the RRn extract as well as rhinacanthin-C against *Streptococcus mutans*, *Propionibacterium acnes*, *Staphylococcus aureus* and *S. epidermidis* was evaluated by microdilution assay [2]. The RRn extract used in this study was standardized by the HPLC method [1] to contain total rhinacantin content not less than 70% w/w. It was found that the RRn extract exhibited potent antibacterial activity against *S. mutans*, and moderate antibacterial activity against *P. acnes*, *S. aureus* and *S. epidermidis* with the MIC and MBC values as shown in Table 1. The antibacterial activity of the RRn extract was almost equal to that of rhinacanthin-C.

Table 1 Antibacterial activity of rhinacanthin rich *Rhinacanthus nasutus* (RRn) extract and rhinacanthin-C

Compounds	*S. mutans*		*P. acnes*		*S. aureus*		*S. epidermidis*	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
RRn extract	4	4	16	32	16	256	8	512
Rhinacanthin-C	2	2	8	> 128	8	> 128	2	> 128
Tetracycline	1	2	0.5	> 32	0.5	> 32	0.25	> 32

Acknowledgements: Prince of Songkla University **References:** [1] Panichayupakaranant, P. et al. (2009) J. Chromatogr. Sci., in press. [2] Wiegand, I. et al. (2008) Nat. Protoc. 3:163 – 175.

PD26 Effect of the marine brown alga *Canistrocarpus cervicornis* on promastigote forms of *Leishmania (L.) amazonensis*

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Leishmaniasis is a disease resulting from infection by protozoan parasites of the genus *Leishmania*. Pentavalent antimonials, used clinically for more than 50 years, are still the first-choice drugs for the treatment of leishmaniasis, but they are toxic, require long-term treatment, and are prone to stimulate drug resistance [1]. Marine brown algae (Phaeophyceae) belonging to the order Dictyotales have emerged as an exceptionally rich source of diterpenoids, which form part of a defensive strategy against herbivores in the marine environment [2]. We have investigated the activity of crude extracts, a fraction, and an isolated compound (4*R*, 9*S*, 14*S*)-4α-Acetoxy-9β,14α-dihydroxydolast-1(15),7-diene of the brown alga *Canistrocarpus cervicornis* against promastigote forms of *Leishmania amazonensis*. The antiproliferative assays showed a dose-dependent effect against promastigotes with IC_{50} values in the range between 20.0 and 80.0 μg/mL for crude extracts, 5.0 μg/mL for the fraction and 2.0 μg/mL for the isolated compound from *C. cervicornis.* We also investigated targets in the parasite by means of electron microscopy. Ultrastructural alterations were mainly observed in the mitochondrion of parasites treated with the isolated compound. Based on the current study, compounds from *C. cervicornis* appear to be an alternative for the development of new antiparasitic chemotherapies. However, further *in vitro* and *in vivo* studies are necessary to elucidate the mechanism of action of this compound. *Acknowledgements: CNPq, FINEP, PRONEX/Fundação Araucária.* **References:** [1] Croft, S.L. et al. (2006) Indian J. Med. Res. 123:399 – 410. [2] Garcia, D.G. et al. (2009) Phytother. Res., in press.

PD27 The chemotherapeutic effectiveness of five Nigerian plants used in treating malaria

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Malaria continues to be one of the greatest health challenges in Africa especially in Nigeria. Resistance of parasites to already existing drugs is leading to unacceptable levels of therapeutic failures globally. There is a growing realization that combination therapy is vital to the optimal control of malaria in developing countries [1]. It has great advantages and latent potentials to be explored over monotherapy. WHO 2001 recommendations of the Artemisinin combination therapy (Acts') is a proven example. The present work focused on the antiplasmodial and cytotoxic effects of five plants commonly used in Nigerian folk medicine either singly (monotherapy) or combined to treat malaria. Ethyl acetate and dichloromethane extracts of two plants exhibited significant activity against chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum* and no significant toxicity against Chinese Hamster Ovarian cell lines. A combination of the extracts of two plants showed a significant enhancement of the activity. A bioassay guided fractionation using solid phase extraction and high performance liquid chromatography revealed three compounds. Two known compounds, linoleic and linolenic acid have been structurally elucidated and characterized using NMR and GC-MS spectrometry methods. These compounds exhibited a good selectivity index against the sensitive and resistant strains of the *Plasmodium falciparum* parasite. No significant *in vitro* toxicity was observed with the compounds. The extract tested *in vivo* at 800 mg/kg was not toxic. Further *in vivo* work of the most active extract and the bioavailability studies of the compounds are in progress. *Acknowledgements: Funding from the University of Cape Town, and the Medical Research Council of South Africa.* **References:** [1] Guerin, P.J. et al. (2002) Lancet Infect. Dis. 2:564 – 573.

PD28 Field study into the efficacy of a medicinal plant based complex for management of Equine Cushings Syndrome in ageing horses

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Pituitary Pars Intermedia Dysfunction (PPID), known as Equine Cushings Syndrome, is characterized by elevated plasma glucocorticoid concentration due to adenoma or hyperplasia of the pars intermedia of the pituitary gland. Clinical signs include hirsutism, hyperhidrosis, muscle atrophy, chronic infections, polyuria, polydipsia and laminitis [1]. The dopamine agonist pergolide mesylate is the conventional therapy of choice. Compliance is poor due to expense, poor prognosis and adverse events [2]. The AIM of this study was to assess effectiveness of a medicinal plant complex based on, though not exclusively, *Vitex agnus-castus* and *Gynostemma pentophyllum*, chosen for their traditional use in PPID and associated laminitis. Anti-ageing herbs, *Gingko biloba* and *Panax ginseng* are also included for mental acruity. METHODOLOGY: Owners of PPID affected horses completed a questionnaire prior to and following supplementation with a medicinal plant based complex. **Results:** Seven animals, mean age 24.71 yrs, were tested. Following one month supplementation a significant ($p < 0.05$) improvement was seen in Body condition, Coat condition and Alertness. Improvement approaching significance ($p < 0.1$) was seen in Lameness, Sweating and Thirst. CONLCUSION: PPID is a relatively common, progressive problem of ageing horses where quality of life is paramount. The plant based complex can improve quality of life for PPID affected horses, providing owners with an acceptable and economical long term management alternative to conventional drug therapy. **References:** [1] Harman, J., Ward, M. (2001) Altern. Med. Rev. 6: Suppl:S4-S16. [2] Sojka, J. (2009) Current Therapy in Equine Medicine. ed Robinson, N.E. and Sprayberry, K.M. Pub: Saunders.

PD29 *In vitro* antileishmanial activity of hydroalcoholic extract, fractions, and compounds isolated from leaves of *Piper ovatum* Vahl against *Leishmania amazonensis*

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The medicinal plant *P. ovatum* is used popularly as an anesthetic [1] and anti-inflammatory [2]. We assessed the biological activity of a crude extract, a mixture of several fractions, and a pure compound obtained from *Piper ovatum* Vahl against promastigote and amastigote forms of *Leishmania amazonensis.* This study included the extraction process and bioassay-guided fractionation by the adsorption chromatography and Sephadex LH-20 method. A progressive increase in the antileishmanial effect was observed in the course of fractionation. The 50% inhibitory concentration (IC_{50}) for dichloromethane-ethyl acetate (1:1 v/v) fraction was 2.1 μg/ml and 24 μg/ml; mixture of piperovatine: piperlongumune (2:3) 0.9 μg/ml and 24 μg/ml; piperovatine (**1**) 9.5 μg/ml and 10 μg/ml; and piperlonguminine (**2**) 2.5 μg/ml and 9.0 μg/ml, for promastigote and amastigote forms, respectively. Cytotoxicity analysis indicated that these toxic concentrations were much higher for J774G8 macrophages and Vero cells than for the protozoans. The mixture of piperovatine:piperlongumune (2:3) showed important antiprotozoal activity against the amastigote and promastigote forms of *L. amazonensis*, and it produced morphological changes in promastigotes and amastigotes at 0.9 μg/ml and 24 μg/ml (50% growth inhibition concentration), respectively, including intense cytoplasmic vacuolization, mitochondrial swelling, and mitochondrial damage, as revealed by transmission electron microscopy. *Acknowledgements The authors are grateful to CNPq for providing a research grant and fellowships.* **References:** [1] Correa, M.P. (1984) Dicionario das Plantas Uteis do Brasil e das Exoticas Cultivadas, vol. 1. Instituto Brasileiro de Desenvolvimento Florestal, Rio de Janeiro. [2] Rodrigues-Silva, D. et al. (2008) J. Ethnopharmacol. 116:569 – 573.

PD30 Inhibition of lipid peroxidation as prognostic biomarkers of wound healing

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Molecular oxygen plays a central role in the pathogenesis and therapy of wounds. Ethanol extracts of six wound healing medicinal plants (*Anthocleista nobilis* G. Don (Loganiaceae), *Entandrophragma utile* (Dawe & Sprague) Meliaceae, *Nauclea latifolia* (Rubiaceae) *Petiveria alliaceae* (Phytolaccaceae), *Treculia africana* Dec'ne (Moraceae) and *Uvaria chamae* P. Beauv. (Annonaceae) identified in an ethnobotanical survey were investigated for free radical scavenging activities and also lipid peroxidation. Free radical scavenging activity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radicals and inhibition of lipid peroxidation was accessed with thiobarbituric acid (TBA) method in a poly unsaturated fatty acid (PUFA) model of *Scomber japonicum* fish homogenate calculated as MDA equivalent/g of tissue. Total phenol and flavonoid contents were determined spectrophotometrically as gallic acid and rutin equivalents, respectively.

Plant Samples	Thiobarbituric Acid Reactive Substances (TBARS) Values (mg/tissue) in fish homogenates		Total Phenols	Total Flavonoid
	Raw	Cooked		
A. nobilis	0.009 ± 0.006	0.007 ± 0.006	10.020 ± 0.016	1.145 ± 0.015
E. utile	0.010 ± 0.001	0.005 ± 0.002	30.101 ± 0.089	17.000 ± 0.008
N. latifolia	0.008 ± 0.002	0.009 ± 0.000	10.411 ± 0.153	15.192 ± 0.006
P. alliaceae	0.005 ± 0.001	0.006 ± 0.000	3.133 ± 0.040	17.474 ± 0.005
T. africana	0.006 ± 0.002	0.004 ± 0.000	3.620 ± 0.020	0.189 ± 0.000
U.chamae	0.017 ± 0.001	0.007 ± 0.000	11.903 ± 0.030	12.233 ± 0.020

Flavonoid content correlated positively with activity. Flavonoids reduce lipid peroxidation by preventing or slowing the onset of cell necrosis and also by improving vascularity. Hence, any extract that inhibits lipid peroxidation will increase the viability of collagen fibrils by increasing the strength of collagen fibres, circulation, prevent cell damage and hasten the process of wound healing by inhibition of lipid peroxidation as prognostic biomarkers.

PD31 UPLC/TOF-MS methodology for the *on-line* identification of secondary metabolites in four *Scleria* species (Cyperaceae): *S. striatonux, S. verrucosa, S. boivinii* and *S. naumaniana*

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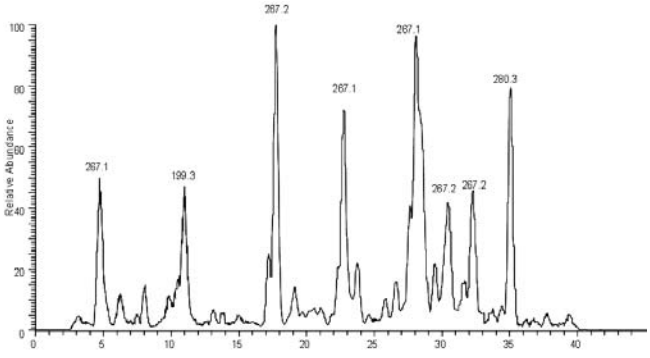
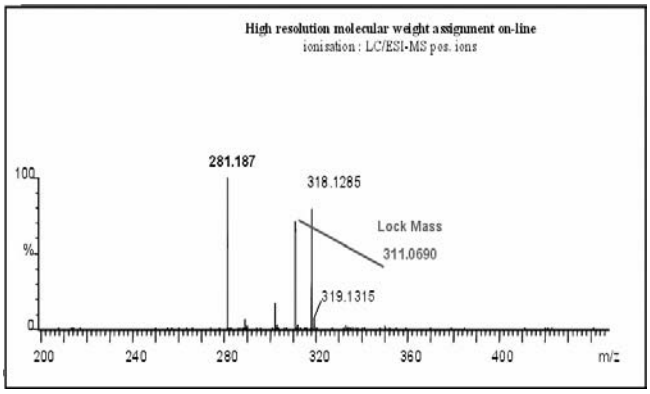
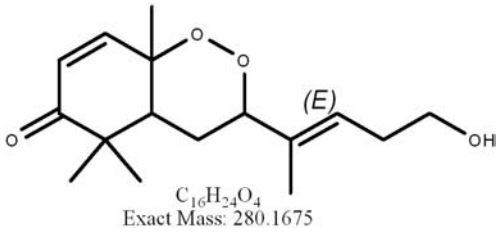
The DCM extract of the rhizome of *S.striatonux* exhibited IC_{50}/IC_{90} values of 0.664 µg/mL/ 1.043 µg/mL (chloroquine sensitive strain D6) and 0.671 µg/mL/ 1.147 µg/mL (chloroquine resistant strain W2) of *Plasmodium falciparum*. Bioassay guided fractionation afforded 4 new sesquiterpenes [1]. The activity was related to one endoperoxyl derivative. Three other species of *Scleria*, *S. bovinii, S. verrucosa and S. naumaniana* were tested for their antimalarial activity on *P. falciparum*. Results revealed activity of *Scleria boivinii* IC_{50} 4.25 µg/mL (chloroquine sensitive, NF54). Combining the separation efficiency of UPLC and high resolution of TOF-MS detector, an analytical method was optimized in order to compare the metabolite profiles of crude extracts from the four *Scleria* species and to track compounds presenting sesquiterpene base structures.







References: [1] Nyongbela, K.D. et al. (2009) Nat. Prod. Comm., in press. [2] Mve-Mba, C.E et al. (1996) J. Ess. Oil Res. 8:59 – 61.

PD32 Phytochemical and antibacterial studies of *Indigofera secundiflora*

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The genus *Indigofera* comprises about 700 species that are distributed geographically in tropical regions including Nigeria, Burkina Faso and India [1]. Various species of *Indigofera* have been used in folkloric medicine, which include antibacterial, antifungal, antisnake venom properties and for tumors in particular the decoction of the aerial parts of *Indigofera secundiflora* is used against bacterial infections, diarrhea and as a cough remedy [2]. In continuation of our phytochemical work into *Indigofera* species of Nigeria flora, the aerial parts of *Indigofera secundiflora* were investigated. The acetone extract was screened for anti-bacterial activity against *E. coli, B. subtilis, P. aeruginosa* and *S. aureus* at concentrations of 5 and 10 mg/ml. The extract inhibits all the test organisms with zones of inhibition ranging from 13 to 23 mm comparable to standard antibiotics gentamycin 10 µg/ml and ciprofloxacin 10 µg/ml. Fractionation of this extract over silica gel open column chromatography, gel filtration over Sephadex LH-20 and preparative TLC gave quercetin, quercetin3-methyl ether, quercetin 3,4-dimethyl ether and kaempferol-3-methyl ether. The structures were elucidated using NMR techniques and compared with those reported in literature [3,4]. The anti-bacterial activity of the isolated flavonoids is discussed. **References:** [1] Dalziel, J.M. (1965) The useful plants of West tropical Africa. A Crown agent for oversea publication. [2] Bakasso, S. et al. (2008)

Pakistan J. Biol. Sci. 11:1429 – 1435. [3] Mabry, T.J., Markham, K.R. (1968) Systematic Identification of Flavonoids. Springer Verlag, Berlin. [4] Agrawal, P.K (1989) Carbon-13NMR of Flavonoids. Elsevier science, Amsterdam.

PD33 Effects of STW 5 and its components on viability of Caco-2 cells

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Herbal preparations like STW 5 (Iberogast®) are widely used in treatment of dyspepsia and motility-related disorders of the gastrointestinal tract. STW 5 is a fixed combination of nine individual plant extracts, containing 15% *Iberis amara* fresh plant extract (STW 6) and showing a very good efficacy and tolerability in a large number of clinical and preclinical studies. In order to characterize the mode of action STW 5, STW 6 as well as cucurbitacines E and I, belonging to the phytochemical constituents of STW 6, were tested on the Caco-2 model to determine cell viability using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The effects were tested time-dependently, 0.5, 1, 3 and 24 hours, after substance application. Short-term incubation of cells with STW 5 (0.5, 1 h) increased cell viability highly in a concentration-dependent manner, but moderately within 3 hours. After long-term incubation (24 h) the viability was maximum stimulated by 256 µg/ml STW 5, whereas high concentrations reduced cell viability concentration-dependently. STW 6 as well as cucurbitacine I (0.01 – 100 µM) did not influence the cell viability. Cucurbitacin E (0.1 – 100 µM) had no effect after short-term incubation (0.5, 1 h) but reduced the cell viability at high concentration (50 – 100 µM) after long-term incubation (24 h). The present data indicate: (1) STW 5 is able to increase cell viability of epithelial cells in vitro, suggesting this mechanism may contribute to the protective effect against morphological changes seen in an experimental model of intestinal inflammation; (2) STW 5 did not affect the integrity of epithelial cells at concentrations of 512 µg/ml and below, not even at long-term incubation; (3) cucurbitacin E had no effect in relevant concentrations. Taken together, these results are in accordance to the well-characterized tolerability of STW 5 and in addition give information on the mechanisms of action involved in its mucosa-protective effects.

PD34 Antitrypanosomal and antileishmanial activities of organic and aqueous extracts of *Artemisia annua*

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Artemisia annua is a herbal drug with profound antimalarial activity, which is ascribed to the unique sesquiterpene lactone artemisinin. Recently, artemisinin has been reported to show efficacy against other parasitic protozoan sp., such as *Trypanosoma* and *Leishmania* [1,2], however trypanocidal and leishmanicidal effects of *A. annua* extracts have remained unstudied. In the current study, we evaluated the *in vitro* growth inhibitory activity of a number of organic and aqueous extracts of a selected high-yield Brazilian cultivar of *A. annua* against three infectious parasitic protozoa, *T. brucei rhodesiense*, *T. cruzi* and *L. donovani.* Artemisinin was also evaluated for its antiparasitic activity for comparison. Artemisinin content of these extracts (obtained by evaporation of the organic solvent or freeze-dried aqueous solutions) was determined by HPLC/DAD/MS. The hexane extract was found to be the richest in artemisinin (3.68%), whereas the toluene extract was the poorest (0.57%) [3]. Among the tested extracts, the acetone- and the *n*-hexane-solubles of *A. annua* were the most potent against *T. b. rhodesiense* with IC_{50} values of 0.30 and 0.455 µg/ml, respectively, whereas the other extracts were ten- to fifty-fold less potent. None of the extracts or artemisinin had trypanocidal activity against *T. cruzi* (IC_{50} > 30 µg/ml). Only the organic extracts of *A. annua* arrested the growth of *L. donovani* with modest IC_{50} values (5.1 to 9.0 µg/ml) comparable to that of artemisinin (IC_{50} 8.8 µg/ml). This study highlights significant variations in the artemisinin content of *A. annua* extracts and underlines the potential of *A. annua* extracts and artemisinin in the treatment of trypanosomal and leishmanial infections. Notes: Percentages are given on the dried extracts obtained by evaporation of the organic solvent or freeze-dried aqueous solutions, and do not reflect the content of artemisinin in the dried herbal drug, which is about 0.52%. **References:** [1] Mishina, Y.V. et al. (2007) Antimicrob. Agents Chemother. 51:1852 – 1854. [2] Sen, R. et al. (2007) J. Med. Microbiol. 56:1213 – 1218.

PD35 Malaria and *Artemisia annua*: Agronomic Research in Switzerland

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Artemisinin, a sesquiterpene lactone extracted from the leaves of *Artemisia annua*, is now under the aegis of WHO, the spearhead of the global fight against malaria. This molecule isolated and characterized in the early seventies, is present only in this Asteraceae and so far not synthesizable. The sudden and very strong growth in demand for artemisinin since 2005 caused a great interest in developing large-scale cultures. Security of supply and lowering the cost of production are the key objectives for this new crop. With the benefit of more than fifteen years of experience in breeding and cultivation of the species, Médiplant was in "pole position" to meet these challenges. This presentation has the objective to give an overview of the research and development activities of Mediplant with *Artemisia annua*. In the recent years, research work is mainly oriented towards the breeding for high levels of artemisinin in the leaves. The cultivars of Mediplant actually contents of 1.5% to 2.0% of artemisinin in the leaves, compared to 0.5% 15 years ago. The development of this new field crop in countries of Africa and South America, interested for producing *Artemisia annua*, allows a direct transfer of knowledge and a very instructive feedback for updating the cultivation problems and research topics.

PD36 Synergistic antiplasmodial activity of artemisinin and olive leaf decoction: the role of the constituents of the phytocomplex

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Within the framework of a larger research project [1] aiming at evaluating the possible synergistic effects in malaria treatment between artemisinins and a variety of plant extracts, the antiplasmodial properties of a dried decoction of olive leaf and its principal constituents, namely phenylpropanoids and flavonoids were tested. Both the extract and constituents were assayed alone and in combination with artemisinin against 3D7 *P. falciparum* strains. The extract was prepared by aqueous extraction of olive leaf and purified with membrane techniques according to PCT/IT2006/47783 [2] and it was characterised by HPLC/DAD as previously reported [3]. Polyphenols represented 33% of olive leaf being oleuropein the principal constituent (20%). The synergism between artemisinin and the decoction or the single constituents on the parasite growth was investigated adding artemisinin, at sublethal doses, ranging from 0.625 to 40 nM, either in the presence (or in the absence) of low concentrations of the decoction or individual compounds. The effects of artemisinin tested at different concentrations olive leaf extract (10 and 50 µg/ml) were synergistic by isobologram analysis. Oleuropein had a moderate activity but only additive effects were found in the presence of artemisinin. Among the other tested compounds verbascoside was the most active (IC50 = 63.73 µg/ml). The observed synergisms of the phytocomplex were exploited to determine the constituents responsible for the synergism in an attempt to design new and/or more effective combination therapies. *Acknowledgments: The Ente Cassa di Risparmio di Firenze and Toscana life Sciences are gratefully acknowleged for generous*

financial supports. **References:** [1] Sannella, A.R. et al. (2007) Biochem. Biophys. Res. Commun. 353:177 – 181. [2] Pizzichini, M. et al. (2007) RM2007A000109. [3] Pinelli, P. et al. (2000) J. Commodity Sci. 39:71 – 83.

PD37 Antiplasmodial activity of papaya leaf decoction and its synergistic effects in combination with artemisinin

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Within the framework of a larger research project [1] aiming at evaluating the possible synergistic effects in malaria treatment between artemisinins-one the most potent antimalarial agents- and a variety of plant extracts and isolated natural constituents, the antiplasmodial properties of a dried decoction of papaya leaf is reported. The antimalarial activity of papaya is mostly anecdotal but in literature there is a report on the efficacy of a crude aqueous extract of papaya leaf on mice infested with malaria parasite *in vivo* [2]. A decoction was prepared with 10 g of "mature" papaya leaf (leaf collected already dried in the plant) from Burundi, according to the traditional preparation described by local healers (boiling for several hours until concentration to half volume). After cooling the solution, leaves were filtered and the decoction was liophylised. The dried extract (2.6 g) was submitted the HPLC analysis to evaluate the content of polyphenols. 1.6% of total flavonoids expressed as rutin were characterised being trisaccharides of kaempferol and quercetin. The extract of papaya inhibited 3D7 *P. falciparum* growth with IC50 values of 166.0 ± 1.23 μg/ml. Isobologram analysis showed that the extract of papaya, 100 or 150 μg/ml, used in combination whith artemisinin, at sublethal doses, ranging from 0.625 to 40 nM, exert a strong sinergistic effect. The crude extract was submitted to a fractionation with Sephadex LH-20 to obtain, among others, three fractions (CPC, CPD and CPE) having strong activity and containing the polyphenol constituents. *Acknowledgments*: *The Ente Cassa di Risparmio di Firenze and Toscana Life sciences are gratefully acknowleged for generous financial supports.* **References:** [1] Sannella, A.R. et al. (2007) Biochem. Biophys. Res. Commun. 353:177 – 181. [2] Berkelar, D. (2002) Echo Tech. Notes 71:1 – 4.

PD38 Medicinal plants used against leucorrhea in several regions of bogra district, Bangladesh

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Leucorrhea denotes a thick, whitish vaginal discharge, which can result from inflammation of the vaginal mucosa or can arise due to various diseases including sexually-transmitted diseases. Leucorrhea is highly prevalent amongst the rural women of Bangladesh. They usually rely on traditional medicinal practitioners (Kavirajes), who administer various decoctions prepared from medicinal plants to treat this ailment. The objective of this study was to conduct an ethnomedicinal survey amongst the Kavirajes of several regions of Bogra district, Bangladesh to collect information on medicinal plants used to treat leucorrhea. Plant specimens as collected from the Kavirajes were identified at the Bangladesh National Herbarium. A total of 12 plant species were found to be used by the Kavirajes. These plant species (with family name and plant parts used given in parenthesis) included *Hygrophila spinosa* (Acanthaceae, whole plant), *Leea indica* (Leeaceae, leaf, seed and fruit), *Morinda citrifolia* (Rubiaceae, leaf, seed and fruit), *Punica granatum* (Lythraceae, leaf, seed and fruit), *Piper betle* (Piperaceae, leaf, stem and root), *Solanum nigrum* (Solanaceae, whole plant), *Glycosmis pentaphylla* (Rutaceae, whole plant), *Sida cordifolia* (Malvaceae, leaf, stem and root), *Cyperus rotundus* (Cyperaceae, tuber), *Phyllanthus emblica* (Euphorbiaceae, leaf, seed and fruit), *Psidium guajava* (Myrtaceae, leaf, bark and fruit), and *Aerva sanguinolenta* (Amaranthaceae, whole plant). Since most rural women are not able to afford allopathic medicinal treatment, Kavirajes play a vital role in providing the necessary health care to treat leucorrhea.

PD39 Medicinal plants used against malaria in several regions of bogra district, Bangladesh

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Malaria is widely prevalent in many countries of the world, including Bangladesh. In recent years, scientific attention has focused on traditional methods for treatment of malaria because of the emergence of drug-resistant forms of this disease. In Bangladesh, malaria is often treated by traditional medicinal practitioners (Kavirajes) who use plant decoctions to treat this disease. The objective of the present study was to collect information amongst the Kavirajes of several regions of Bogra district, Bangladesh on medicinal plants used to treat malaria. Kavirajes were interviewed and plant specimens as pointed out by them were collected and identified at the Bangladesh National Herbarium. A total of 23 plant species was found to be used by the Kavirajes for treatment. These plant species (with family name given in parenthesis) included *Daucus carota* (Apiaceae), *Momordica charantia* (Cucurbitaceae), *Mallotus paniculatus* (Euphorbiaceae), *Smilax china* (Smilacaceae), *Thevetia peruviana* (Apocynaceae), *Michelia champaca* (Magnoliaceae), *Lantana camara* (Verbenaceae), *Carissa carandas* (Apocynaceae), *Kaempferia galangal* (Zingiberaceae), *Adansonia digitata* (Bombacaceae), *Siegesbeckia orientalis* (Asteraceae), *Hyptis suaveolens* (Lamiaceae), *Drynaria quercifolia* (Polypodiaceae), *Helminthostachys zeylanica* (Ophioglossaceae), *Calotropis procera* (Apocynaceae), *Camellia sinensis* (Theaceae), *Swietenia mahagoni* (Meliaceae), *Eucalyptus globules* (Myrtaceae), *Bixa orellana* (Bixaceae), *Delonix regia* (Leguminaceae), *Tectona grandis* (Lamiaceae), *Alstonia scholaris* (Apocynaceae), and *Piper nigrum* (Piperaceae). Taken together, the plants can prove to be potentially important for isolation of components, which are active against drug-resistant forms of malaria.

PD40 Quercetin-3-rutinoside, a flavonol glycoside from *Teucrium polium* suppresses advanced glycation end products (AGEs) formation: A structural study

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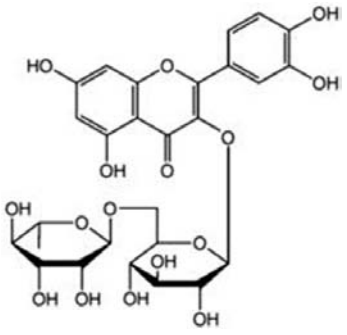
In this study, isolation and structure elucidation of the high antioxidant and antiglycation compound(s) from *Teucrium polium* were performed. Based on our results, rutin (quercetin-3-rutinoside), a flavonol glycoside isolated from *T. polium* exhibited high antioxidant activity compared to the other isolated compounds from *T. polium.*



The protein glycation inhibitory activity of rutin was also evaluated *in vivo* using various models [1, 2, 3]. In the early stage of protein glycation rutin showed a moderately inhibitory activity on HbA_{1C} formation, which were similar to that of aminoguanidine, a well-known inhibitor for advanced glycation endproducts (AGEs). For the middle stage, rutin developed more significant inhibitory effect on methylglyoxal-medicated protein modification, and in the last stage of glycation, rutin was

found to be potent inhibitor of both the AGEs formation and the subsequent cross-linking of proteins. Furthermore, the effect of rutin on preventing oxidative protein damages including effect on protein carbonyl (PCO) formation and thiol oxidation which are believed to form under the glycoxidation process was achieved. Rutin inhibited high glucose induced oxidative damages to protein by decreasing PCO formation and preventing thiols group from oxidation. In addition, the structural changes of human serum albumin with glucose, in the presence of rutin were evaluated by circular dichroism and fluorescence techniques. Regarding enhancing the helicity of the protein and prevents helix decrement in the secondary structure of human serum albumin in the presence of glucose, it can be concluded that rutin may be act as an antiglycation agent for human serum albumin. *Acknowledgements: This research work was supported by the Research Council of Shahid Beheshti University (G.C), Tehran, Iran. We also extend our thanks to Mrs. M. Ashorzadeh for her instrumental assistance.* **References:** [1]Rahbar, S. et al. (2000) Mol. Cell Biol. Res. Commun. 3:360 – 366. [2] Lee, C. et al. (1998)J. Biol. Chem. 273:25272 – 25278. [3] Nagarai, R.H. et al. (1996)J. Biol. Chem. 271:19338 – 19345.

PD41 Antioxidant capacity against peroxyl free radicals of various edible fruits from Bosnia

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The aim of this study was to determine the antioxidant capacity (AC) of various edible fruits from Bosnia. AC measurements were performed on the fruits of bilberry, cranberry, cherry and wild cherry, strawberry, black and white mulberry, black and red currant, and raspberry. The AC was determined using modified Oxygen Radical Absorbance Capacity (ORAC) assay, previously described by Cao et al. [1]. The ORAC assay is based on the propensity of the fluorescence emitted by fluorescein to be quenched when exposed to free radical action. The AC of the analysed fruits was in the following order (expressed in mmol trolox equivalents per gram fresh fruits, $mmol_{TE}/g_{f.f.}$): bilberry 12.61, cranberry 10.54, wild cherry 8.95, black currant 8.42, black mulberry 7.84, red currant 3.96, strawberry 2.91, white mulberry 2.25, cherry (1.71 for *Prunus cerasus* L. and 1.41 for *Prunus avium* L.), and raspberry 0.68. The results are in good correlation with total content of phenols (TCP) and anthocyanins in the same fruits from Bosnia reported by Toromanovic et al. [2]. In fruits with higher TCP it was found higher AC. The analysis of the AC of various fruits can be useful for the characterisation of overall antioxidant status in biological samples. **References:** [1] Cao, G. et al. (1995) Clin. Chem. 41:1738 – 1744. [2] Toromanovic, J. et al. (2008) Planta Med. 74:1181.

PD42 High performance liquid chromatographic analysis of rutin in Tarragon extracts

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Preparations from tarragon (*Artemisia dracunculus* L., Asteraceae) are widely used as prophylactics and as treatments for various diseases. The most important classes of biologically active substances present in the herbage and leaves of tarragon are the essential oil, coumarins, flavonoids and phenolic acids. Some studies of cultivated tarragon show that the herbage contains up to 4.9% of flavonoids, included quercetin, luteolin, camphorol, isorhamnetin and their glycosides. Samples of wild-growing plants were also found to have flavonoid contents varying from 0.5 to 1.9%. Objectives: In this study, using HPLC-ED system, quantitative analysis of rutin was carried out in different extracts of tarragon. Hot, cold and ultrasonic types of water extracts of tarragon leaf were prepared. The drug (1 g) was powdered and extracted with HPLC water (10 ml). Afterward 1 ml of that extract was decanted and centrifuged, obtaining supernatant which was used for further analysis. The standard solution was rutin (Merck, Germany), dissolved in isopropyl alcohol. HPLC conditions were following: Mobil phase methanol-acetonitrile-HPLC water-acetic acid (20+10+70+1); electro-chemical detector with range 50nA, potential +0.840 V, filter 0.02 Hz; flow rate 0.8 ml/min; temperature 25 °C. **Results:** Determination of rutin was based on a comparison of retention-times obtained from different extracts of tarragon by the ED detector. The highest amount of rutin was obtained with ultrasonic extraction, 6.5 mg/g. Applying cold extraction of tarragon leaves, the amount of calculated rutin was 6 mg/g and the lesser amount of rutin was obtained with hot extraction, 5.5 mg/g. Conclusion: The presence of rutin, the rhamnoglucoside of the flavonoid quercetin, give more importance to tarragon as potential medicinal plant to improve microcirculation. **References:** [1] Aglarova, A.M. et al. (2008) Pharm. Chem. J. 42:81 – 86. [2] Shahriyary, L. et al. (2007)J. Ethnopharmacol. 114:194 – 198. [3] Lopes-Lutz, D. et al. (2008) Phytochemistry 69:1732 – 1738.

PD43 Comparative analysis of total phenols and sulfur content in some plant organs of ramsons and two garlic species

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The aim of this study was to compare total phenols and sulfur content in ramsons and two garlic species, autumn- and spring-garlic. Harvesting time for ramsons was May and for garlics was June. Total phenol content was determined by the Singleton-Rossi method, which is based on phenol oxidation using Folin-Ciocalteu reagent and spectrophotometric quantification of reduced blue-colored products. For total sulfur analysis, all sulfur molecular species were oxidised to the stable sulfate form, which was quantified by ion chromatography (HPIC). The quantity of phenolic compounds (mg phenols/g fresh sample) was the highest for the leaves of autumn-garlic (1.97 mg/g), followed by leaves of spring-garlic (1.49 mg/g) and ramsons (1.28 mg/g). A lower phenol content was found in the bulbs: spring-garlic bulb (0.80 mg/g), autumn-garlic bulb (0.48 mg/g) and ramson bulb (0.46 mg/g). The highest sulfur level (mg sulfur/g fresh sample) was found in spring-garlic leaf (1.10 mg/g) while the quantity of sulfur for other samples were: ramsons bulb (0.93 mg/g), ramsons leaf (0.74 mg/g), spring-garlic bulb (0.70 mg/g), autumn-garlic leaf (0.66 mg/g) and autumn-garlic bulb (0.63 mg/g). Levels of sulfur compounds and total phenol content in the bulbs and leaves correlated with the age of the plant. Garlic leaves can be used as a significant source of organosulfur compounds for middle to late spring.

PD44 Effect of strawberry dietary supplement over IL-10 and IL-12 in TNBS model of rat ulcerative colitis

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In the last few years the study of the IBDs has shown an interaction between the immune response and the intestinal flora. In the ulcerative colitis we note a dysregulation in the immune response, and this is one of the causes of the evolution of the inflammatory process [1]. A group of molecules has been reported in the participation of this inflammatory process, such as the pro-inflammatory interleukin 12 (IL-12) and anti-inflammatory interleukin 10 (IL-10). Strawberry already has been reported as a source of some molecules groups with anti-inflammatory and antioxidant activity, such as hydrolizable tannins and flavan-3,4-diol, and others [2]. The aim of this study was to evaluate the effect of strawberry on the diet of TNBS model of rat colitis. Three groups of rats were used (n = 8); non-colitic (NC) and control groups (C) did not receive treatment, and the treated groups were given a diet with lyophilized fruit 5 g/kg of a diet with 17% of proteins. After two weeks, colitis was induced by intracolonic administration of TNBS (30 mg), and fed for one more week. Biochemical parameters (IL-10 and IL-12) were evaluated. The administration of the strawberry diet has shown an intestinal immunoregulatory activity. This diet significantly increased production of IL-10 (136 ± 19.8 versus 93. 8 ± 4 pg/g tissue; $p < 0.01$) when compared with TNBS group. But there's no alteration on IL-12 levels. *Acknowledgements: CNPQ and Capes.* **References:** [1] de la Lastra, C.A., Villegas, I. (2007) Biochem. Soc. Trans. 35:1156 – 1160. [2] Seeram, N.P. et. al. (2006) Food Chem. 97:1 – 11.

PD45 The contents of therapeutically effective compounds of cowslip (*Primula veris* L.) from various stands of Levocske Mountains in eastern Slovakia

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Cowslip (*Primula veris* L.) is a relaxing, sedative remedy, indicated in states of insomnia, tension, nervous excitability. The high saponin content accounts for its reputation in the treatment of pertussis and bronchitis, while the salicylates explain its use in the treatment of arthritic conditions. Also it is thought to be mildly diuretic and to slow blood clotting. The flavonoids are active constituens which exhibit anti-inflammatory and antispasmodic activity. They inhibit the release of histamine and act as free radical scavengers [1]. Two methoxylated dietary flavonoids which are constituents in leaves of *Primula veris* L. potentially decrease adverse biological effects, such as mutagenesis and tumor formation [2]. The saponin content in *flos* and *herba* of cowslip growing in the selected parts of Levocske Mountains in eastern Slovakia was evaluated using the densitometric method. Total composition of saponine in primrose was accounted as beta-escin by using spectrophotometric method [3]. Beta-escin in cowslip from six parts of Levocske Mountains was in range of 1.2 – 1.7 ± 0.1 %. *Aknowledgements: MS SR VEGA project 1/4365/07.* **References:** [1] Kimakova, T. (2003) Zivotne podmienky a zdravie. UK. Bratislava. [2] Tsuji, P.A., Walle, T. (2006) Carcinogenesis 27:1579 – 1585. [3] Deutches Arzneibuch (1999). [4] Foubert, K. (2008) Curr. Org. Chem. 12:629 – 642. [5] Burt, S.A., Reinders, R.D. (2003) Lett. Appl. Microbiol. 36:162 – 167.

PD46 Evaluation of dissolution rates of physical mixtures of rutin with β-cyclodextrin

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Rutin have been reported to exert numerous biochemical and pharmacological activities. Oral administration of rutin has been limited by its poor water solubility. Cyclodextrins have been recognized as potential candidates to overcome the poor solubility of rutin. Formulation with a physical mixture rather than a complex was desirable from a manufacturing viewpoint [1,2]. The aim of this study was to compare the dissolution profiles of rutin, alone or in the combination with β-cyclodextrin (β-CD). The samples used for the dissolution study were rutin alone or in the combination with β-cyclodextrin (rutin: β-CD molar ratio: 1:20, 1:4, 1:2, 1:1.5, 1:1). The inclusion complexes of rutin with β-cyclodextrin were prepared by direct mixing in dissolution vessel (*in-situ* complexation). Fixed volumes of the dissolution medium were withdrawn at 0.5, 1, 4, 8 and 14 hours. Dissolution tests were performed on the USP Apparatus 2 (Dissolution tester ERWEKA DT 800; rotating speed 100 rpm at 37 ± 0.5°C, 500 mL distilled water). Quantification of rutin in solutions was performed by UV/VIS spectrophotometric method at the absorption maximum around 258 nm. The dissolved amount of both alone or complexed rutin rapidly increases within 1 h, folloved by a slower dissolution until it reaches a plateau after about 4 h. The dissolved amounts of rutin in combination with β-cyclodextrin at the end of testing were increased in range of 11.48 to 58.69 % The *in-situ* complexation of rutin with β-cyclodextrin in all cases led to an increased dissolution rate and can be used to modify release rates of rutin in controlled-release devices. **References:** [1] Calabro, M. L. et al. (2005) J. Pharmaceut. Biomed. 36:1019 – 1027. [2] Carrier, R.L. et al. (2007) J. Control. Release 123:78 – 99.

PD47 Cysteine sulphoxides, amino acids and alliinase activity of *Allium nigrum*

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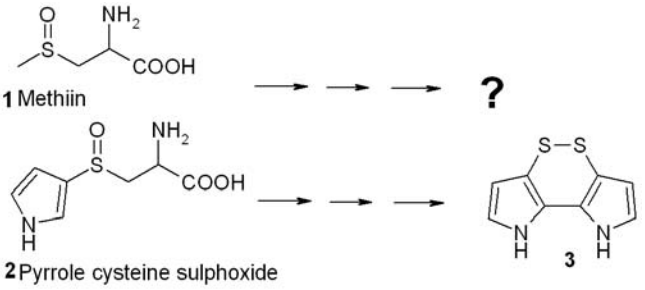
Allium nigrum L. belongs to the subgenus *Melanocrommyum* of the genus *Allium*. This subgenus is widely distributed in Southwest and Central Asia. However, *A. nigrum* is a typical plant of east Mediterranean islands. Many species of this subgenus were traditionally used. *A. nigrum* is reported to be active against helminthiasis. Typical for the genus *Allium* is a rather high content of cysteine sulphoxides. Most abundant is the cysteine sulphoxide methiin (**1**). Recently, an unusual cysteine sulphoxide containing a pyrrole ring system (**2**) could be reported [1]. Both compounds were also present in *A. nigrum*. Amino acid derivatives were analysed as corresponding *o*-phthaldialdehyde derivatives by means of HPLC-MS/MS. For methiin (**1**) and the pyrrole derivative (**2**), average concentrations of 0.04 % and 0.01 %, respectively, were found (concentrations related to the fresh weight of bulbs). If these cysteine sulphoxides would be incubated with the enzyme alliinase, a number of thiosulphinates can be expected. Enzymatic incubations were also performed for the low molecular weight extract of *A. nigrum* and resulting compounds were analyzed by HPLC-MS/MS. Interestingly, only the pyrrole derivative (**3**) could be detected. It is completely unclear in which manner methiin (**1**) reacts with the alliinase of *A. nigrum*. It can be supposed that this alliinase acts different from the well described alliinase of *A. sativum* (garlic).



Reference: [1] Jedelská, J. et al. (2008) J. Agric. Food Chem. 56:1465 – 70

PD48 Anti-allergic activity of Thai medicinal plants

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Nineteen Thai medicinal plants which were used in Thai traditional medicine preparation for treat a cold, ashma and as antipyretic drug. They are *Amomum testaceum*, *Anethum graveolens*, *Angelica dahurica*, *Angelica sinensis*, *Artemisia annua*, *Atractylodes lancea*, *Cuminum cyminum*, *Dracaena loureiri*, *Foeniculum vulgare*, *Kaempferia galanga*, *Lepidium sativum*, *Ligusticum sinense*, *Mammea siamensis*, *Mesua ferrea*, *Mimusops elengi*, *Myristica fragrans*, *Nelumbo nucifera*, *Nigella sativa* and *Syzygium aromaticum* [1]. The objective of this research is to investigate on anti-allergic activity of these plants. They were extracted by ethanol, ethanol-water and water which imitated the use in Thai traditional book [1]. These extracts were examined for antiallergic activity by determination of inhibitory activities on the release of β-hexosaminidase from RBL-3H3 cells [2]. The results were found that the ethanolic (EtOH) extract of *Mammea siamensis* exhibited the most potent anti-allergic effect against antigen-induced β-hexosaminidase release as a marker of degranulation in RBL-2H3 cells, with an IC_{50} value of 8.476 μg/ml, followed by the ethanolic extract of *Dracaena loureiri* and *Myristica fragrans* (Mace) (IC_{50}= 9.912 and 11.205 μg/ml, respectively). The water and ethanol-water extracts of all plants were apparently inactive (IC_{50} > 100 μg/ml). These results can support using Thai traditional plants for cold and asthma. **References:** [1] Foundation of resuscitate and encourage Thai Traditinal Medicine (2005) Thai Pharmaceutical Book Pikanate Printing Center Cooporation 225 – 226. [2] Tewtrakul, S., Subhadhirasakul, S. (2007) J. Ethnopharmacol. 109:535 – 538.

PD49 Primary preventive effects of Kinginka tea on metabolic syndrome (Part 2)

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Metabolic syndrome, which has been increasing rapidly, complicates lifestyle related disease such as obesity, hypertension, hyperlipidemia and diabetes. We previously developed an *in vivo* assay method to search for primary preventive substances of the metabolic syndrome, monitors the decrease of peripheral blood flow due to the onset of the metabolic syndrome in SHR/NDmcr-cp/cp (SHR/cp) rats of a model [1]. Using this method, we previously found that Kinginka tea (the buds of *Lonicera japonica* L.) may reduce the risk factors of metabolic syndrome by preventing and improving the circulatory system (peripheral blood flow and blood pressure) if consumed daily [1]. In this study, we reported active mechanisms and compounds of Kinginka tea. Kinginka tea significantly inhibited elevated serum level of lipid peroxide (LPO) and 8-hydroxydeoxyguanosine (8-OHdG), such as oxidative stress markers, 3-nitrotyrosine (3-NT) and 3-chlorotyrosyne (3-Cl), such as inflammatory markers in SHR/cp rats. The increase of 3-NT and 3-Cl are caused by the activation of the macrophage and neutrophilic leukocyte by the oxidation stress. Thus, one active mechanism of Kinginka tea may be preventive effects on vascular damage induced oxidative stress. Furthermore, by bioassay-directed fractionation of Kinginka tea, chlorogenic acid (**1**), luteolin (**2**), luteolin 7-glucoside (**3**), loganin (**4**), swerside (**5**) and secoxyloganin (**6**) were isolated. Compound **1**, a major constituent of this tea significantly improved the decrease of peripheral blood flow in SHR/cp rats. Some various bioactivities of compound **1** on lifestyle-related disease, such as antioxidant action [2,3] have been reported. However, to our knowledge, this is the first report of the primary preventive activity of compound **1** on metabolic syndrome using SHR/cp rats. **References:** [1] Oku, H. et al. (2007) Clin. Exp. Pharmacol. Physiol. 34:S40 – 42. [2] Sakamoto, W. et al. (2003) Toxicology 183:255 – 263. [3] Nakajima, Y. et al. (2007) Life Sci. 80:370 – 377.

PD50 Effects of an aqueous extract from dried mature unripe fruit of *Morinda citrifolia* (L.) and its biomarker scopoletin on acute reflux esophagitis and gastritis in rats

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The dried mature unripe noni fruit (*Morinda citrifolia* L.) has been safely used for centuries as a traditional folk medicine for the treatment of various gastrointestinal disorders in Thailand. Recently, noni fruit extract has shown anti-inflammatory [1] and antioxidative activities [2] in several *in vitro* test systems. The present study was carried out to evaluate the preventive effect of an aqueous extract from dried mature unripe noni fruit and its biomarker scopoletin on acute acid reflux esophagitis and ethanol-induced acute gastritis in rats. The potency of an aqueous fruit extract and scopoletin was compared with those of ranitidine and lansoprazole. Results indicated that aqueous fruit extracts (0.625 – 5.0 g/kg), scopoletin (0.5 mg/kg), ranitidine (50 mg/kg) and lansoprazole (1 mg/kg) significantly prevented the formation of acute acid reflux esophagitis by 80.52 – 86.65, 78.13, 87.87 and 85.71% inhibition, respectively and reduced the formation of ethanol-induced acute gastric lesions by 77.65 – 88.39, 74.94, 89.68 and 89.63% inhibition, respectively. The results indicate that an aqueous extract from dried mature unripe noni fruit and its biomarker scopoletin can effectively prevent acute reflux esophagitis and gastritis. The antiulcerogenic mechanism seemed to be closely associated with its ability to decrease gastric acid secretion and might also increase the mucosal defensive mechanism through its antioxidant properties [2,3,4,] and ability to inhibit cytokine-mediated inflammation [1,5,6]. *Acknowledgements: This work was supported by a grant from Prince of Songkla University of Thailand.* **References:** [1] Xu, J. et al. (2006) J. Vet. Intern. Med. 20:756. [2] Zin, Z.M. et al. (2002) Food Chem. 78:227 – 231. [3] Shaw, C.Y. et al. (2003) Phytother. Res. 17:823 – 825. [4] Ikeda, R. et al. (2009) Food Chem. 113:1169 – 1172. [5] Kim, H.J. et al. (2004) Fitoterapia 75:261 – 266. [6] Moon, P.D. et al. (2007) Eur. J. Pharm. 555:218 – 225.

PD51 *Ex vivo* absorption of STW 5 and some of its components using a new HPLC method

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STW 5 (Iberogast®) is a combination of nine herbal drugs. The fresh whole plant extract of STW 6, a characteristic component of STW 5, contains flavonoids, glucosinolates and low amounts of cucurbitacins. STW 5 and STW 6 as well as cucurbitacins E and I were able to affect protectively inflammatory processes in an *in-vitro* model. STW 6 (24.1 µg/ml) as well as cucurbitacin E (10 µM) increased the gene expression of the anti-inflammatory agent cytokine IL-10. As they therefore may contribute to the pharmacological properties, there it is of relevance to analyze the absorption of these cucurbitacins in the gastrointestinal tract. Therefore, an absorption chamber and a refined HPLC method for quantification of cucurbitacins were developed. For the HPLC an isocratic eluent was used to detect the two cucurbitacins into the same sample. The experiments were done with untreated and inflamed (0.01 M TNBS, 30 min) intestinal preparations from rats. The test substance was applied in the donor compartment. The concentrations of the cucurbitacins in the donor and acceptor compartments and in tissue preparations were analysed using solid phase extraction columns followed by HPLC. Fluorescein was used as negative control. Using untreated tissue preparations, no cucurbitacins were detected neither in the acceptor compartment nor in the intestinal preparation after application of commercially available cucurbitacin E and I (0.01 – 10 µM). Low amount of cucurbitacin E and I penetrated into the acceptor compartment after application of STW 5 and STW 6. Comparable results were found when the experiments were conducted with preparations preincubated with TNBS. Our results indicate that cucurbitacins from STW 5 and STW 6 do not penetrate the gastrointestinal wall under normal or inflamed conditions in relevant concentrations. Analysis of the tissue preparations focuses on the assumption that cucurbitacins might be metabolised rapidly in the intestine. Therefore further studies are needed to characterize their potential pharmacological relevance.

PD52 Impact of extraction methodology on microbiological screening of *Coptis chinensis* Franch for antimicrobial activity

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Coptis chinensis Franch (CCF) is commonly used in herbal remedies in Traditional Chinese Medicine (TCM) owing to its observed antimicrobial and anticancer effects in clinical medicine [1]. As an individual herb, CCF has exhibited potent inhibition on a number of bacteria including *Escherichia coli in vitro* conditions. The alkaloid components in CCF have been suggested to be the bioactive ingredients. The aim of our study was to investigate: 1) if there is any inhibitory effect on food and animal pathogens such as *E. coli, Listeria* and *Mycobacterium smegmatis*, 2) the efficiency of our routine extraction procedures for removal of the known maker alkaloids from CCF. *Coptis chinensis* Franch (10 g) were extracted using a successive Soxhlet procedure (a) with a series of solvents (Hexane, Dichloromethane, Methanol and water) or a successive solvent and water procedure (b) in a beaker at room temperature. Crude extracts were tested by microbiological procedure (broth or plate), for possible antimicrobial effect on the three selected microorganisms and their growth inhibition effects were compared with the conventional antibiotics. No inhibitory effect on *E. coli was* observed from extracts of Soxhlet procedure except the methanol extract showed weak effect. The hexane extract (Soxhlet) and acetone extract from procedure (b) also exhibited weak effect on *Listeria monocytogenes*. Interestingly, none of the water extracts from two procedures demonstrated inhibition on neither *E. coli* nor *Listeria monocytogenes.* However, the water extract of Soxhlet was found to partially inhibit the growth of *M. smegmatis.* These results suggest that synergistic effect of different components of *Coptis chinen-*

sis Franch may be important for its antimicrobial effect. **References:** [1] Birdsall, T.C. et al. (1997) Altern. Med. Rev. 2:94. [2] Lin, S. et al. (2006) Food Chem. Toxicol. 44:2078 – 2085.

PD53 Bioactive compounds, antimicrobial and antioxidant activities of endemic *Origanum hypericifolium* O.Schwartz & P.H. Davis in Turkey

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The chemical composition, total phenolic content, antioxidant and antimicrobial activities with oxidant status of the essential oils from Turkish endemic species, *Origanum hypericifolium*, were investigated. Steam distillation was used to isolate the essential oils, and the chemical analyses were performed by GC-MS. The antimicrobial activity was tested by agar disc diffusion method against *Morganella morganii*, *Micrococcus flavus*, *Micrococcus luteus* NRLL B-4375, *Proteus vulgaris* RSKK 96026, *Escherichia coli* ATCC 11230, *Escherichia coli* ATCC 25922, *Yersinia enterocolitica* RSKK 1501, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25933, *Staphylococcus aureus* ATCC 12598, *Staphylococcus aureus* (clinic isolate), MRSA 1 (clinic isolate), MRSA 2 (clinic isolate), MRSA 3 (clinic isolate), and MRSA 4 (clinic isolate). The major compounds found in volatiles of *O. hypericifolium* were p-cymene, carvacrol and γ-terpinene. Results showed that *O. hypericifolium* had a potential of being used in food and medicine because of its antioxidant and antibacterial activity. **Reference:** [1] Skerget, M. et al. (2005) Food Chem. 89:191 – 198.

PD54 A survey of medicinal plants used to treat cattle diseases in satkhira district, Bangladesh

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Cattle are primarily owned by small-scale farmers in Bangladesh. Most households have one or two cows, which are used either for plowing or to obtain milk. Since these farmers cannot afford to visit modern veterinarians, they rely mostly on traditional medicinal practitioners, who administer various medicinal plants for treatment of cattle ailments. We conducted an ethnobotanical survey amongst the traditional medicinal practitioners to learn more about the plants used to treat cattle diseases. All plant specimens were identified at the Bangladesh National Herbarium. Some of the medicinal plants used (with ailments treated given in parentheses) included *Triticum aestivum* (to aid cattle birth), *Coriandrum sativum* (to aid cattle birth), *Scirpus groβus* (stomach ache), *Acorus calamus* (coughs), *Smilax china* (weakness), *Piper cubeba* (whitish discharge in urine), *Phoenix sylvestris* (helminthiasis), *Allium sativum* (wounds), *Tagetes patula* (to stop bleeding), *Euphorbia tirucalli* (to increase lactation), *Mangifera indica* (to increase strength, antidote to poisoning), *Cynodon dactylon* (to stop bleeding, swelling of throat), *Crataeva religiosa* (helminthiasis), Ficus *religiosa* (tongue lesions), *Solanum tuberosum* (burns), *Syzygium aromaticum* (abscesses), *Citrus grandis* (stomach ache), *Bambusa arundinaceae* (diarrhea), *Streblus asper* (fever, coughs), *Trigonella foenum-graecum* (to increase strength, to fatten cattle), *Oxalis lobata* (stomachache), *Datura stramonium* (abscesses, fractures, sprains, pain), *Basella alba* (burns), *Trapa bispinosa* (post-delivery aid), *Cassia fistula* (bloating, dysentery, tongue lesions), *Zizyphus mauritiana* (diarrhea), *Leea macrophylla* (fractures, sprains), *Ficus hispida* (coughs, stomach disorders), *Clerodendrum viscosum* (fever), *Cuscuta reflexa* (to increase lactation), *Alternanthera sessilis* (stomach ache, fractures, dysentery, diarrhea), *Chenopodium ambrosioides* (appetite stimulant), and *Hyptis suaveolens* (skin infections, dog, snake and insect bites).

PD55 Investigation of *Valeriana officinalis* L. from Iran

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Valeriana officinalis L. is a member of the Valerianaceae family. It is a perennial plant native to Europe, North and South America as well as parts of Northern Asia. Valerian bushes reach from 1 to 1.5 m height, growing in humid woods and coasts of streams and rivers. The root and rhizome of the valerian plant is used medicinally for its sedative properties with indications including nervous tension, insomnia, anxiety and stress. Nowadays, valerian ranks at the 12th place among the top-selling herbal dietary supplements. It is cultivated in different regions of the world [1,2]. The aerial parts of *Valeriana officinalis* grown at Karaj in the north-west part of Iran were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.7% (w/w) of green yellow oil. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial at +4 °C until analysis. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [3]. Twenty six compounds were characterized in the essential oil of *Valeriana officinalis*, representing 97.11% of the oil, of which borneol acetate (18.47), valerenal (15.77%), logifolene aldehyde (13.04), β- gurjunene (9.99) and 8S,14-cedran-diol were found to be the major components. *Acknowledgements: The authors acknowledge the financial contribution from the Research and Technology Deputy of ACECR (Academic Centre for Education Culture & Research) for supporting this research.* **References:** [1] Houghton, P.J. (1998) J. Pharm. Pharmacol. 51:505 – 512. [2] Blumenthal, M. (2005) *HerbalGram* 66:63. [3] Adams, R.P. (2001) Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass spectroscopy. Allured Publishing Crop. Carol stream, IL.

PD56 Antioxidant and hepatoprotective activities of terpenoids isolated from *Salvia multicaulis* Vahl

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Salvia multicaulis is a perennial shrub belonging to family Lamiaceae [1]. Candesalvone A, candesalvone B, with two triterpenes have been isolated from the aerial parts, the structure was elucidated by means of spectral data [2]. The isolated compounds were tested for their antioxidant and hepatoprotective activities [3,4]. Almost all the compounds (at 25 – 40 μg/ml) provoked significant hepatoprotection (95%), comparable to silymarin (98%). The diterpenoids (A, B) exhibited strong antioxidant activity (92%), compared to Trolox (97%), while the triterpenoid compounds showed only negligible activity (70%). **References:** [1] Boulos, L. (2000) Flora of Egypt. 3:12. [2] Abd El-Mohsen, M. (2007) Ph D Thesis, Pharmacognosy Dept., Faculty of Pharmacy, Cairo University, Egypt . [3] Cai, Y. et al. (2004) Life Sci. 74:2157 – 2184. [4] Kiso, Y. et al (1983) J. Nat. Prod. 46:841 – 847.

PD57 Medicinal plants used against gastrointestinal tract disorders by traditional medicinal practitioners of Bangladesh

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Gastrointestinal (GI) -tract disorders like diarrhea and dysentery are endemic throughout Bangladesh because of periodic floods and the poor sanitary conditions of the predominantly rural population. Since the rural population relies mainly on traditional medicinal practitioners

(Kavirajes) for their health needs, we conducted an ethno-medicinal survey amongst the Kavirajes of Bangladesh to identify medicinal plants used as remedy for GI-tract disorders. Report on an ethno-medicinal survey in Bangladesh for plants used for GI tract disorders. 29 plants were identified (which were identified at the Bangladesh National Herbarium) but not ethno-pharmacological work was done. These plants include (with family name in parenthesis) *Polyanthes tuberosa* (Amaryllidaceae), *Spondias dulcis* (Anacardiaceae), *Daucus carota* (Apiaceae), *Tabernaemontana divaricata* (Apocynaceae), *Areca catechu* (Arecaceae), *Phoenix sylvestris* (Arecaceae), *Calendula officinalis* (Asteraceae), *Parthenium hysterophorus* (Asteraceae), *Chenopodium album* (Chenopodiaceae), *Terminalia belerica* (Combretaceae), *Terminalia chebula* (Combretaceae), *Ipomoea batatas* (Convolvulaceae), *Momordica charantia* (Cucurbitaceae), *Swertia chirata* (Gentianaceae), *Gloriosa superba* (Liliaceae), *Abelmoschus moschatus* (Malvaceae), *Nymphaea nouchali* (Nymphaeaceae), *Averrhoa carambola* (Oxalidaceae), *Cedrus deodara* (Pinaceae), *Piper nigrum* (Piperaceae), *Bambusa arundinacea* (Poaceae), *Rosa damascena* (Rosaceae), *Citrus acida* (Rutaceae), *Citrus grandis* (Rutaceae), *Feronia elephantum* (Rutaceae), *Solanum tuberosum* (Solanaceae), *Curcuma zedoaria* (Zingiberaceae), *Elettaria cardamomum* (Zingiberaceae), and *Zingiber officinale* (Zingiberaceae). Whole plant or plant parts are used to treat ailments like indigestion, flatulency, anti-helminthes, constipation, diarrhea, dysentery, piles, and fistula.

PD58 Medicinal plants used against rheumatoid arthritis by traditional medicinal practitioners of Bangladesh

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Rheumatoid arthritis is a chronic, systemic autoimmune disorder that causes the immune system to attack the joints, which can be disabling and painful. The disease has a worldwide distribution with an estimated prevalence of 1 to 2%. This prevalence increases with age and can approach 5% in women over age 55. Since this disorder is also present in Bangladesh, we conducted an ethnomedicinal survey amongst the traditional medicinal practitioners (Kavirajes) of Bangladesh to gather information on medicinal plants used by them to treat this disorder. The rural populations of Bangladesh, often lacking access to modern medicinal facilities rely on Kavirajes, who possess in them an incredible knowledge of medicinal properties of plants and often use such plants with success in treating various ailments. Plant samples were collected from the Kavirajes and identified at the Bangladesh National Herbarium. The plants mostly used to treat rheumatoid arthritis (with family name given in parenthesis) include *Cananga odorata* (Annonaceae), *Scindapsus officinalis* (Araceae), *Tylophora indica* (Asclepiadaceae), *Cleome viscose* (Capparaceae), *Mesua ferrea* (Clusiaceae), *Argyreia speciosa* (Convolvulaceae), *Thuja orientalis* (Cupressaceae), *Euphorbia antiquorum* (Euphorbiaceae), *Cinnamomum iners* (Lauraceae), *Crinum latifolium* (Liliaceae), *Michelia champaca* (Magnoliaceae), *Spathoglottis plicata* (Orchidaceae), *Piper cubeba* (Piperaceae), *Zanthoxylum simulans* (Rutaceae), *Madhuca indica* (Sapotaceae), *Stemona tuberose* (Stemonaceae), *Boehmeria nivea* (Urticaceae), and *Kaempferia galanga* (Zingiberaceae). Since modern medicine is unable to cure this disease but merely addresses the symptoms associated with the disease like pain, the above plants can be of potential importance for further scientific studies leading to complete cure of this debilitating disease.

PD59 Phytochemical analysis of *Ballota aucheri* Boiss. of Iran

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The genus *Ballota* consists of about 33 species of flowering plants in the family Lamiaceae, native to temperate regions of Europe, north Africa and western Asia, with the highest diversity in the Mediterranean region. In Iran only three species are available, *B. aucheri* Boiss., *B. nigra* L. and *B. platyloma* Rech. f., of which *B. aucheri* and *B. platyloma* are endemic plants. *Ballota* species are used in folk medicine as antiulcer, antispasmodic, diuretic, choleretic, antihemorrhiodal and sedative agents as well as for treatment of wounds, burns, cough suppression and upper respiratory inflammation [1,2,3]. The aerial parts of *B. aucheri* grown at Shiraz in the south of Iran were hydrodistilled for 4 hours, using a Clevenger-type apparatus to yield 2.6% (w/w) of yellowish color oil. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial at +4 °C until analysis. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [4]. Twelve compounds were characterized in the essential oil of *B. aucheri*, representing 96.74% of the oil, of which phytol (55.96%), cetal (16.52%) were found to be the major components. *Acknowledgements: The authors acknowledge the financial contribution from the Research and Technology Deputy of ACECR (Academic Centre for Education Culture & Research) for supporting this research.* **References:** [1] Rechinger, K.H. et al. (1982) Flora Iranian Labiatae. Akademische Druck and Verlagsanstalt, Graz, Austria. [2] Mozaffarian, V. (1996) A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran. [3] Citoglu, G. et al. (1998) Planta Med.64:484. [4] Adams, R.P. (2001) Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass spectroscopy. Allured Publishing Crop. Carol stream, IL.

PD60 Phytochemical analysis of essential oil from *Lavandula angustifolia* L. of Iran

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The genus *Lavandula* is an important member of Lamiaceae family, comprises about 20 species and over 100 varieties in the world. *Lavandula* species are widely distributed in the Mediterranean region and cultivated in different regions of the world. One of these species *Lavandula angustifolia* is the most commercially important aromatic plants. *L. angustifolia* is used in aromatherapy as a holistic relaxant and is said to have carminative, sedative, spasmolytic, antiflatulence, anticolic, antiviral and antibacterial properties [1,2,3]. The aerial parts of *L. angustifolia* grown at Shiraz in the south of Iran were hydrodistilled for 4 hours, using a Clevenger-type apparatus to yield 1.14% (w/w) of pale yellow oil. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial at +4 °C until analysis. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [4]. Thirty-eight compounds were characterized in the essential oil of *L. angustifolia*, representing 96.1% of the oil, of which 1,8-cineole (43.07%), camphor (19.98%), isobornyl formate (7.51%) were found to be the major components. *Acknowledgements: The authors acknowledge the financial contribution from the Research and Technology Deputy of ACECR (Academic Centre for Education Culture & Research) for supporting this research.* **References:** [1] Da Porto, C., Decorti, D. (2008) Planta Med. 74:182 – 187. [2] Kim, N.S., Lee, D.S. (2002) J. Chromatogr. A. 982:31 – 47. [3] Lis-Balchin, M., Hart S. (1999). Phytother. Res.13:540 – 542. [4] Adams, R.P. (2001) Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass spectroscopy. Allured Publishing Crop. Carol stream, IL.

PD61 Protective effects of ligustilide, a natural product from *Aaugellica sinensis* (Oliv.) Diels, in a rabbit model of endotoxic shock

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Tumor necrosis factor-α (TNF-α) is one of the biological mediators that play a critical role in endotoxic shock [1]. Ligustilide, isolated from the rhizome of *Aaugellica sinensis* (Oliv.) Diels, has been shown to inhibit lipopolysaccharide (LPS)-induced TNF-α production in the monocytes [2]. In this study, we investigated the effects of Ligustilide in the rabbit model of LPS-induced endotoxicity. We randomly separated 42 New Zealand rabbits into 6 groups: normal group, model group, dexamethasone group (5 mg/kg), and ligustilide groups (10 mg/kg, 20 mg/kg, and

40 mg/kg). The LPS infusion (0.3 mg/kg) was administered to the rabbits, and the abovementioned doses of dexamethasone and ligustilide were intravenously injected in the rabbits of the respective groups. The respiratory rate, heart rate, mean arterial pressure (MAP), and rectal temperature (RT) were recorded throughout the experiment. The TNF-α and IL-1β levels were measured by radioimmunoassay every 30 minutes during the first hour, and then every 60 minutes till the end of experiment. The levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), creatine kinase (CK), blood urea nitrogen (BUN), and serum creatinine (Scr) were measured at 0, 120, and 300 minutes. *The administration of LPS caused a significant increase in the serum levels of* TNF-α, IL-1, ALT, AST, ALP, GGT, LDH, CK, BUN, and Scr *and a marked decrease in MAP and RT.* Treatment with ligustilide (20 mg/kg and 40 mg/kg) significantly attenuated the reduction in MAP and RT, suppressed the release of the proinflammatory cytokines (TNF-α, IL-1), and decreased the levels of the above mentioned markers of organ injury. These results show that ligustilide affords protection against LPS-induced shock in rabbits. **References:** [1] Karima, R. et al. (1999) Mol. Med. Today 5:123 – 132. [2] Liu, L. et al. (2005) Planta Med. 71:808 – 813.

PD62 Medicinal preparations on the basis of vegetable phenolic compounds

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Azotemic preparation Flaronin was produced on the basis of individual flavonoid glycoside – robinin which is kaempferol 3-O-β-robinobiosyl-7-O-α-L-rhamnopyranoside isolated from *Astragalus falcatus.* Flaronin stimulates the nitrogen releasing function of kidneys, reduces the amount of residual nitrogen, urea and creatinine in blood, and promotes diuresis. Flaronin is successfully used for treatment of nephratonia complicated with pyelonephritis and other nephritic diseases [1]. The leaves of *Pueraria hirsute* are proposed as another vegetable raw material for production of Flaronin [2]. The phenolic compounds of the Caucasian endemic *Rhododendron ungernii,* consisting of flavonoids, catechins and leucoanthocyanins, completely inhibited the herpes virus in experiment. From the pure sum of phenolic compounds, preparation Rhodopes was produced in the form of ointment. The clinical examinations showed high therapeutic efficiency of Rhodopes. The preparation is recommended for treatment of all types of herpes simplex and chickenpox, herpes zoster, genital herpes, primary and recurrent herpetic stomatitis etc. [3]. From *Satureja hortensis* hypoglycemic preparation Saturin was produced. It consists of flavonoids and phenylpropanoids, mainly of luteolin glycosides and rosmarinic acid. Saturin reliably decreases the blood sugar. It is used as a food supplement in mild and medium cases of non-insulin-dependent diabetes independently or in combination with appropriate antidiabetes medication [4]. **References:** [1] Alania, M. et al. (2002) Flavonoids of some species *Astragalus* L. from Georgia. Tbilisi, Mecniereba. [2] Kemertelidze, E. et al. (2008) Chem. Farm.J. 6(42):28 – 31. [3] Kemertelidze, E. et al. (2007) Chem. Farm.J. 1(41):10 – 13. [4] Kemertelidze, E. et al. (2004) Chem. Farm.J. 6(38):33 – 35.

PD63 Investigation of anti-inflammatory and antinociceptive activities of *Hymenocardia acida*

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Hymenocardia acida Tul. (Hymenocardiaceae) locally called Orupa, is a small tree shrub about 6 m high, gnarled and twisted with characteristic rough rusty-red bark [1]. In Nigeria, the leaf is traditionally used for the treatment of inflammation, including arthritis, rheumatic pain and toothache. In this study, the potential anti-inflammatory and antinociceptive activities of the aqueous leaf extract of this plant was evaluated in animal models. The extract (50, 100 and 200 mg/kg) significantly ($P < 0.05$) and dose dependently inhibited carrageenan and egg albumin- induced rat paw oedema compared with control group. At three hours post-carrageenan, the highest dose of the extract 200 mg/kg inhibited the oedema by 66.67%, while indomethacin (10 mg/kg, p.o), used in this assay as a reference, gave an inhibition of 72.22%. The inhibitory activity shown by the aqueous leaf extract of *H. acida* over a period of 3 h in egg albumin induced paw inflammation was quite similar to that exhibited by the group treated with cyproheptadine (10 mg/kg) used as reference drug in this model. The extract elicited a significant analgesic activity in tail immersion test as evidenced by increase in latency time in seconds as compared with the control at the end of 20 minutes. In the acetic acid induced writhing model, the extract showed a dose-dependent reduction in the number of writhes at 50, 100 and 200 mg/kg when compared to the control group. The 200 mg/kg dose gave a complete protective effect, as no abdominal constriction was observed. The results provide some justification for the folkloric uses of *H. acida* as a remedy for relieving pain and inflammation. **Reference:** [1] Burkill, H.M., (1994) Useful plants of West Tropical Africa. Vol. 2. Families E-I. Royal Botanical Gardens, Kew.

PD64 Medicinal plants used against tuberculosis by traditional medicinal practitioners of Bogra district, Bangladesh

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Tuberculosis is an age-old contagious disease, which often leads to fatality if not treated properly. Recently, there has been increasing concerns because the organism causing this disease has become multi-drug resistant. As a result, searches are underway throughout the world for discovery of novel compounds, which can be used successfully to treat multi-drug resistant tuberculosis. Since this disease is prevalent in Bangladesh and is often treated with herbal medicines by the traditional medicinal practitioners (Kavirajes), we undertook an ethnomedicinal survey of Kavirajes in Bogra district, Bangladesh to gather information on medicinal plants used to treat this disease. Plants were collected from the Kavirajes and identified at the Bangladesh National Herbarium. The collected information indicates that the following plants (with family name in parenthesis) are used to treat tuberculosis: *Adhatoda vasica* (Acanthaceae), *Andrographis paniculata* (Acanthaceae), *Centella asiatica* (Apiaceae), *Catharanthus roseus* (Apocynaceae), *Holarrhena antidysenterica* (Apocynaceae), *Colocasia esculenta* (Araceae), *Pistia stratiotes* (Araceae), *Aloe vera* (Asphodelaceae), *Calendula officinalis* (Asteraceae), *Shorea robusta* (Dipterocarpaceae), *Ricinus communis* (Euphorbiaceae), *Swertia chirata* (Gentianaceae), *Ocimum sanctum* (Lamiaceae), *Allium sativum* (Liliaceae), *Hibiscus rosa sinensis* (Malvaceae), *Swietenia mahagoni* (Meliaceae), *Tinospora cordifolia* (Menispermaceae), *Eucalyptus globules* (Myrtaceae), *Piper longum* (Piperaceae), *Cymbopogon citratus* (Poaceae), *Zizyphus mauritiana* (Rhamnaceae), *Morinda citrifolia* (Rubiaceae), and *Vitis vinifera* (Vitaceae). The anti-tubercular effect of *Adhatoda vasica* (mediated through chemical components of the plant like vasicine and vasicinone, and their semi-synthetic derivatives like bromhexine and ambroxol) has already been reported. It is important that modern scientific studies be conducted on other plants towards isolation and identification of compounds through which multi-drug resistant tuberculosis can be effectively treated.

PD65 A survey of medicinal plants used to treat diabetes mellitus in two northern districts of Bangladesh

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Diabetes mellitus (DM), a disease characterized by abnormalities in insulin secretion and consequent hyperglycemia, affects millions of people world-wide. It has been estimated that 3.8% of the rural population and

a larger percentage of the urban population of Bangladesh suffers from this disease, which over the years can lead to hypertension, cardiovascular disorders and diabetic nephropathy, to mention only a few. Modern allopathic medicine has no known cure for DM. On the other hand, traditional medicinal practitioners (TMPs) are known in Bangladesh to treat DM with concoctions made from medicinal plants. It is also claimed by the TMPs that their treatment can completely cure DM. We accordingly conducted an ethnomedicinal survey of TMPs in two northern districts of Bangladesh, namely Dinajpur and Panchagar to find out about medicinal plants used by them to treat DM. Interviews were conducted with the help of a semi-structured questionnaire and plant specimens as pointed out by TMPs were collected and identified at the Bangladesh National Herbarium. The names of 14 plant species were obtained. These plant species (with family name given in parenthesis) included *Catharanthus roseus* (Apocynaceae), *Coccinia grandis* (Cucurbitaceae), *Psidium guajava* (Myrtaceae), *Cassia occidentalis* (Fabaceae), *Fragaria vesca* (Rosaceae), *Coccinia cordifolia* (Cucurbitaceae), *Murraya koenigii* (Rutaceae), *Aegle marmelos* (Rutaceae), *Abroma augusta* (Sterculiaceae), *Berberis asiatica* (Berberidaceae), *Cryphaea glomerata* (Cryphaeaceae), *Saccharum spontaneum* (Gramineae), *Hyptis suaveolens* (Lamiaceae), and *Tinospora cordifolia* (Menispermaceae). Plants like *Catharanthus roseus*, *Psidium guajava*, and *Coccinia cordifolia* have already been reported in scientific studies to have considerable hypoglycemic potential. It is expected that more studies on the other plants can lead to identification of novel compounds to treat DM.

PD66

A survey of medicinal plants used to treat rheumatoid arthritis in two northern districts of Bangladesh

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Rheumatoid arthritis (RA) is a debilitating disease leading to chronic pain, disability and often joint destruction, deformity and loss of function. The estimated prevalence of the disease is 1 – 2% worldwide and approaches 5% in women over age 55. Modern medicines can only alleviate some of the symptoms associated with the disease but cannot cure the disease. On the other hand, alternative medicine practitioners (AMPs) of various countries claim to cure this diseases with formulations prepared from a single or a combination of medicinal plants. RA is widely prevalent in Bangladesh and is present in both rural as well as urban segments of the country's population. The objective of the present study was to conduct an ethnomedicinal survey amongst the AMPs of two northern districts of Bangladesh, namely Dinajpur and Panchagarh to collect information on medicinal plants used for treatment of RA. Interviews with AMPs were done with the help of a semi-structured questionnaire and plant species pointed out by them were collected and identified at the Bangladesh National Herbarium. A total of 17 plant species were found to be used by the AMPs to treat RA. These species included (with family name given in parenthesis) *Aerva sanguinolenta* (Amaranthaceae), *Crinum asiaticum* (Amaryllidaceae), *Hemidesmus indicus* (Apocynaceae), *Scindapsus officinalis* (Araceae), *Carica papaya* (Caricaceae), *Gloriosa superba* (Colchicaceae), *Costus speciosus* (Costaceae), *Erythrina variegata* (Fabaceae), *Tinospora crispa* (Menispermaceae), *Mimosa pudica* (Mimosaceae), *Piper chaba* (Piperaceae), *Piper cubeba* (Piperaceae), *Bacopa monnieri* (Scrophulariaceae), *Smilax china* (Smilacaceae), *Smilax macrophylla* (Smilacaceae), *Leea macrophylla* (Vitaceae), and *Zingiber officinale* (Zingiberaceae). Due to loss of natural habitat, a number of the above plants are rapidly becoming endangered. It is imperative to conduct scientific studies on these plants towards finding a better cure for RA.

PD67

Stilbenoids constituents in *Welwitshia mirabilis* 2

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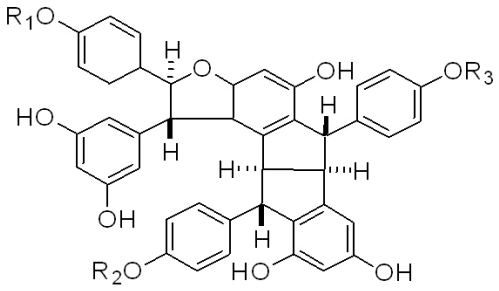
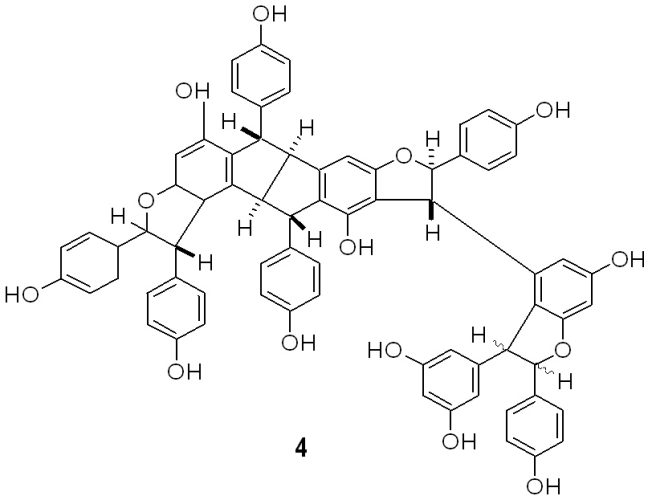
Welwitschia mirabilis is an endangered and unique gymnosperm of Namib Desert of South West Africa. It is a monotypic member of the Genus Wellwitschia. In our previous work we have reported the isolated and identification of sevaral stilbenoids from a cultivated *W. Mirabilis*. In the present study we report the isolation and structure elucidation of five new stilbene derivatives including the first incidence of a stilbene pentamer from the family. The structures of the new compounds were assigned by spectroscopic analysis. The apoptotic activities of the stilbenoids were also investigated.[1]



1: $R_1 = R_2 = R_3 = OH$ (Gnetin I)
2: $R_1 = R_2 = R_3 = Glc$
3: $R_1 = R_3 = H$, $R_2 = Glc$



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References: [1] Iliya, I. et al. (2006) Biol. Pharm. Bull. 29(7):1490 – 1492.

PD68 Cytotoxic and antibacterial activity of Laevifonol from the Stem Bark of *Vatica odorata*

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Laevifonol, a dimerstilbene from *Vatica odorata* was isolated for the second time from *Vatica* sp. This compound is a unique oligostilbene formed from a condensation between e-viniferin [1] and ascorbic acid, and was firstly isolated from *Shorea laevifonia* [2] and recently from *Vatica umbonata* [3]. In this work the structure of laevifonol was established on the basis of its spectral data, including UV, IR and NMR spectra and also in comparison with the previously reported data. Cytotoxic properties of laevifonol were evaluated against murine leukimia P-388 cells and *Artemia salina*. The results showed that laevifonol moderately inhibit P-388 cell line with IC_{50} value of IC_{50} and appeared inactive towards *Artemia salina* (IC_{50} > 796.2 μM). Antibacterial activity of this dimerstilbene was screened against two gram positive bacteria (Bacillus *subtilis* and *Staphylococcus aureus*) and one gram negative bacteria (*E.coli*). The antibacterial testing was carried out by using the disc diffusion method. Blank disc of 6 mm diameter were loaded with 1000 μg/ml of the laevifonol and applied to the inoculate plate. The compound showed moderate activity against all the bacteria with inhibition zones of 0.5 cm against *E.coli* and *Bacillus subtilis* and 0.1 cm against *Staphylococcus aureus* compared to positive control (erythromycin 60 μg). The present investigation is apart of our ongoing studies on the oligostilbenoids of Malaysian Dipterocarapcaeae in which no phytochemical data was recorded on *Vatica odorata*. **References:** [1] Sotheeswaran, S. and Pasupathy, V. (1993) Phytochemistry 32:1083 – 1092. [2] Hirano, Y. et al. (2001) J. Wood Sci. 47:308 – 312. [3] Atun, S. et al. (2005) Biochem. Syst. Ecol. 32:1051 – 1053.

PD69 Chemical constituents and *in vitro* antistaphylococcal activities of endemic *Salvia cedronella* and *S. fruticosa* naturally distributed in Denizli (Turkey)

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The genus *Salvia* (sage) is an important genus of the Lamiaceae family and comprises about 900 species, widespread throughout the world. Some members of this genus are also cultivated to use as flavouring agents in perfumery, cosmetics as well as food. There are about 90 species of Salvia in the Turkish flora, of which 45 are endemic [1]. The species of *Salvia*, known as "adacayi" in Anatolia, are used as antiseptics, stimulants, diuretics and for wound healing in Turkish folk medicine and for herbal teas and food flavoring [2]. The essential oils isolated from *S. cedronella* and *S. fruticosa* were determined by GC/MS and 32 and 27 constituents were identified, respectively. The results show that major constituents of *S. cedronella* and *S. fruticosa* oils were α-pinene (16.1 and 18.9%), eucalyptol (15.3 and 20.1%), camphor (6.6 and 10.0%), α-thujene (8.7 and 6.8%) and borneol (5.2 and 8.3%), respectively. *In vitro* antibacterial activities of crude extracts were tested against *Staphylococcus aureus* ATCC 25923 and *Cowan liyofili* by broth microdilution method. *S. aureus* was found more sensitive microorganism than *C. liyofili* to essential oil of *S. cedronella* and *S. fruticosa* (having MIC values from 80 to 120 μg/ml). In conclusion, the results indicate that the oils of *S. cedronella* and *S. fruticosa* have the capacity to inhibit the growth of pathogenic microorganisms. Therefore they could be suitable for using as antimicrobial agents in the food industry. **References:** [1] Guneş, A. et al. (2000) Flora of Turkey and the East Aegean Islands (Vol. 11). [2] Newall, C.A. et al. (1996) Herbal Medicines. A Guide for Health Care Professionals. London: The Pharmaceutical Press.

PD70 Anthraquinone glycosides from *Cassia roxburghii* and antioxidant activity of its extract

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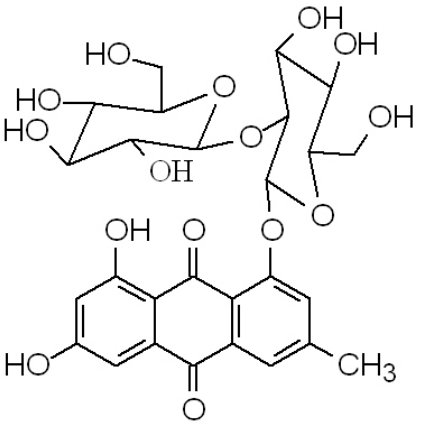
The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs [1]. The present study deals with the isolation and identification of anthraquinones from *Cassia roxburghii* leaves and evaluation of antioxidant activity of the extract. The aqueous alcoholic extract (MeOH:H_2O 7:3) of *Cassia roxburghii* leaves was subjected to extensive repeated column chromatography on polyamide, and Sephadex LH-20 resulted in two new anthraquinone glycosides named emodin 1-*O*-β- glucoside (2→1) glucopyranoside (**1**) and aloemodin 8-*O*-β- glucoside (6→1) glucopyranoside (**2**) as well as aloemodin 8-*O*-β-glucoside, emodin and aloemodin. Structures of the isolated compounds were established by chromatography, UV, HRESI-MS and 1D/2D $^1H/^{13}C$ NMR spectroscopy.



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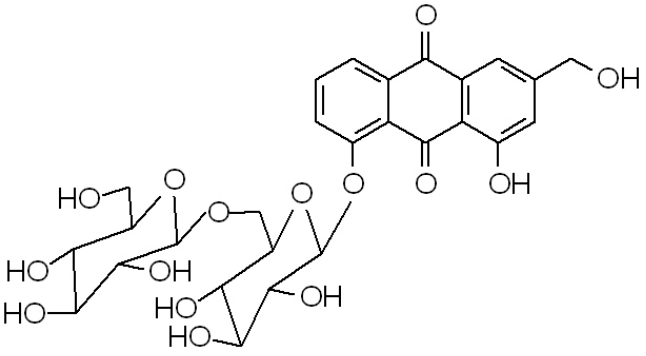

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The radical scavenging activity of the extract was quantified spectrophotometrically, using DPPH radical. The effective dose 50 (ED50) of the extract was compared with that of standard antioxidants as vitamin C. The free radical scavenging properties were assayed with complementary methods, as electron paramagnetic resonance (EPR) spectroscopy and other UV-visible absorbance based assays. **References:** [1] Farnsworth, N.R., Bingel, A.S. (1997) New natural products and plant drugs with pharmacological, biological, or therapeutic activity, Springer, New York.

PD71 Ethno medicinal approach to drug development: present status and future prospects

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The genera *Homalomena* belongs to the family Araceae of the group Monocotyledon. There are about 140 spices in tropical Asia and South America; two species in India; one in Mizoram, i.e., *Homalomena aromatica* Schott. The plant is very popular among the *Mizo*-tribal communities. The boiled petiole is used as vegetable, rhizome as aromatic stimulant, powdered rhizome as gun-powder, burnt smoke of rhizome as mosquito repellant and infusion of the plant for easy labor. The juice of whole plant is used as lotion in skin diseases. Besides these, the plant contains strong antimicrobial activity. The minimum cidal concentration (MCC) of the oil against some common human pathogenic fungi was found to be 1.2 to 1.8 µl/ml, which contains heavy inoculums density. The oils toxicity persists up to 80°C and also autoclavable, with a broad fungi toxic spectrum. The pure oil kills the test pathogenic fungi just within a minute; however, its MCC takes 5.30 to 6.30 hrs to kill all the test fungi. Besides this, while comparing the MECs of the oil with some synthetic antifungal drugs, the MECs of the oil were found to be more active than MECs of Dactrine, Nizaral and Tenaderm. Further, during pre-clinical investigations, the efficacy of oil contains 60 – 80% cure of the skin diseases. Based on these findings as well as after detailed *in vitro, in vivo*, clinical as well as multi-central clinical investigations, formulations can be transferred to the pharmaceutical companies. *Acknowledgements: 1. All India Institute of Medical Sciences, Microbiology Div. New Delhi, Dr. Uma Banerjee. 2. MLN Medical College, Allahabad, Dermatology Department, Prof. A.K. Bajaj.* **References:** [1] Shahi, S.K. et al. (1997) Proc. of the 4th Int. Symposium on Diagnosis and Identification of Plant Pathogens, (Eds. H.W. Dehne et al). Kluwer Academic Publishers, Netherlands. Shahi, S.K. et al. (2000) Skin Pharmacol. Appl. 13:60 – 64.

PD72 Free radical scavenging and antibacterial activities of medicinal plants used in Eastern Botswana

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Roots and leaves of *Ozoroa paniculosa* are being extensively used to treat a number of diseases including hypertension, asthma and backache [1,2,3]. Powdered root decoctions of DMT1* are used by rural dwellers in Eastern Botswana to alleviate painful menstruations, treat vaginal superficial infections, penile sores (lesions), gastrointestinal infections and diabetes mellitus related neuropathy. DMT1 root powder in petroleum jelly is known to alleviate chronic chest pains and asthma. This study is part of an ongoing project to search for health benefiting agents from natural sources. **Methods:** The free radical scavenging potency of the methanolic and dichloromethane extracts of DMT1 and water extract of *Ozoroa paniculosa* leaves were evaluated using the DPPH (Diphenypicrylhydrazyl) free radical scavenging assay. The antibacterial activities of the extracts were assessed against five Gram positive and four Gram negative typed cultures (WARD'S) of bacteria using the Agar Well Diffusion assay. **Results:** At 25 µg/ml the scavenging potencies of the extracts were as follows: DMT1 methanolic (89%), *O. paniculosa* leaves water (73%), DMT1 organic extract (56%). The scavenging powers of polar extracts of DMT1 and *O. paniculosa* were comparable to controls ascorbic acid and epicatechin (89 and 90%), respectively. None of the tested plant extracts showed any antibacterial activity. **Conclusions:** The results of this study suggest that the presence of antioxidant compounds can account for their health benefiting properties as advocated in traditional medicine. **DMT1: Voucher specimen code for the studied plant) Acknowledgements: Traditional Healers for supplying the plants.* **References:** [1] Motlhanka, D.M. et al. (2008) Planta Med. 74:928. [2] Motlhanka, D.M. (2008) Pakistan J. Biol. Sci. 11:805 – 808. [3] Motlhanka, D.M. et al. (2005) J. Pharmacol. 57:S7.

PD73 Bioactive constituents from *Bergia suffruticosa*

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In vitro antibacterial investigation of the various extracts of *Bergia suffruticosa* leaf belonging to the family Elatinaceae: a plant used in Sudanese folk medicine to treat skin wounds [1] was evaluated against 72 strains of standard and clinical isolates of Gram positive and Gram negative bacteria. Six known compounds were isolated from methanolic extract, which was the most active fraction. The isolated compounds were Gallic acid methyl ester; Daucosterol; 1,2,3,6-Tetra-O-galloyl - β-glucose; 1,2,3,4,6-Penta-O-galloyl- β-glucose; Kaemferol-3-O-rhamnoside and Quercetin-3-O-rhamnoside. Their identification were based on their spectroscopic data (UV, IR, 1H & 13CNMR and MS). Gallic acid methyl ester was found to have MIC 25 µg/ml against *Staphylococcus aureus* and *Escherichia coli*, whereas 1,2,3,6-Tetra-O-galloyl - β-glucose and 1,2,3,4,6-Penta-O-galloyl- β-glucose were found to be 50 µg/ml against *S. aureus* and 100 µg/ml against *E. coli*, the results suggested that the antibacterial effect of these two compounds is due to the presence of galloyl group. The MIC of other three compounds displayed no antibacterial activity against both organisms at 200 µg/ml. Ampicillin and Gentamicin were used as reference antibacterial activity. In an early study, conducted the antibacterial activity of *B. suffruticosa* whole plant reported that, its methanolic extract showed significant inhibition of the four tested micro-organisms [2]. There is no phytochemical report encountered on the plant species undertaken in this study. This result justifies the traditional therapeutic use of the plant. **References:** [1] El Ghazali, G. et al. (1997) Medicinal Plants of Northern Kordofan. Sudan. [2] Farouk, A. et al. (1983) Fitoterapia 54:103.

PD74 Garlic Biochemistry in Mushrooms

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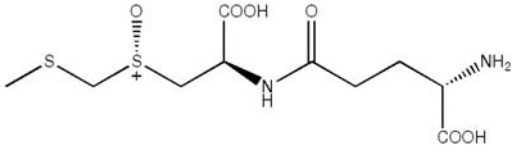
The genus of onions (*Allium*) has a diverse pattern of distinct sulphur compounds, which are responsible for the remarkable aroma profiles of garlic (*A. sativum* L.) and related species. Cysteine sulphoxides of these plants are converted by the enzyme alliinase into the corresponding thiosulphinates, *e.g.*, the thiosulphinate allicin is produced from alliin. Besides onions, also some mushrooms of the class of *Basidiomycetes* exhibit a strong garlic-like smell and taste. Remarkable are the shiitake mushroom [*Lentinus edodes* (Berkeley) Pegler] and the garlic parachute *Marasmius alliaceus* Jacq. Fr.. The cysteine sulphoxide lentinic acid could be already isolated from *Lentinus edodes* [1]. Similar compounds were assumed for the genus *Marasmius*. In the actual investigation, *Marasmius alliaceus* was screened by HPLC-MS/MS for possible atypical cysteine sulphoxides. At least one new sulphur containing compound could be identified. Structure elucidation showed that this substance is a new cysteine sulphoxide with a methylthiomethyl moitey and a second amino acid, glutamate (Figure). In earlier investigations, alliinase of *Allium* species show no reaction with substrates of *Marasmius alliaceus*. Consequently, alliinase of *Marasmius alliaceus* must have unique kinetic properties, which enables the observed rapid cleavage into volatile sulphur compounds. These will be subjects of the ongoing research.



(*S*)-2-amino-5-((*R*)-1-carboxy-2-((*R*)-methylthiomethylsulfinyl)ethylamino)-5-oxopentanoic acid

References: [1] Yasumoto, K., Iwani, K. (1987) Meth. Enzymol. 143:434 – 439.

PD75 Chemical and Biological Evaluation of *Myoporum laetum*

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Myoporum laetum (Myoporaceae) is an evergreen ornamental shrub. Fractionation and isolation of the butanol extract yielded five major flavonoids, luteolin 4-O-rhamnosid, 5-methoxy-lutelin 7-O-arabinoside, 5'-hydroxy-luteolin 7-O-glucoside, luteolin and apigenin. Their structures were determined by spectroscopic methods. The hepatoprotective and antioxidant activities of the butanol extract against liver injury induced by repeated doses of the hepatotoxicant, profenofos, were investigated. Hepatotoxicity of liver tissues was indicated by abnormal liver functions as shown by elevated levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin with concomitant decrease in albumin level in relation to normal group. The pesticide induced oxidative hepatopathy ensured by a pronounced decrease in the activities of hepatic antioxidant enzymes namely, catalase (CAT), glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G-6-PDH) accompanied by an increase in the oxidative stress marker, malonaldehyde (MDA, index of lipid peroxidation) versus normal ones. Oral supplementation of butanol extract to profenofos treated animals successfully modulated the hepatotoxicant induces deviation in the liver function markers, liver oxidative and antioxidant markers, indicating its potential hepatoprotective and antioxidant abilities.

PD76 Bioactive phenolic compound from *Hymenocrater calycinus*

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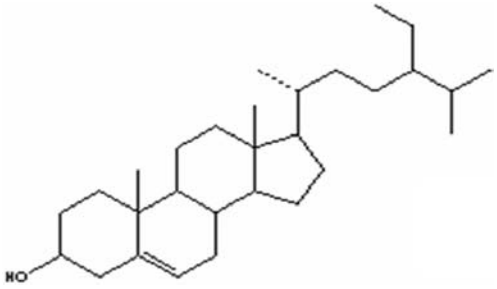
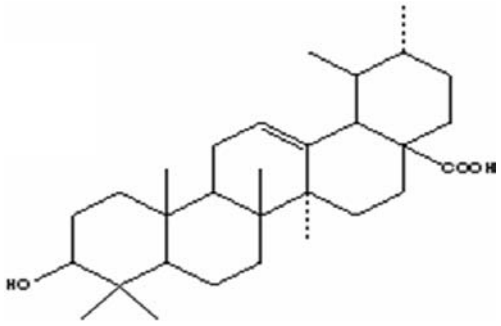
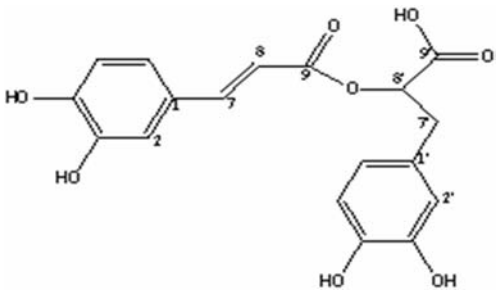
Four compounds were isolated from ethyl acetate and methanol extracts of the flowered aerial parts of *Hymenocrater calycinus* (Lamiaceae) using chromatographic methods and identified by spectroscopic data (MS, ^{1}H- and ^{13}C-NMR, HMQC, HMBC and ^{1}H-^{1}H COSY). Antifungal and antibacterial effects of rosmarinic acid, the main component, were determined against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* within the broth dilution method. Isolated compounds were identified as β-sitosterol (1), ursolic acid (2), rosmarinic acid (3) and quercetin 3 – 0- rutinoside (4) for the first time in *Hymenocrater* genus. The results of our assay against bacteria and fungi represent that rosmarinic acid has an antifungal property against *Candida albicans* (MIC, 250 μg/ml).



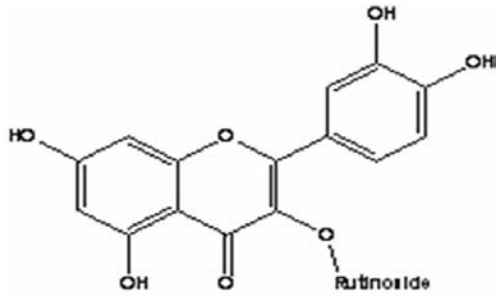
β-Sistosterol



Ursolic acid



Rosmarinic acid



Rutin

PD77 Response of *Grindelia camporum* Greene vegetative growth, flowering and resin content to the growing media variation

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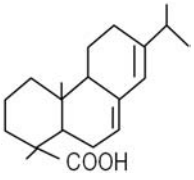
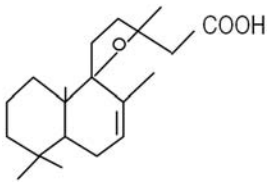
Gum weed or gum plant (*Grindelia camporum* Greene, family Asteraceae) is a conspicuously resinous, herbaceous perennial medicinal plant native to the arid regions [1]. Flowers were used clinically for the treatment of asthma, bronchitis, and poison ivy rash [2]. Its resin content is similar to the resin acid (abietic acid) that constitutes rosin (pine resin) and would have the same uses as a principal product in industry. Furthermore, gum weed is an arid adapted plant that grows well under harsh desert condition in low level of irrigation, thus it appears to satisfy the requirements established for new crops in arid environment [3].



A- Abietic acid



B-Grindelic acid

Series groups of arranged pots were separately filled by equal and homogeneous quantities from the growing media separately or in combinations as follow: Loam soil – Loam: sand at rate of 1:1 -Loam: sand: Beat moss at rate of 1:1:1 – Sand. The loam soil seems to be the best medium for producing the tallest plants and the heaviest weight of herb

and the biggest flower numbers. Further, the highest values from crude resin % (CR%), resin acid number (AN), resin acid (RA%) and crude resin gm/plant were achieved in almost cases from leaves and flowers of plants grown in sand soil as well as combination between loam and sand soil, respectively. **References:** [1] Mahmoud, S. (2002) Acta Hort. 576. [2] Lust, J. (1974) The Herb Book. Bantam books, Inc. New York, USA. [3] Timmermann, B.N. et al. (1987) Biochem. Syst. Ecol. 15:401 – 410.

PD78 Antioxidant activity of *Geranium robertianum* concentrated extracts by ultrafiltration process

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This paper describes the efficiency the integrated membrane process for obtain of concentrated medicinal plant extract as alternative to the traditional vacuum evaporation. The attention focused on the vegetal extracts obtained from *Geranium robertianum L.*, herb Robert (Geraniaceae) is justified by their use in the traditional medicine for the treatment of human and animal diseases [1]. *Geranium robertianum* concentrated extract by ultrafiltration process was examined for antioxidant properties. The antioxidant capacities and total polyphenols contents of the extracts were evaluated using ABTS and DPPH scavenging methods [2,3] and the total polyphenolic content was determined using the Folin-Ciocalteu method [4]. The air-dried ground aerial parts of the *Geranium robertianum* were extracted with two solvents: distilled water (8% w/v). After filtration, the extract was processed by microfiltration (MF) through Millipore membrane with 0.45 μm pores, followed by concentration using the ultrafiltration process (UF). The ultrafiltration process was performed using two types of membranes with a cut-off 10,000Da: UF1 with cellulose regenerated membrane and UF2 with polysuphone membrane. The results show that even low molecular mass compounds like polyphenols pass through membranes, the content of polyphenols in retentate (4.22 mg/L – UF1 and 4.68 mg/L – UF2) was higher than all the permeates (3.15 mg/L – UF1 and 3.52 mg/L – UF2). The data were sustained by TEAC values obtained for retentates (1247.3 μmol Trolox equivalent – UF1 and 2634.4 μmol Trolox equivalent – UF2) and permeates (711.4 μmol Trolox equivalent – UF1 and 1372.5 μmol Trolox equivalent – UF2). The values obtained by the DPPH assay varied from 77.6% DPPH inhibition for the *G.robertianum* aqueous extract to 95.3% DPPH inhibition for the UF1 concentrated extract and 92.5% DPPH inhibition for the UF1 concentrated extract. The results of this study show the performance of ultrafiltration membranes for the medicinal plants concentration and that the aqueous of *Geranium robertianum* extracts have a high antioxidant activity and can be considered as good source for further medicinal applications. *Acknowledgements: This work was financially supported by the Romanian National Center for Program Management – PN62076/2008 and PN71025/2007.* **References:** [1] Chevallier, A. (1996) The Encyclopedia of Medicinal Plants, Dorling Kindersley Limited, London. [2] Rice-Evans, C., Miller, N.J. (1994) Meth. Enzymol. 234:279 – 293. [3] Litescu, S., Radu, G.L. (2000) Eur. Food Res. Technol. 211(3):218 – 221. [4] Waterhouse, A.L. (2002) Current Protocols in Food Analytical Chemistry. John Wiley&Sons. New York.

PD79 Trypanocidal, leishmanicidal and cytotoxic effects of anthecotulide type linear sesquiterpene lactones from *Anthemis auriculata*

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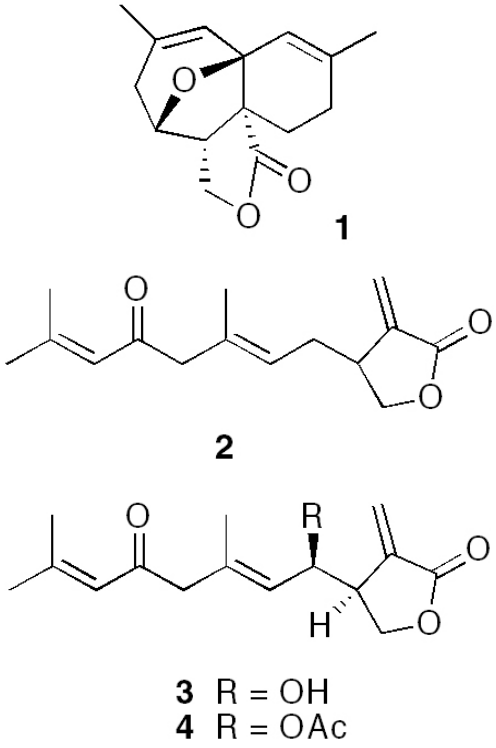
Trypanosomiasis and leishmaniasis pose major public health threats for many tropical countries. We recently reported the antiprotozoal activiy of anthecularin (**1**), a minor sesquiterpene lactone (SL) with a novel ring system from Greek *Anthemis auriculata* [1]. In the current study, we evaluated the *in vitro* antiprotozoal and cytotoxic potential of anthecotulide (**2**), 4-hydroxyanthecotulide (**3**) and 4-acetoxyanthecotulide (**4**), irregular, linear SLs biosynthetically related to anthecularin, also obtained from the same plant [2]. Trypomastigote forms of *Trypanosoma brucei rhodesiense* and *T. cruzi* and axenic amastigotes of *Leishmania donovani* were used for testing. Cytotoxic potential of the compounds were also assessed against mammalian (rat) skeletal myoblasts (L6 cells). All compounds showed potent trypanocidal and leishmanicidal activity, which enabled us to draw some valuable SARs. Notably, 4-hydroxyanthecotulide (**3**) appeared to be the most active compound against all parasites, particularly towards *T. b. rhodesiense* (IC_{50} 0.56 μg/ml), whereas 4-acetoxyanthecotulide (**4**) was the least active. However, the compounds possessed toxicity (IC_{50} 5.14 – 38.3 μg/ml), which might limit their use as antiprotozoal agents.



References: [1] Karioti, A. et al. (2007) J. Org. Chem. 72:8103 – 8106. [2] Theodori, R. et al. (2006) J. Nat. Prod. 69:662 – 664.

PD80 Antimicrobial activity of pentacyclic triterpenes isolated from *Berkheya bergiana*

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The use of medicinal plants in the world and especially in South Africa, contributes significantly to Primary Health Care[1]. The genus *Berkheya* belongs to the family Astereceae[2]. *B. bergiana* leaves and stem are used as traditional medicine. Decoction of leaves and roots are used for the treatment of coughs, gonorrhoea, rheumatism and abdominal disorders especially for pains after eating. It is also used as anti-emetics[3]. Unusual sesquiterpenoids and thiophene derivatives have been isolated from *Berkheya* species[3]. The aim of the study was to provide scientific rationale for the use of the plant in traditional medicine through bioassay-guided fractionation of *B. bergiana* leaves. Bioactivity testing was done against selected microbes using disc diffusion technique as outlined in Clinical Laboratory Standard Institute (CLSI). Structure elucidation of the isolated compounds was based primarily on 1D and 2D NMR analyses, including HMQC, HMBC and NOESY correlations. Fractionation yielded some triterpenoids; 20(29)-Lupene-1,3-diol, 3-Methoxy-20(29)-lupene and 17-Epilupenyl acetate. The compounds were active against 25 bacterial strains both standard and isolates and were active against *P. aeruginosa* ATCC 7700, *P. vulgaris* ATCC 6830, *S. marscens* ATCC 9986, *E. coil* ATCC 8739 *S. epididirmis, Salmonella* spp, *E. faecalis* etc. These results explain the support the use of *B. Bergiana* leaves for the treatment of infectious diseases in traditional South Africa medicine. It also shows that the antimicrobial activity is concentrated in the triterpenoid fractions. *Acknowledgements: The authors are grateful to the NRF, South Africa*

and University of Zululand Research Committees for financial support **References:** [1] Van der Watt, E., Pretorius, J.C. (2001) J. Ethnopharmacol. 76:87 – 91. [2] Van Wyk, B.K., Gericke, N. (2000) People's Plants. Briza Publications, Pretoria, South Africa. [3] Hutchings, A. et al. (1996) Pietermaritzburg, University of Natal Press.

PD81 Determination of total anthocyanins and anthocyanine glycosides in of various edible fruits from Bosnia

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The aim of this study was to determine total anthocyanins and anthocyanine glycosides in various fruits of different species and cultivars of sweet cherry *Prunus avium* L. (Rosaceae), and sour cherry *Prunus cerasus L.* (Rosaceae). Total anthocyanins were estimated with a spectrophotometric pH differential method. Cyanidin -3- galactoside served as a standard. Anthocyanins were measured by High Pressure Liquid Chromatography with dioda array detection (HPLC-DAD). HPLC-DAD was performed with a Zorbax StableBond-C18 column (250 x 4.6 mm, 5 µm), mobile phase was A: acetonitrile 100% and B: 10% (v/v) acetic acid and 1% phosphoric acid in water. Supernatants of fresh fruits were hydrolysed with 2 M HCl. As standards for HPLC-DAD were used pelargonidine chloride ($C_{15}H_{11}O_5Cl$), malvidine chloride ($C_{17}H_{15}ClO_7$), delphinidine chloride ($C_{15}H_{11}O_7Cl$), cyanidin-3-galactoside chloride ($C_{21}H_{21}ClO_{11}$), naringenine-7-glycoside ($C_{21}H_{22}O_{10}$), peonidine chloride ($C_{16}H_{13}O_6Cl$), peonidine-3-o-glycoside chloride ($C_{22}H_{23}ClO_{11}$), petunidine chloride ($C_{16}H_{13}ClO_7$) and malvidine-3-o-galactoside chloride ($C_{23}H_{25}ClO_{12}$). The samples were monitored at 517/525 and 220/254 nm. **Results:** Total content of anthocyanins were 2.36 mg/g of fresh weight of wild cherry, 0.10 mg/g of sweet cherry and 0.17 mg/g of sour cherry. Next, using HPLC-DAD in wild cherry the following aglucone were found: malvidin, peonidin, pelargonidin, delphinidin and petunidin. In sweet and sour cherry only two aglucone, peonidin and pelargonidin were found.

PD82 HPLC determination of certain flavonoids in *Ginkgo biloba* L.

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Ginkgo, *Ginkgo biloba* L. (Ginkgoaceae, Ginkgoales), is probably the oldest living tree one earth (190 – 200 million years) and is commonly referred to as a living fossil. The herbal material was gathered from two different locations in Sarajevo in the period from April until November 2008 (location A – Park at City centre and Location B – Botanical Gardens at National Museum of Bosnia and Herzegovina). It was air-dried and pulverized in a grinder for drugs just before the analysis. This study investigated the flavonoids, which include three main aglycone (isorhamnetin, kaempferol, quercetin) derivates. Flavonoids are the major active constituents in *G. biloba*. The specific concentrations of these substances in the leaves vary by season. The aim of this work was separation and assay flavonoids by HPLC method of *Ginkgo biloba* extracts. Reversed-phase liquid chromatography method with ODS column, and mobile phases 0.3% H_3PO_4 pH 2.0 (A solvent) and methanol (B solvent) were recommended to separate this kind of substances. At a flow rate of 1 ml/min, a column temperature 30 °C and UV detector was set at λ= 370 nm. At the location A in period from April until November the highest content of flavonoids was 0.50%, and at location B, in the same period, it was 0.17%. Conclusion: HPLC method, carried out for determination of various flavonoids in *Ginkgo biloba*, is very precise and suitable. **References:** 1. Hasler, A., Sticher, O. (1992) J. Chromatogr. 605:41 – 48. 2. European Pharmacopoeia, 6th ed. Monograph 01/2008:1828.

Topic E: Anti-cancer agents

PE1 New compounds from *Metaxya rostrata* (Kunth C. Presl)

Kainz KP[1], Virtbauer J[1], Marian B[2], Kaehlig H[3], Donath O[1], Reznicek G[1], Krenn L[1]
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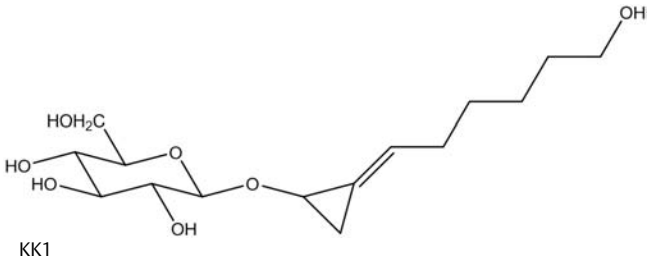
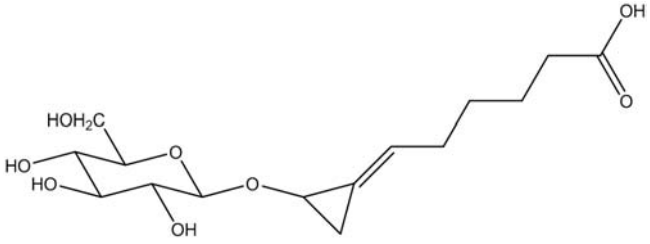
Two new compounds were isolated from the tree fern *Metaxya rostrata*, *Metaxyaceae*, a Costa Rican traditional herbal remedy against intestinal diseases. Until now only two polyphenols (cinnamtannin B-1 and aesculitannin B), two glycosides of phenolic acids (4-O-ß-D-glucopyranosyl-caffeic-acid and 4-O-ß-D-glucopyranosyl-p-trans-coumaric-acid), sugars and common sterols have been isolated from this plant [1,2]. Dried rhizomes were extracted by sonification with hot water and methanol. The lyophilisate was extracted sequentially with ethylacetate, butanol and methanol. The fractions were subjected to vacuum liquid chromatography (VLC) on silica gel using EtOAc/MeOH/H_2O mixtures of increasing polarity as mobile phases to obtain 15 fractions [1]. Fraction 12, cytotoxic to SW 480 colorectal carcinoma cells, was subjected to gel permeation chromatography on Sephadex LH-20. From the resulting fraction A compounds KK1 (58 mg) and KK5 (3 mg) were isolated. By detailed NMR and MS experiments the compounds were identified as (2E)-2-(6-hydroxyhexyliden)cyclopropyl-β-glucopyranoside (KK1) and (6E)-6[2-(β-glucopyranosyloxy)cyclopropylidene]hexanoic acid (KK5). The substances did not show cytotoxic activity. Thus, the cytotoxic activity of fraction 12 obviously is due to other compounds.



KK1



KK5

References: [1] Virtbauer, J. et al. (2008) Z. Naturforsch. 63c:469 – 475. [2] Kainz, K.P. et al. (2009) Sci. Pharm. 77:in press

PE2 *In vivo* cancer chemopreventive activity of umbelliprenin

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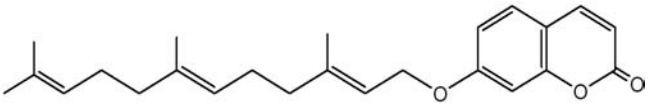
Umbelliprenin is a prenylated compound which belongs to the class of sesquiterpene coumarins. In continuation of our previous *in vitro* finding [1], we determined to assess the cancer chemopreventive activity of umbelliprenin *in vivo* by using a two-stage carcinogenesis assay of mouse skin tumors induced by peroxynitrite as an initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter. In this assay, treatment with umbelliprenin along with peroxynitrite/TPA inhibited papilloma formation up to week 9 and the percentage of papilloma

bearers was approximately 86.6% at week 20. The average number of papillomas formed per mouse was only 3.9 even at week 20 which was significantly reduced compared to the control group ($p < 0.05$). The results of the *in vivo* two-stage mouse skin carcinogenesis test revealed that umbelliprenin possessed a pronounced chemopreventive activity and its activity was comparable to that of curcumin, a well-known chemopreventor. Therefore, umbelliprenin might be valuable as a cancer chemopreventive agent.



References: [1] Iranshahi, M. et al. (2008) Planta Med. 74:147 – 150.

PE3 Isolation and cytotoxic activity of buchariol from *Salvia leriifolia* Benth.

Loizzo MR[1], Tundis R[1], Menichini F[1], Bonesi M[1], Conforti F[1], Statti GA[1], Nadjafi F[2], Nicoletti M[3], Menichini F[1]
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The search of natural products for cancer therapy represents an area of great interest in which plants have been the most important source. In the continuing search for cytotoxic compounds from plants in the present investigation we reported the cytotoxic activity of sesquiterpenoid 4,10-epoxy-6alpha-hydroxyguaiane, named buchariol, isolated from *S. leriifolia* Benth. The genus *Salvia* (Lamiaceae) comprises about 700 herbs and shrubs, growing in the temperate and warmer zones of the world [1]. Plants belonging to this genus show high diversity in their secondary metabolites [2] as well as in pharmacological effects. *Salvia leriifolia* aerial parts collected in Sabzewar (Iran) were extracted with MeOH at room temperature. The extract was dissolved in H_2O and partitioned with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. Fractionation of dichloromethane extract led the isolation of buchariol (20.06 mg).The cytotoxicity was evaluated using the sulforodamine B (SRB) assay [3]. The test is based on the estimation of cell number indirectly by providing a sensitive index of total cellular protein content which is linear to cell density. Buchariol exhibited a strong cytotoxic activity against COR-l23 and C32 cell lines with IC_{50} value of 0.5 and 0.6 μg/mL, respectively. Moreover, the sesquiterpenoid buchariol inhibited the proliferation of A549 cell line with an IC_{50} value of 4.6 μg/mL.

References: [1] Chadefaud, M., Emberger, L. (1960) Traité de Botanique Systematique, Masson, Paris [2] Lu, Y. et al. (2002) Phytochemistry 59:117 [3] Monks, A. et al. (1991) J. Nat. Cancer Institute, 83:757 – 66.

PE4 Pitfalls in testing saponins for their anti-angiogenic activity: comparison of test systems

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Angiogenesis is a major component in the progression of various diseases such as cancer, psoriasis and rheumatoid arthritis. Besides their virucidal, haemolytic and molluscicidal activity, the saponins of *M. lanceolata*, displayed anti-angiogenic activity in the chick chorioallantoic membrane (CAM) assay [1,2]. This latter activity was further investigated in an *ex-vivo* test. The growth of the microvessels in the rat aorta ring assay was compared with the sprouting in the human placental vein assay during 20 days, while different concentrations of serum were added to the test. In both *ex-vivo* assays suramin was tested as positive control. A mixture of maesasaponins and several individual saponins were tested in the rat aorta ring assay (10 – 100 μg/mL). Based on the growth curves, the tests with suramin in both *ex-vivo* assays and literature the rat aorta ring assay was chosen as most preferable *ex-vivo* test for angiogenesis. Although the tested maesasaponins showed anti-angiogenic activity in the CAM assay at a concentration of 1 – 10 μg/mL, assay activity was only found at 25 – 50 μg/mL in the rat aorta ring assay. This could be due to the concentration locally obtained with the pellets used in the CAM assay or the influence of a non specific inflammatory reaction in the *in-vivo* test which is not present in the *ex-vivo* test.

References: [1] Apers, S. et al. (1998) J. Nat. Prod. 61:585 – 590. [2] Apers, S. et al. (2002) J. Pharm. Belg. 57:47 – 49.

PE5 New jaspamide derivatives from the marine sponge *Jaspis* sp.

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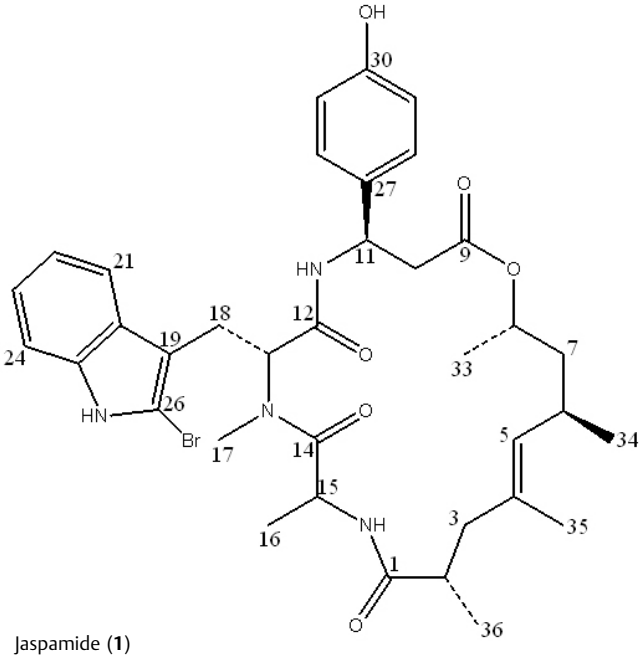
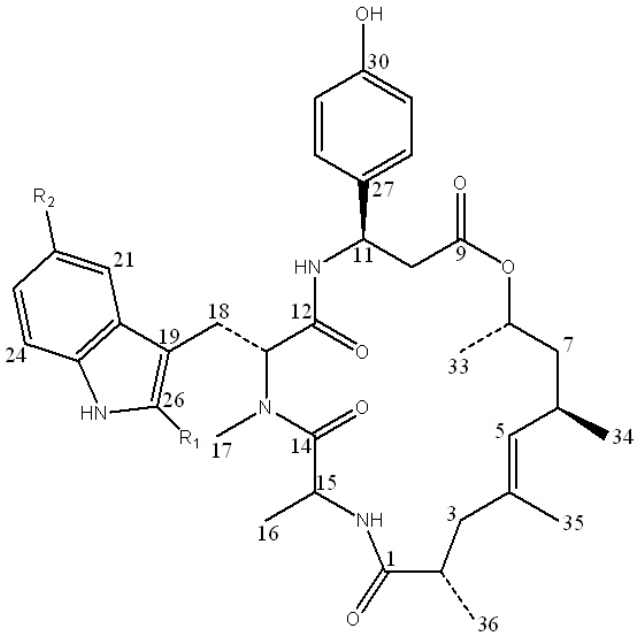
Jaspamide (jasplakinolide, **1**), a cyclodepsipeptide isolated from marine sponges of the genus *Jaspis*, is known for its pronounced biological activities which include antifungal, insecticidal, and cytotoxic activity against 36 human solid tumor cell cultures. The biological properties and structural features of jaspamide stimulated numerous efforts aiming at a total synthesis and structural modification. As a part of our ongoing studies on bioactive natural products from marine sponges we investigated a specimen of *Jaspis* sp. collected at Kalimantan (Indonesia). The crude methanolic extract exhibited considerable *in vitro* cytotoxic activity against mouse lymphoma L5178Y cells. Chromatographic separation the extract yielded jaspamide (**1**) as the major constituent. Through a detailed bioactivity-guided chemical investigation of the ethyl acetate souble fraction minor analogues of jaspamide, including the new natural products jaspamide Q (**2**) and jaspamide R (**3**) were obtained. The structures of the new compounds were unambiguously elucidated based on 1D and 2D NMR spectral data, mass spectrometry and comparison with the parent compound jaspamide. Details on the structure elucidation of the new jaspamide analogues will be presented.



Jaspamide (**1**)



Jaspamide Q (**2**) R_1, R_2= H, Jaspamide R (**3**) R_1, R_2= Br

PE6

Aryltetralin lignans from *Anthriscus sylvestris* L. (Hoffm.)

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Anthriscus sylvestris (L.) Hoffm. (Apiaceae) is a common wild plant in Northwest Europe that accumulates considerable amounts of lignans. Deoxypodophyllotoxin as the main attractive constituent can be used as a precursor for the production of podophyllotoxin. Podophyllotoxin is currently receiving great attention as one of the most important aryltetralin-lignan in related to human health. It is used as a semisynthetic precursor for anticancer drugs: Etoposide®, Teniposide®, and Etopophos® to treat various types of neoplasms [1]. To date, podophyllotoxin is obtained by isolation from the plant. In the future, the availability of podophyllotoxin from this source is likely to become a major bottleneck. *Podophyllum* species have now been listed on the endanger species list, proving that the increasing demand of podophyllotoxin is a serious threat for the plant [2]. An alternative source of podophyllotoxin may be obtained by (biotechnological) hydroxylation of deoxypodophyllotoxin at the C7position. Deoxypodophyllotoxin is much more abundant in the plant kingdom than podophyllotoxin. A better insight in the occurrence of deoxypodophyllotoxin combined with profound knowlegde of its biosynthestic pathway(s) will help to develop alternative sources for the desired lignans. We found several lignans in *Anthriscus sylvestris* that may be involved in the biosynthetic pathway of deoxypodophyllotoxin using HPLC and Electrospray tandem mass spectra techniques. Podophyllotoxone, α-peltatin, and β-peltatin that have not been previously reported to be present in *A. sylvestris* could be identified based on the mass spectra, UV spectra and retention times compared with pure reference compounds. Deoxypodophyllotoxin, yatein, and anhydropodorhizol were also present in the extracts. The presence of these compounds in *A. sylvestris* has been reported earlier. Podophyllotoxone, anhydropodorhizol and deoxypodophyllotoxin were the major compounds, while α-peltatin and β-peltatin, were present in lower concentration. Yatein is an earlier precursor leading to deoxypodophyllotoxin formation, while β-peltatin is the product of the metabolization of dexypodophyllotoxin according to the hypothetical biosynthetic pathway of lignans as reported [3]. **References:** [1] Ayres, D.C., Loike, J.D., (1990) Chemistry and Pharmacology of Natural Products. Lignans: Chemical, Biological and Clinical Properties. Cambridge University Press, Cambridge. [2] Nayar, M.P., Sastry, A.P.K., (1990) Red Data Book of Indian Plants. Botanical Survey of India, Calcutta. [3] Federolf, K. et al. (2007) Phytochemistry 68:1397 – 1406.

PE7

Absolute structures of Salvileucalin A and B from *Salvia leucantha* Cav.

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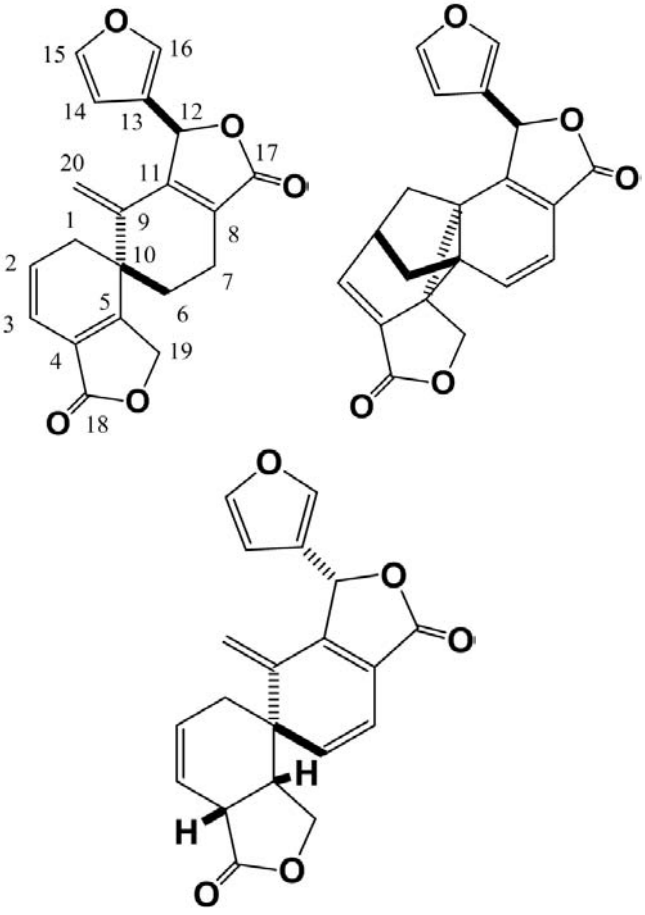
From the aerial parts of *Salvia leucantha* of the subgenus Calosphace (Labiatae), an evergreen herbaceous perennial in the frost-free climate area, some rearranged neoclerodane diterpenes were isolated by Esquivel et al. [1], and another highly rearranged diterpene, spiroleucantholide (**3**), having a spiro skeleton in the molecule, by Takeda et al. [2]. In our study, salvileucalin B (**2**), having an unprecedented rearranged neoclerodane skeleton, was isolated from the aerial parts of this plant along with salvinoleucalin A (**1**) [3]. The absolute structures were elucidated by spectroscopic analysis, X-ray crystallographic analysis and vibrational circular dichroism (VCD). Compound 2 represents a novel clerodane diterpene, characterized by a tricycle[3.2.1.$0^{2,7}$]octane substructure incorporating the exocyclic C-20 methylene of **1**. Salvileucalin B (**2**) exhibited cytotoxicity against A549 and HT-29 cells with IC_{50} values of 5.23 and 1.88 μg/mL, respectively.



References: [1] Esquivel, B. et al. (1994) Tetrahedron 50:11593 – 11600. [2] Narukawa, Y. et al. (2006) J. Nat. Med. 60:206 – 209. [3] Aoyagi, Y. et al. (2008) Org. Lett. 10:4429 – 4432.

PE8 New cucurbitacin derivatives from *Bryonia aspera* Stev. ex Ledeb

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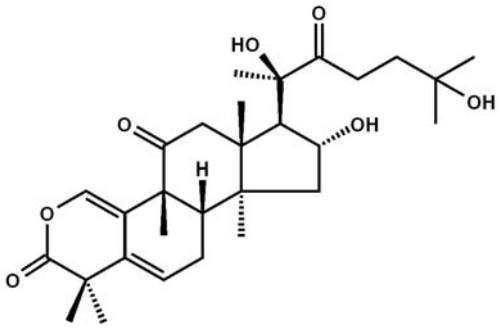
Bryonia aspera Stev. ex Ledeb from the Cucurbitaceae family is native to Iran. This plant has been described in an ethnobotanical study as being used as a treatment of dermal wounds, cancer and digestive disorders [1], whereas no phytochemical investigations have been reported up to now. The isolation of bioactive compounds from this plant seemed to be of interest because a chloroform extract showed cytotoxic activity. Therefore a phytochemical investigation of the root extract has been undertaken and yielded 11 compounds. Their structures were elucidated by spectroscopic means (1D- and 2D-NMR spectroscopy, ESI-MS). The majority of the compounds turned out to be 23,24-dihydro-cucurbitacin derivatives.



23,24-dihydro-neocucurbitacin B

Three substances- 23,24-dihydro-neocucurbitacin B, 23,24-dihydro-7-hydroxy-cucurbitacin D, and 23,24-dihydro-25-O-glucopyranosyl-cucurbitacin D – were new natural products. **References:** [1] Ghorbani, A. (2005) J. Ethnopharmacol. 102:58 – 68.

PE9 A petrol ether extract of *Onosma paniculatum* Bur. & Franch. shows strong anti-proliferative activity and induces apoptosis in human cancer cell lines

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In Chinese medicine, the roots of *Onosma paniculatum* Bur. & Franch. are traditionally used for cancer treatment. For initial investigations, the dried roots have been successively extracted with petrol ether (PE) and methanol (MeOH). In a pharmacological screening, the extracts were tested against human CCRF-CEM leukaemia cells, human MDA-MB-231 breast cancer cells, human HCT 116 colon cancer cells and human U251 glioblastoma cells at a final concentration of 10 µg/ml. For quantification of cell proliferation and viability, a XTT based colorimetric assay was used. Whereas the MeOH extract showed no activity against these cell lines, the PE extract strongly inhibited cell proliferation and reduced cell viability. To further investigate the active extract, we determined the effect on four different melanoma cell lines, primarily isolated from different stages of melanoma progression: SBcl2, WM35, WM9 and WM 164. After exposure for 48 h, more melanoma cells were detached and less cell density was observed in comparison to un-exposed cells. In addition, changes in cell cycle regulation and caspase-3 activity were determined by flow cytometry. At 10 µg/ml, significant alterations in cell cycle and cleaved caspase-3 could be detected. These results indicate that the PE extract of *Onosma paniculatum* induces apoptosis in human melanoma cell lines in a caspase dependent manner and changes cell cycle. In further experiments, the active compounds of the extract will be isolated and identified and their growth inhibitory and apoptosis-inducing properties investigated in more detail. *Acknowledgements: This work was supported by the "Fonds zur Foerderung der Wissenschaftlichen Forschung" P21114.*

PE10 Diterpenoids from *Andrographis paniculata* as natural chemosensitizers

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Extracts of the medicinal plant *Andrographis paniculata* Nees (Acanthaceae) are described in literature as showing anticancer properties in leukaemic cell lines [1,2]. The aim of this study was to isolate the main constituents of a commercially available phytotherapeutic preparation of *A. paniculata* and to determine their chemosensitizing potential using the Nicoletti assay [3]. Chromatographic separation steps resulted in the isolation of the diterpenes andrographolide (**1**), 14-deoxy-11,12-didehydroandrographolide (**2**) and the diterpene glucoside neoandrographolide (**3**). Whereas the individual effects of suboptimal concentrations of the chemotherapeutic etoposide (500 nM) and 20.8 µM of **3** showed only weak effects in S-Jurkat cells (15% and 8% apoptotic cells [AC], respectively), their combination strongly induced cell death (64% AC). In contrast, **1** and **2** showed no increase of AC. In order to specify the chemosensitizing effect, we tested the compounds also in X-linked inhibitor of apoptosis proteins (XIAP)-overexpressing Jurkat cells. XIAP overexpression protects Jurkat cells from etoposide-induced apoptosis. Although the combination of etoposide and **2** showed no synergism in S-Jurkat cells, an increased percentage of AC was observed in XIAP-overexpressing cells. For **3** (20.8 µM, 3% AC), the chemosensitizing effect could be confirmed (37% AC). We enzymatically cleaved the glucose-moiety of **3** obtaining the diterpene andrograpanin (**4**). When used in combination with etoposide, a distinct loss of activity was observed, which indicates a major impact of the sugar-moiety on the bioactivity. As expected from a XIAP inhibitor, we found that **3** potentiates the caspase-3 like activity. In conclusion, this study enriches the pharmacological profile of the medicinal plant *A. paniculata* and elucidates compound **3** as potent, naturally derived small-molecule chemosensitizer in a leukaemic cell line. **References:** [1] Matsuda, T. et al. (1994) Chem. Pharm. Bull. 42:1216 – 1225. [2] Cheung, H.Y. et al. Planta Med. (2005) 71:1106 – 1111. [3] Nicoletti, I. et al. (1991) J. Immunol. Methods 139:271 – 279.

PE11 Anthraquinones and naphthopyrones from the marine echinoderm *Comanthus* sp.

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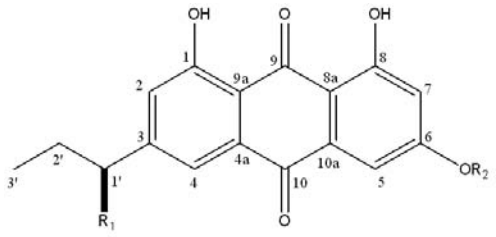
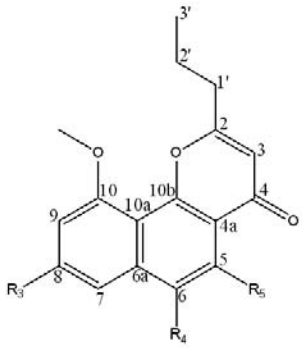
A detailed analysis of a Philippine specimen of the marine echinoderm *Comanthus* sp. yielded fifteen compounds including four anthraquinones identified as 1'-deoxyrhodoptilometrin (**1**) along with its 6-*O*-sulfate derivative (**3**), and rhodoptilometrin (**2**) with its 6-*O*-sulfate derivative (**4**). In addition five naphthpyrones including comaparvin (**5**), 6-methoxycomaparvin (**6**), 6-methoxycomaparvin-5-methylether (**7**), 6-methoxycomaparvin-5-methylether-8-*O*-sulfate (**8**), and 6-hydroxycomaparvin-8-*O*-sulfate (**9**) were likewise isolated and identified. Further compounds include steroids and a nucleoside derivative. The structures of the isolated compounds were unambiguously elucidated based on HRESIMS analysis, 1D and 2D NMR, and by comparison with the literature. For compounds **2** and **4** the absolute configurations were identified for the first time using the Mosher reaction. Both compounds are (*S*)-(-) enantiomers. All isolated compounds were evaluated for their cytotoxic

activities against cancer cells using the (MTT) assay and compared to the well known marine cancer drug candidate kahalalide F (EC_{50}= 6.3 μg/mL). 1'-Deoxyrhodoptilometrine (**1**) and an unseparable mixture of comaparvin (**5**) and 6-methoxycomaparvin (**6**) exhibited pronounced cytotoxicity against mouse lymphoma L5178Y cells with EC_{50} values of 2.3 and 5.2 μg/mL, respectively.



	R_1	R_2
1:	H	H
2:	OH	H
3:	H	SO_3^-
4:	OH	SO_3^-



	R_3	R_4	R_5
5:	OH	H	OH
6:	OH	OCH_3	OH
7:	OH	OCH_3	OCH_3
8:	SO_3^-	OCH_3	OCH_3
9:	SO_3^-	OH	OH

PE12 Novel insights into the mechanism of action of grayanotoxin III

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Grayanotoxins are natural products that occur in species belonging to the Ericaceae family and contribute to plant toxicity [1]. They bind to ion channels and cause membrane depolarization [2]. Ion channels are implicated in the progression of cancer [3,4,5]. Therefore, we investigated the effect of grayanotoxin III (GTX III) on cell viability and induction of apoptosis, as well as the underlying mechanisms of GTX III triggered cell death in the HL-60 leukemia cell line. Cell viability decreased as evaluated by WST-1 assay and Western blot analyses indicated caspase cleavage after GTX III exposure. In addition, p38 MAP kinase was phosphorylated pointing to a p38 regulated apoptosis pathway. Preincubation with BAPT AM (cell permeable calcium chelator), Ruthenium Red (blocker of Ca^{2+} uptake and release from mitochondria), MDL 28170 (calpain inhibitor) and dibucaine (voltage-gated sodium channel blocker) before GTX III treatment decreased the cleavage of caspase-9 to its active form. These data suggest an increase in intracellular Ca^{2+} following GTX III exposure, as well as the implication of ion channels and Ca^{2+} in GTX III-induced cell death signaling. Thus, our findings show that GTX III decreased cell viability and induced p38 MAP kinase, Ca^{2+} and voltage-gated sodium channel dependent apoptosis in the HL-60 cell line. **References:** [1] Kinghorn, A.D. et al. (1978) J. Chrom. 147:299 – 308. [2] Cestele, S., Catterall, A. (2000) Biochem. 82:883 – 892. [3] Anderson, J. et al. (2003) Mol. Cancer Ther. 2:1149 – 1154. [4] Brackenbury, W.J. et al. (2008) Neuroscientist 14:571 – 583. [5] Kondo, S. et al. (1995) J. Neurosurg. 82:469 – 474.

PE13 A new type of phytotherapeutic approach with angiosperms from arid zones of northern Mexico in patients with malignant and benign tumors

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Certain angiosperms from the arid regions from northern Mexico have shown antineoplastic activity [1,2]. We enrolled a cohort of patients with malignant and benign tumors seen from 2005 to 2009. They received phytotherapy as only causal treatment. Cases: 42 -year-old woman with papillary thyroid carcinoma and cervical nodal metastases, evolved without evidence of malignant growths. Two female patients with meningioma of the brain 71 and 72 years of age developed calcified meningioma. A female, 38 years of age, with invasive cervical squamous cell carcinoma with metastases in abdominal cavity, her condition was getting better. 44-year-old male, with tumor of clear cells in the right kidney with lung metastases are diminishing. 96-year-old male with bladder transitional cell carcinoma, evolved with no signs of tumor. This therapeutic approach has demonstrated no adverse reactions or clinical and laboratory events, improving quality of life and survival. **References:** [1] López, M.C.A. et al. (2000) International Symposium: Oncology. Schliersee, Deutschland. [2] Arizawa, M. et al. Planta med. (1985) 6:544 – 545.

PE14 Cell death and impairment of mitochondrial functions induced by *Phyllanthus virgatus*: a comparison study

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The genus *Phyllanthus* consists of several species in the family Euphorbiaceae [1]. In Thailand, *Phyllanthus virgatus* and other two species, *P. amarus* and *P. urinaria*, are closely related in appearance, phytochemical structure and have the same common local name. Several activities of *P. amarus* and *P. urinaria*, such as anti-inflammatory and hepatoprotective effects, have been reported [2,3]. However, information on biological activities of *P. virgatus* is very limited. In this study, the pharmacological activities of the extract of three *Phyllanthus* species were compared. We found that the methanolic extract of *P. virgatus*, containing more phenolic compounds than that of the other species, showed the highest free radical scavenging activity and highest inhibition of peroxidation in linoleic acid system. Furthermore, *P. virgatus* extract showed the strongest cytotoxic effect to human hepatoma HepG2 cells. All of the extracts caused morphology changes and stimulated oxygen consumption of HepG2. With isolated rat liver mitochondria it was found that *P. virgatus* extract was the most active in stimulating mitochondrial state 4 respiration, in consonant with its effect on HepG2 cells. In addition, the extract also depressed state 3 respiration and respiratory control ratio. Thus, the extract impairs hepatic energy metabolism by acting as mitochondrial uncoupler and inhibitor of oxidative phosphorylation. These mitochondrial effects may intimately involve in the cytotoxic action of *P. virgatus* extract on HepG2 cells. **References:** [1] Jain, N. et al. (2008) Planta Med. 74:296 – 301. [2] Harish, R., Shivananda, T. (2006) Food Chem. 95:180 – 185. [3] Lee, C.Y. et al. (2006) Am. J. Clin. Med. 34:471 – 482.

PE15 Search for new natural ligands of the antiapoptotic protein Bcl-xL from Malaysian plants

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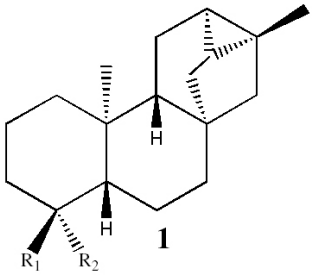
Bcl-xL is an antiapoptotic protein of the Bcl-2 family located in the membrane of the mitochondria of eukaryote cells. Its involvement in the programmed cell death through the activation of caspase pathway was widely studied and discussed [1,2]. The overexpression of Bcl-xL in cancer cells was reported to have an antiapoptotic effect on tumour and to confer a multidrug resistance [3]. Therefore, the study of the interaction between Bcl-xL and some new ligands appeared to be a very good strategy in the search for new anticancer drugs. Consequently, a biological screening was carried out on 1476 ethyl acetate extracts from various parts of 670 Malaysian plants. The binding activity against Bcl-xL was evaluated using an affinity displacement assay based on Bcl-xL/Bak (BH3 domain) interaction (fluorescence polarization assay). Only 18 extracts revealed a noticeable activity. Among them, the bark extract of *Xylopia sp*. (Annonaceae) exhibited a significant binding activity: 31% at 10 µg/mL. The phytochemical study of the plant was undertaken and bioguided fractionation using silica gel chromatography and HPLC led to the isolation of several *ent*-trachylobane terpenoids (**1**), identified by spectroscopic and crystallographic methods. Access to chemodiversity was attempted through bioconversion of the major compound **1** (*ent*-trachyloban-18-oic acid) using a panel of microorganisms. This work describes biological activity of this chemical series and their structure-activity relationships. Thanks to this screening program of Malaysian plant extracts on Bcl-xL, we were able to discover natural compounds that might be good candidates for further studies in the oncological domain.



Acknowledgement: This work was supported by an ICSN-CNRS grant to one of us (A.L.). References: 1. Danial, N.N. (2007) Clin. Cancer Res. 13:7254 – 7262. 2. Adams, J.M., Cory, S. (1998) Science 281:1322 – 1326. 3. Castilla, C. et al. (2006) Endocrinology 147:4960 – 4967.

PE16 Thai medicinal plants and the search for new anti-inflammatory and anticancer agents

Siriwatanametanon N[1], Fiebich BL[2], Prieto JM[1], Efferth T[3], Heinrich M[1]
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Nine plant species with anti-inflammatory effects were selected from Thai textbooks. It is expected that long history of their uses might offer opportunities for the discovery of novel anti-inflammatory and/or anticancer agents. In this study, anti-inflammatory NF-κB inhibitory activities were determined by luciferase assay and effects on LPS-induced pro-inflammatory cytokines PGE2, IL-6, IL-1β, and TNFα were assessed by ELISA [1]. Cytotoxicity activities were examined by the MTT test in HeLa cells, and the XTT test in leukaemia CCRF-CEM cells including their multidrug-resistant CEM-ADR5000 subline [2]. Among all the tested extracts, *Gynura pseudochina* (L.) DC. *var. hispida* Thv. (Asteraceae) (ME) and *Oroxylum indicum* (L.) Kurz. (Bignoniaceae) (EA) showed the greatest NF-κB inhibitory effects with the lowest IC_{50} values (41.96 µg/ml and 47.45 µg/ml, respectively). While *G. pseudochina var. hispida* (ME) inhibited the release of IL-1β (IC_{50} 2.46 µg/ml), *O. indicum* (EA) also inhibited the release of PGE_2 (IC_{50} 26.98 µg/ml). *Muehlenbeckia platyclada* F., Muell., Meisn. (Polygonaceae) (EA and ME) did not inhibit NF-κB activation but inhibited the release of IL-6, IL-1β and TNF-α with the lowest IC_{50} values ranged from 0.28 – 8.67 µg/ml. *Pouzolzia indica* (L.) Gaudich. (Urticaceae) (PE) show the strongest antileukemic effects on both CCRF-CEM cells and the multidrug resistant subline at 10 µg/ml (90.25 ± 0.29% and 89.52 ± 0.12% cell dead, respectively). The active compounds isolated from *G. pseudochina var. hispida* (ME), the strongest NF-κB inhibitory extract, were identified as the known compounds quercetin-3-rutinoside (IC_{50} = 24.78 µg/ml) and quinic acid (IC_{50}= 49.18 µg/ml). *Acknowledgement: The Royal Thai Government for funding.* References: [1] Bremner, P. et al. (2004) Planta Med. 70:1 – 5. [2] Efferth, T. et al. (2002) Blood Cell. Mol. Dis. 28:160 – 168.

PE17 Diterpenoids with antitumor activity from *Euphorbia esula* L.

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Euphorbia is the largest genus in the family Euphorbiaceae, comprising about 2000 species. Hitherto many secondary metabolites with specific types of diterpene skeletons were isolated from these plants. Euphorbia diterpenes possess a number of interesting biological activities, such as antiproliferative, antiviral, multidrug resistance (MDR) reversing activities [1,2,3]. *E. esula* can be regarded as a promising source of diterpenes, since ingenane, lathyrane and jatrophane polyesters were isolated previously from the root, seed and herb, including compounds with cytotoxic, MDR modifying, and anti-herpes simplex activities [4,5,6]. In continuing our search for biologically active compounds from *E. esula*, a new jatrophane diterpene (**1**) was isolated from the CH_2Cl_2 extract of the aerial part by means of multistep chromatographic purification. The compound was identified as a jatrophane tetraester acylated with acetic and isobutanoic acids. The structure elucidation was carried out by extensive spectroscopic analysis, including 1D and 2D NMR and HRESIMS experiments. The isolated compound was tested for its MDR-reversing activity on mouse lymphoma cells using the standard functional assay with Rhodamine 123, and found to be effective in modulating the efflux-pump activity. Furthermore, compound **1**, together with twelve jatrophane diterpenes obtained in our earlier experiment, were tested for their cytotoxic activity on human tumor cell lines (HeLa, Ishikawa and MCF7). In the assay, the highest effect was demonstrated by **1**, but moderate or weak activities were detected for some other compounds, too. *Acknowledgements: This work was supported by Hungarian Scientific Research Fund (OTKA PD 78145).* References: [1] Engi, H. et al. (2007) Anticancer Res. 27:3454 – 3458. [2] Valente, C. et al. (2004) J. Nat. Prod. 67:902 – 904. [3] Betancur-Galvis, L. (2003) Planta Med. 69:177 – 178. [4] Mucsi, I. et al. (2001) Planta Med. 67:672 – 674. [5] Lu, Z.Q. et al. (2008) Phytochemistry 69:812 – 819 [6] Seip, E.H. et al. (1982) Planta Med. 46:215 – 218.

PE18 Antiproliferative sesquiterpenes and flavonoids from *Anthemis ruthenica* L.

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As a part of a continuing search aimed at the discovery of novel compounds with antiproliferative activity from Hungarian plants belonging to the Asteraceae family, it was found that the herbs of *Anthemis ruthenica* M. Bieb. exert high antiproliferative activity against cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF7) and skin epidermoid carcinoma (A431) cells using the MTT assay [1]. Previous publications dealing with this species reported only the composition of the volatile oil obtained from the plant [2]. The present paper reports the isolation of a new eudesmanolide sesquiterpene, sivasinolide-6-O-angelate, and the known compounds chrysanin, tanacin, eupatolide, centauridin, and

centaureidin from the aerial parts of *A. ruthenica*. The compounds were isolated using bioactivity guided fractionation from the $CHCl_3$ extract of the herb, which displayed high tumor cell proliferation inhibitory activity. The structures were determined by UV, HRESI-MS, high-field 1D and 2D NMR spectral analyses, affording complete ^1H and ^{13}C NMR assignments for all isolated compounds. The antiproliferative activity of the sesquiterpenes and flavonoids was assessed against three cell lines mentioned above, and found that besides the extremely active centaureidin, all isolated compounds exert high or moderate antitumor effect. The germacranolide taxillin and 3β-hydroxycostunolide displayed higher activity, while the eudesmanolides sivasinolide-6-O-angelate and chrysanin were marginally active. *Acknowledgements: Financial support by the Hungarian Research Fund Agency (OTKA grant K72771) is gratefully acknowledged. The authors thank Dr. Pál Szabó (Chemical Research Centre, Hungarian Academy of Sciences, Budapest) for the mass spectral measurements.* **References:** [1] Réthy, B. et al. (2007) Phytother. Res. 21:1200 – 1208. [2] Vujisic, L. et al. (2006) Flavour Fragr. J. 21:458 – 461.

PE19 Cytotoxicity, antioxidant and composition of the essential oil of *Dracocephalum surmandinum* Rech. from Iran

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The chemical composition of hydrodistilled essential oil from the aerial flowering parts of *D. surmandinum* was analyzed for the first time by GC and GC-MS. Monoterpenoids including oxygenated and hydrocarbons comprising 63.4 and 33.9% were the principal compound groups of the essential oil, respectively. In total, 25 constituents accounting for 97.8% of the oil were identified [1]. Perilla aldehyde (54.3%) and limonene (30.1%) were characterized as the main components. In addition, our results indicated that the essential oil of *D. surmandinum* (5 – 100 μg/ml) possesses a marked antioxidant and radical scavenging activity using different model systems including Trolox equivalent antioxidant capacity, β-carotene-linoleic acid blenching and 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assays [2]. The cytotoxicity activity was also carried out using the MTT assay [3]. Results showed that the essential oil of *D. surmandinum* has a good cytotoxic activity against Human breast adenocarcinoma cell line (MCF-7) and Human erythromyeloblastoid leukemia cell line (K562) with an IC_{50} value of 14 and 16 μg/ml, respectively. However, the cytotoxic potential of *D. surmandinum* essential oil against Rat adrenal pheochromocytoma cell line (PC 12) was weak (IC_{50} of > 100 μg/ml). *Acknowledgements: We are grateful to Shahid Beheshti University Research Council for financial support of this work.* **References:** [1] Adams, R. (2007) Identification of essential oil components by gas chromatography/quadropole mass spectroscopy. Allured Publishing Corporation, Carol Stream. [2] Blois, M.S. (1958) Nature 181:1199 – 1200. [3] Vistica, D.T. et al. (1991) Cancer Res. 51:2515 – 2520.

PE20 Elastase inhibitors and cancer preventive potential agents from *Calophyllum inophyllum* (L.) grown in French Polynesia

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Elastase is a well-known serine-endoproteinase responsible for extracellular matrix degradation and inflammation processes. It is also linked to COPD and cystic fibrosis [1]. Furthermore, overactivation of elastase and down-regulation of its natural inhibitor elafin are associated with increased levels in constitutively active low molecular weight form cyclin E. This correlates with poor diagnosis and morbidity increase in certain types of breast cancer [2]. *Calophyllum inophyllum* (Clusiaceae) is a pantropical species considered as a sacred tree by traditional Polynesian people. Nowadays, *C. inophyllum* oil is still widely used for skin treatments, wound healing and included in cosmetic formulations. Previous studies have shown the presence of pyranocoumarin derivatives in this oil. Some of these compounds, like calophyllolide, have been reported to have interesting bioactive properties such as anti-HIV1 and cancer chemopreventive effects [3]. Results presented herein show promising activity of fractions from *C. inophyllum* oil in an enzymatic screening assay for elastase inhibitory compounds. Therefore, phytochemical studies led to the isolation of elastase inhibiting dipyranocoumarin compounds from *C. inophyllum* oil grown in French Polynesia. Thus, these compounds should provide an interesting scaffold for the design of new potential anticancer agents. To the best of our knowledge, this is the first report showing elastase inhibitory activity of compounds extracted from *C. inophyllum* grown in French Polynesia. **References:** [1] Barnes, P.J., Hansel, T.T. (2004) The Lancet 364:985 – 996. [2] Akli, S., Keyomarsi, K. (2004) Br. Cancer Res. 6:188 – 191. [3] Laure, F. et al. (2008) Anal. Chim. Acta 624:147 – 153.

PE21 *Mangifera pajang* kernel crude extract induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cell lines

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Ingestion of fruits and their products have been associated with a decreased cancer risk. Phytochemicals present in fruits and their products (i.e. polyphenols, anthocyanins, carotenoids) have been linked to the anticancer activity. *M. pajang* kernel extract has been found to contain high polyphenol and flavonoid content and displayed superior antioxidant properties compared to the peel and flesh extracts of the fruit [1]. This research was conducted to investigate the anticancer potential of the kernel extract of *M. pajang*. The results showed that the kernel crude extract induced cytotoxicity in MCF-7 (hormone-dependent breast cancer) cells and MDA-MB-231 (non-hormone dependent breast cancer) cells with IC_{50} values of 23 and 30.5 μg/ml, respectively. The kernel extract induced cell cycle arrest in MCF-7 cells at Sub-G_1 (apoptosis) in a time-dependent manner. Interestingly, for MDA-MB-231, the kernel extract induced strong G2-M arrest in cell cycle progression at 24 hours, resulting in the high Sub-G1 (apoptosis) arrest after 48 and 72 hours of incubation. This apoptosis appears to be caspase-2 and caspase-3 dependent in MCF-7; and caspase-2, caspase-3 and caspase-9 dependent in MDA-MB-231 as studied using ELISA method. These findings suggest *M. pajang* kernel extract has potential as a potent cytotoxic agent in both hormone and non-hormone dependent breast cancer cell lines. The mechanisms for the cytotoxic effects might be associated with caspases activation and G2-M cell cycle arrest leading to the induction of apoptosis. *Acknowledgements: Universiti Malaysia Sabah, Universiti Putra Malaysia, University of Nottingham, UK and Ministry of Science, Technology and Innovation of Malaysia (MOSTI).* **Reference:** [1] Abu Bakar, M.F. et al. (2009) Food Chem. 113:479 – 483.

PE22 Chemical constituents of *Croton ensifolius* leaves

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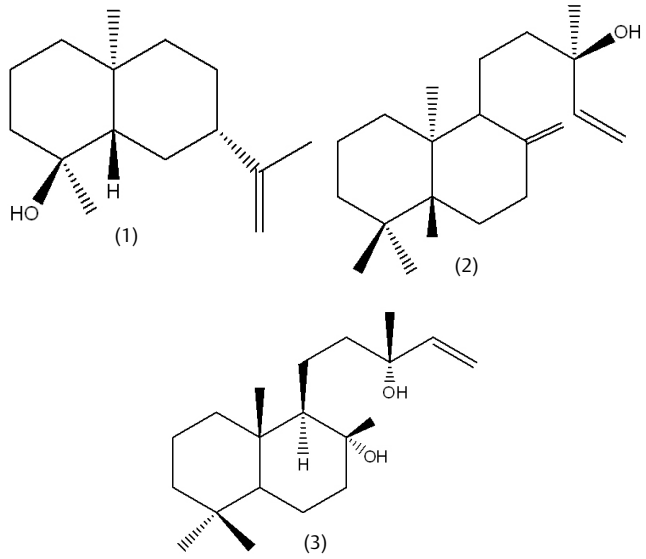
A phytochemical study was carried out on the leaves of *Croton ensifolius* (Euphorbiaceae) which showed cytotoxic activity against HL-60 cell lines (IC_{50} 28.17 μg/ml). Five compounds have been successfully isolated from its active fraction (90%-MeOH fraction, IC_{50} 17.27 μg/ml) including (+)-selin-11-en-4α-ol (**1**), ent-13-epimanool (**2**) and 13-episclareol (**3**) and two other triterpenoids. This recent study was the first report of chemical constituents of this plant.



PE23

Mechanisms underliying the antiproliferative effects of *Citrus bergamia* juice on HepG2 human hepatocellular carcinoma cell line

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Citrus bergamia Risso et Poiteau (bergamot) is a small tree belonging to the family Rutaceae, cultivated almost exclusively along the southern coast of Calabria region (Italy). Due to the growing interest concerning the antiproliferative activities of several fruit juices and vegetable extracts, the object of the present study is to evaluate the effects of C. *bergamia* juice (BJ) on the HepG2 human hepatocellular carcinoma cell line proliferation. C. *bergamia* fruits were hand-squeezed and small aliquots were stored at -20 °C. Then, BJ was defrosted, filtered, adjusted to pH 7.4 and diluted in culture media until the desired concentrations just prior to use. The incubation of HepG2 cells with increased dilution of BJ ranging from 0.5% and 10% for 24, 48 and 72 hours, reduces cell proliferation in time and concentration-dependent manner, as assessed by MTT test. This data was confirmed by the cell growth curves analysis. The reduction of HepG2 growth rate seems to be correlated, at least in part, to a cytotoxic action elicited by BJ, as suggested by the trypan blue assay that shows a significant cell death induced by BJ 10%. Moreover, Annexin V staining suggests that BJ is able to activate the programmed cell death, and shows that apoptotic population increase in relationship with the time incubation and BJ concentration, reaching the 65% of apoptotic cells after 72 hours of treatment with BJ 10%. Western blot analysis suggests that apoptosis of HepG2 cells induced by BJ could be due to a decreased expression of Bcl2 and BclXL. Furthermore, BJ increase the expression of P53 that may contribute to the pro-apoptotic activity of BJ and may be responsible for the HepG2 cell cycle arrest in G2 phase. Our data indicate the ability of BJ in reducing the growth of HepG2 human hepatocellular carcinoma cell line by both a pro-apoptotic mechanism and cell cycle arrest, suggesting a promising role as anticancer drugs.

PE24

Extracts from *Cistus albidus* are effective antioxidants and inhibitors of cell proliferation *in vitro*

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The aim of this study was to assess the antioxidant, antiproliferative, cytotoxic and apoptotic effects of aqueous and methanol extracts from the Western Mediterranean species *Cistus albidus* L. (Cistaceae). Antioxidant activity was evaluated by three different assays: Folin-Ciocalteau, trolox equivalent antioxidant capacity and oxygen radical antioxidant capacity. Antiproliferative activity and cytotoxicity were evaluated on HeLa cells by the crystal violet and WST-1 assays, respectively. The distribution of normal and apoptotic cells in the various phases of cell cycle was analysed by flow cytometry. The data showed that both *C. albidus* extracts exhibit interesting antioxidant properties, although slightly higher in methanol. Extracts inhibited cell proliferation and reduced cell viability in a time and concentration dependent manner. Methanol extract at 136 μg ml^{-1} and aqueous extract at 169 μg ml^{-1} reduced 50% of cell proliferation after 72 h. In the cytotoxicity assay, IC_{50} values increase to 353 and 389 μg ml^{-1}, respectively for methanol and aqueous extracts. The sub-G1 population significantly increased in cells treated with 300 μg ml^{-1} of methanol and aqueous extract, indicating apoptotic-associated chromatin degradation. Moreover, in the non-apoptotic population the HeLa cells treated with methanol extract seem to accumulate in the G_2/M phase and, on the other hand, the aqueous extract seems to cause the accumulation of cells in the G_0/G_1 phase. In conclusion, this study demonstrates the strong antioxidant potential and anticancer activity, through the inhibition of cell proliferation and induction of apoptosis on cancer cells, of methanol and aqueous extracts from *C. albidus*. *S. Gonçalves acknowledges a grant from Portuguese Science and Technology Foundation (FCT, Grant SFRH/BPD/31534/2006).*

PE25

Antioxidant compounds from *Solenostemon monostachys (*P.Beauv.) Briq. (Lamiaceae)

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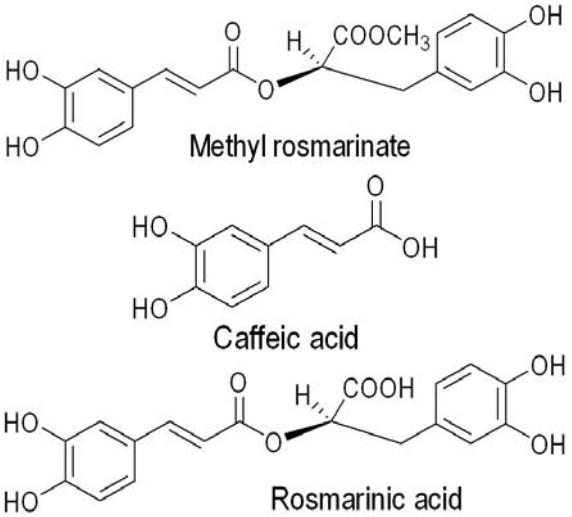
*Solenostemon monostachys (*P.Beauv.) Briq. (Lamiaceae) is used in ethnomedicine to treat convulsion, epilepsy, infection, rheumatism and other inflammatory conditions [1]. The crude 50% aqueous ethanolic extract of the aerial parts of the plant gave an inhibition of 68.8% in the carrageenan induced oedema of the rat paw test as against 81.3% by Indomethacin. Repeated chromatographic separation of the crude 50% aqueous ethanolic extract on silica gel and Sephadex LH-20 monitored by DPPH bioautographic assay led to the isolation of three compounds whose structure were determined using ^1H NMR, ^{13}C NMR (1D, 2D, APT) spectroscopy and comparison with literature values. The compounds were identified as caffeic acid (CA), rosmarinic acid, (RA) and methyl rosmarinate (MR). These compounds are reported for the first time in this plant. The Ferric Reducing Antioxidant Power assay gave 19.180, 5.740 and 9.730 mMol Fe^{2+}/mg of RA, CA, and MR respectively against 4.40 and 7.90 mMol Fe^{2+}/mg of BHT and Vit. C respectively. In the radical scavenging assay of the compounds using DPPH, the IC_{50} for the compounds were 43.3, 51.34 and 56.2 μg/L for RA, MR and CA respectively against 61.45 μg/L for gallic acid. Since reactive oxygen species are linked with inflammation, the presence of these compounds in *Solenostemon monostachys* P.Beauv. (Briq.) (Lamiaceae) may justify some of the ethnomedicinal uses of the plant.



Acknowledgement: Central Science Laboratory, Obafemi Awolowo University, Ile Ife, Nigeria for the acquisition of the U.V and NMR spectra data.

References: [1] Burkill, H.M. (1995) The useful plants of West Tropical Africa, 2nd Edition, Vol.3 Families J-L. Royal Botanical Gardens, Kew. United Kingdom.

PE26 Anticancer activities of Thai medicinal plant recipes

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Some Thai traditional recipes for anticancer treatment are still found in the markets and used for remedies in household when the modern medicines are not effective. The objective of this study was to investigate the anticancer activity of the anti-cancer recipes which were surveyed and collected during June 2007 to December 2008 from one hundred Thai traditional healers in 4 region of Thailand (North, Northeast, Central and South) with 25 healers in each region. The total of 201 recipes were collected with 46, 43, 61, 51 recipes from the North, the Northeast, the Central and the South respectively. The five highest frequency plants found were *Smilax glabra* Wall.ex Roxb, *Smylax peguana, Rhinacanthus nasutus* Kurz, *Stemona tuberosa* Lour and *Suregada multiflorum* Bail. which had the frequency of 96, 95, 39, 39 and 33 respectively. Twenty four recipes with high evidences for anticancer treatment were selected to test for the growth inhibitory activity on human mouth epidermal carcinoma (KB) cell lines by Sulforhodamine B (SRB) method. The recipe numbers 1, 2, 7, 8, and 9 exhibited growth inhibit activity against KB cell lines with the GI_{50} values of 10.92, 7.66, 14.97, 13.78, and 4.77 μg/ml, which were less than 30 μg/ml according to the NCI criteria indicating having anti-cancer activity. However, their activities were lower than doxorubicin ((GI_{50} = 0.02 μg/ml)) of 546, 383, 748.5, 689 and 238.5 times respectively. The results from this study have indicated the beneficials of the Thai folklore wisdom in cancer therapy. *Acknowledgements: Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health for funding and folk doctors for data.* **References:** [1] Saetung, A. et al. (2005) Songklanakarin J. Sci. Technol. 27(Suppl. 2):469 – 478. [2] Wattanapiromsakul, C. et al. (2005) Songklanakarin J. Sci. Technol. 27(Suppl. 2):479 – 487. [3] Skehan, P. et al. (1990) J. Natl. Cancer Inst. Jul. 82:1107 – 1112.

PE27 Chemical composition of some *Sargassum* species and their cytotoxic and antimicrobial activities

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The comparative study of the chemical composition of the brown algae *Sargassum Asperifolium*, *Sargassum dentifolium* and *Sargassum linifolium* (Family: Sargassaceae) from the Red sea at Hurghada, Egypt was investigated. The volatile constituents obtained by hydrodistillation [1] as well as unsaponifiable matters and the fatty acids were analyzed qualitatively and quantitatively by GC/MS technique. The analysis of the volatile fraction led to identify sexual pheromones, terpenes, phenolic compounds, free fatty acids and esters. The most abundant sterol was fucosterol and cholesterol in all algae. Palmitic acid was found in all studied algae as major fatty acid. Cytotoxicity of the isolated crude extracts have been carried out *in vitro* on different human cell lines using method of Skehan *et al.* [2] Furthermore, antimicrobial activity of the volatile constituents, successive extractive, unsaponifiable matters and fatty acids has been tested on 11 different micro-organisms using antibiotic assay method [3]. The obtained biological screening proved that the tested algae have various cytotoxic and antimicrobial activities. **References:** [1] Macleod, A.J., Cave, S.J. (1975) J. Sci. Food Agric. 26:351 – 360. [2] Skehan, P. et al. (1990) J. Nat. Cancer Inst. 82:1107 – 1112. [3] Gnanamanickam, S.S., Mansfield, J.W. (1981) Phytochemistry, 20:997 – 1000.

PE28 Cytotoxic activities of different extracts of *Euphorbia boissieriana* (Woron.) Prokh.

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Introduction*: Euphorbia* is the main genus of Euphorbiacea family which includes more than 2000 species. Of about 82 species of this genus grown in Iran some have been used to treat arthritis and wart in Iranian traditional medicine. This genus also has attracted much of interest for its cytotoxic characteristic. Therefore, we aimed to study the cytotoxic activity of the aerial parts of an endemic *Euphorbia* species, *Euphorbia boissieriana* (Woron.) Prokh. **Methods:** Wild samples of *E. boissieriana* were collected in Semirom area, Isfahan Province, Iran, in June 2007. Aerial parts of plant were air dried, powdered and extracted by different solvents including: Methanol-water (70 – 30), methanol, ethylacetate, acetone, dichloromethane and hexane. The cytotoxicity of various concentrations of each extract was studied on Hela cells using the colorometric MTT assay *in vitro*. **Results:** The acetone, ethylactate, dichloromethane, methanolic and hexanoic extracts of *E. boissiariana* exhibited cytotoxic activities in a decrease manner, respectively. However the hydroalcoholic extract possessed no cytotoxic activity at all concentrations tested. Discussion: Exhibition of higher cytotoxic activities of acetone, ethylactate, dichloromethane extracts where compared with methanolic or hydroalcoholic extracts (both being more polar than other solvents) and hexaonic extract (being the most non polar solvent of the group) may indicate that major cytotoxic compounds of this plant have low to moderate polarity. Further phytochemical studies are being conducted to isolate and elucidate the individual compounds responsible for this activity. *Acknowledgements: This work was supported by Research council of he Isfahan Unvisity of Medical Sciences, Isfahan, Iran. (Research project No. 386252*).

PE29 Mutagenic and antimutagenic effects of the traditional phytoestrogen-rich herbs, *Pueraria mirifica* and *Pueraria lobata*

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This study aimed to evaluate the mutagenic and antimutagenic potentials of *Pueraria mirifica* and *Pueraria lobata* by the Ames test preincubation method in the presence and absence of rat liver S9 mixture for metabolic activation in *Salmonella typhimurium* strains TA98 and TA100. The cytotoxicity of the two plant extracts to the two *S. typhimurium* indicator was evaluated. Both plant extracts at a final concentration of 2.5, 5, 10 or 20 mg/plate exhibited only mild cytotoxic effects. At a final concentration of 2.5, 5, or 10 mg/plate in the presence and absence of S9 mixture, both were negative in the mutagenic Ames test. Both plant extracts were positive in the antimutagenic Ames test towards either one or both of the tested mutagen: 2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide (AF-2) and benzo(a)pyrene. Both the absence of mutagenic and the presence of antimutagenic activities of the two plant extracts were confirmed in *rec*-assays. Micronucleus test of both plant extracts exhibited no significant micronucleus formation in the tested rats. The overall tests confirmed the non-mutagenic but reasonably antimutagenic activities of the two plant extracts thus supporting their current prescribed as safe dietary supplements and cosmetics.

PE30 Anti-proliferation activity and phytochemical screening of Thai medicinal plant recipes

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Thai medicinal plant recipes were surveyed and divided into two groups including the books of Thai traditional medicine and Thai traditional healers. 12 recipes were found from the books of Thai traditional medicine and 28 recipes from 14 questionnaires. 10 recipes were selected from 40 recipes of the books and questionnaires to test the anti-proliferation activity on human mouth epidermal carcinoma (KB) cell lines by Sulforhodamine B (SRB) assay. The anti-proliferation activity and phytochemical screening of 9 herbs in recipe 9 was investigated. The results found that *Hydnophyum formicarum* exhibited growth inhibit activity against KB cell lines with GI_{50} value 5.54 μg/ml, which were about 1/277 times of doxorubicin (GI_{50} = 0.02 μg/ml). *Hydnophyum formicarum* contain tannin, alkaloid, flavonone and xanthone. The results from this study will be beneficial for further research and development of anticancer drug. **References:** [1] Manosroi J. et al. (2006) Cancer Letter 235:114 – 120. [2] Saetung, A. et al. (2005) Songklanakarin J. Sci. Technol. 27(Suppl. 2):469 – 478. [3] Wattanapiromsakul, C. et al. (2005) Songklanakarin J. Sci. Technol. 27(Suppl. 2):479 – 487. [4] Skehan, P. et al. (1990) J. Natl. Cancer Inst. 82:1107 – 1112.

PE31 Cationic nanoliposomes enhance cytotoxicity activity of curcumin

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Curcumin (diferuloylmethane) is a promising natural product with pleiotropic pharmacological activities. Among these, potential anticancer properties are stirring up the research interest all around the world [1]. Therapeutic applications of curcumin would imply its proper formulation into a suitable pharmaceutical form which ideally would enhance its activity by maximising delivery to the cancer cells. We here report the effects of several nanoliposomal formulations loaded with curcumin using a modified ethanolic proliposome method [2]. Cationic nanoliposomes were produced from ethanol-dimethyl sulfoxide-based egg phosphatidylcholine with cationic surfactant dimethyl dioctadecyl ammonium bromide (DDA) containing curcumin by addition of isotonic sodium chloride. Their affects on the viability of cervical cancer cells (HeLa cells) were measured after 48 hours incubation by an MTS assay [3]. Free curcumin had an LC_{50} 90 μM, whilst two different nano liposomes bearing a positive net charge in their surface were able to dramatically lower the LC_{50} down to 2 – 1.5 μM). The other nanoliposomal formulations exhibited the same LC_{50} as free curcumin. The results indicate that positively charged nanoliposomes formulations enhanced the *in vitro* cytotoxicity activity of curcumin in cervical cancer cells and therefore they could be a promising delivery system for this potent natural product. **References:** [1] Anand, P. et al. (2008) Planta Med. 74 : 1560-1569. [2] Taylor, K.M. et al. (2006) J. Pharm. Pharmacol. 58: 887-894. [3] Mosmann, T. (1983) J. Immunol. Methods 65: 55-63

PE32 Monoterpene indole alkaloids from the leaves of *Tabernaemontana elegans* induce apoptosis activity in human hepatoma cells

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Apoptosis (programmed cell death) is a natural mechanism to eliminate unwanted or cancerous cells and virtually all the anticancer drugs currently utilized can induce apoptosis in susceptible cells [1]. Morphologically, this process is characterized by plasma membrane blebbing, cell shrinkage, and chromatin condensation followed by disassembly of the cell into multiple membrane-enclosed fragments, which are then engulfed by neighbouring cells or professional phagocytes. In our search for molecules with apoptosis inducing activity from medicinal plants, we have isolated three known and a new corynanthe type monoterpene indole alkaloids from the methanol extract of leaves of *Tabernaemontana elegans*. The structures of these compounds were elucidated by a series of spectroscopic experiments. The identification of the known alkaloids tabernaemontanine, vobasine, and dregamine, was corroborated by comparison of their spectroscopic data with those reported in literature [2]. The isolated monoterpene indole alkaloids were studied for their apoptosis induction activity in human hepatoma (HuH-7) cells. Methodology for apoptosis detection included cell viability assays, nuclear morphology evaluation, and general caspase-3-like activity assessments. Tabernaemontanine and vobasine showed the most promising apoptotic induction profile in HuH-7 cells, inducing 41 and 44% of apoptosis, respectively, after 24 h of exposure. Caspase activity assays confirmed these results. Our data suggest that monoterpene indole alkaloids from the leaves of *Tabernaemontana elegans* may be considered as significant apoptosis inducers and should be further studied in other cell lines. *Acknowledgements: This study was supported by a fellowship from FCT, Portugal (reference number BPD/30492/2006).* **References:** [1] Kaufmann, S.H. et al. (2000) Exp. Cell Res. 256:42 – 49. [2] Bombardelli, E. et al. (1976) J.C.S. Perkin I:1432 – 1438.

PE33 Novel β-carboline indole alkaloids from the leaves of *Tabernaemontana elegans*

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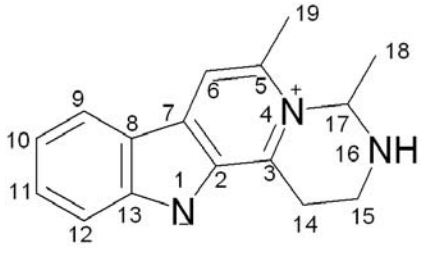
The genus *Tabernaemontana* (Apocynaceae) has a wide distribution and plants belonging to this genus are used in traditional medicine to treat cancer [1]. These plants are characterized to produce indole alkaloids of unusual structures as well as novel bioactivity. We have isolated three β-carboline indole alkaloids (**1-3**) from the MeOH extract of the leaves of *Tabernaemontana elegans*. The chemical structures of these novel entities were established by means of spectroscopic techniques including 2D NMR spectroscopic experiments.



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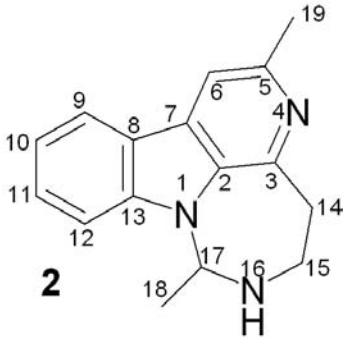

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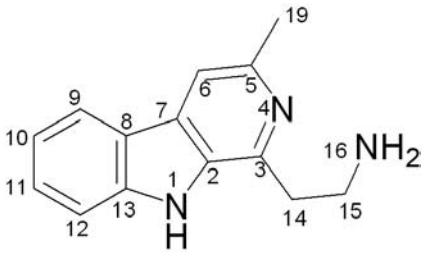

3

The new skeletal features of compounds **1** and **2** were the presence of a two-carbon unit, attached to a structurally related β-carboline skeleton [2], resulting in the formation of additional six and seven-membered new rings in **1** and **2**, respectively. To the best of our knowledge, it appears to be the first report on the isolation of β-carboline indole alkaloids from the genus *Tabernaemontana.* Compounds **1**-**3** were evaluated for their potential P-glycoprotein modulating properties using the rhodamine-123 assay, in both MDR1-gene transfected and parental mouse lymphoma cell lines. Compounds **1** and **3** exhibited a weak activity. *Acknowledgements: This study was supported by a fellowship from FCT, Portugal (reference number BPD/30492/2006).* **References:** [1] Graham, J. et al. (2000) J. Ethnopharm. 73:347 – 377. [2] Sandler, J.S. et al. (2002) J. Nat. Prod. 65:1258 – 1261.

PE34 Determination of rosmarinic acid and rutin in *Hymenocrater bituminosus* by using TLC

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A simple, rapid, precise, accurate and repeatable thin-layer chromatographic (TLC) method has been established for the determination of rosmarinic acid and rutin in whole plant powder of *Hymenocrater bituminosus* Fisch. and C.A. Mey. Rosmarinic acid and rutin have been reported to have strong antioxidant properties and also anti-diabetic, antithrombotic, anti-inflammatory and anti-carcinogenic activity [1,2]. The aqueous methanolic extract of aerial parts of plant powder was prepared using Sonication Extraction Method (SEM). The concentration of rosmarinic acid and rutin in the whole plant powder were found to be 1.37 and 0.5% (w/w) respectively. Separation was performed on TLC aluminum sheets silica gel 60 F254 plates with ethyl acetate-methanol-distilled water-formic acid (7.2:0.8:1.3:0.7) for rutin and acetone-toluene-formic acid (4:5:1) for rosmarinic acid as mobile phase. Sample solutions for TLC analyses were applied by means of a CAMAG Linomat 5 automated spray-on band applicator. The determination was carried out using the densitometry absorbance mode at 366 nm using a CAMAG TLC Scanner 3. The linear range for rutin and rosmarinc acid were 50 – 450 ng with correlation coefficient (r-value) of 0.991 and 150 – 600 ng with correlation coefficient 0.991 respectively. The variability of the method was expressed as intra-day and inter-day precision **References:** [1] Petersen, M., Simmonds M.S.J. (2003) Phytochemistry 62:121 – 125. [2] Lacopini, P. et al. (2008) J. Food Comp. Anal. 21:589 – 598.

PE35 Antiproliferative activity: Extract versus isolated active constituent

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Recently, there have been extensive efforts to evaluate the chemopreventive role of substances present in natural products. We compared the antiproliferative potency (measured by WST-1 assay) of four plant extracts from the gum resin of *Boswellia serrata* Roxb., *Hypericum perforatum* L., *Cimicifuga racemosa* L.and *Salix* sp. to their biologically active compounds boswellic acid (AKBA), hypericin (HY), hyperforin (HP), triterpene glycosides (TTG), cinnamic acid esters (CAE) and salicin (SAL) using different cancer cells. Substances were compared by their GI_{50} values. In three leukaemia cell lines (K562, U937 and MOLT-4) the effect of the crude extract of *B. serrata* was 2.3 – 3.3 times more potent than AKBA [1].The antiproliferative effect of *H. perforatum* extract depends on light activation of HY. However, in the dark, the effect of *H. perforatum* extract on K562 and U937 cells was found 10-times more potent than HY, but 2-times less potent than HP [2]. Furthermore, HY and HP acted synergistically on cell growth inhibition in the dark [3]. The effect of two main classes of compounds, TTG and CAE on breast ER^+ MCF-7 and ER^- MDA MB231 cancer cells was 2.2 – 3 times and 4 – 6 times less potent in comparison to their parent isopropanolic *C. racemosa* extract [4]. Effect of willow bark extract, its fraction of salicylic alcohol derivates F1 [5] and SAL was investigated on the cell growth of COX-2-proficient HT-29 and COX-2-deficient HCT 116 colon cancer cells. There were differences of about two decades of logarithmic scale for favour of extract in comparison to the single compound SAL and the parent extract was 3-times more potent than the isolated fraction F1. Natural products as a multiple compounds mixtures possess the potential to attack cancer cells more effectively than their single active constituents. **References:** [1] Hostanska, K. et al. (2002) Anticancer Res. 22:2853 – 2862. [2] Hostanska, K. et al. (2003) J. Pharm. Pharmacol. 55:973 – 980. [3] Hostanska, K. et al. (2003) Eur. J. Pharm. Biopharm. 56:121 – 132. [4] Hostanska, K. et al. (2004) Biol. Pharm. Bull. 27:1970 – 1975. [5] Hostanska, K. et al. (2007) Cancer Detect. Prev. 31:129 – 139.

PE36 Inhibition of P-glycoprotein activity by curcubitane-type triterpenes and their interaction with doxorubicine on resistant cancer cells

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The overexpression of P-glycoprotein (P-gp) is one of the mechanisms of multidrug resistance (MDR), responsible for the failure of cancer treatment. One strategy to restore the effectiveness of the anti-cancer drugs is to co-administer compounds that are not toxic themselves, but inhibit these efflux pumps. These compounds have been called MDR inhibitors, MDR modulators, MDR reversal agents or chemosensitizers. In recent years, several compounds have been reported as MDR modulators, obtained either from natural origin or by synthesis. However, in spite of the great number of MDR inhibitors known, no effective modulator without side effects is still available for the clinical practice [1]. In our search for biologically active compounds from *Momordica balsamina* L., a climber extensively cultivated and used in tropical and subtropical countries to treat various diseases [2], we have isolated three new curcubitane-type triterpenes, named balsaminagenin A and B, and balsaminoside A, together with the known curcubitacine karavelagenin C. Moreover, karavelagenin C was derivatized using several reagents, to afford five new mono- and diacylated compounds. All the structures were deduced from their physical and spectroscopic data, including 2D NMR experiments (COSY, HMQC, HMBC and NOESY). The ability of these curcubitane-type triterpenes to inhibit P-gp activity was investigated by flow cytometry, in a rhodamine-123 exclusion test using human MDR1 gene-transfected mouse lymphoma cells. Verapamil was used as positive control. Some of the tested triterpenes have shown to enhance strongly drug retention by inhibiting the efflux pump activity mediated by P-gp. Furthermore, in the model of combination chemotherapy, the interaction between doxorubicin and the most active compounds was studied *in vitro*. All of them synergistically enhance the effect of the anticancer drug in combination. *Acknowledgements: The authors wish to thank the Science and Technology Foundation, (FCT, grant SFRH/BD/22321/2005).* **References:** [1] Szakács, G. et al (2006) Nat. Rev. Drug Discov. 5:219 – 234. [2] Flymen, M.V., Afolayan, A.J. (2007) Int. J. Food Sci. Nutr. 58:419 – 423.

PE37 Dioscoreanone-induced growth arrest and apoptosis in lung carcinoma cells

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Dioscoreanone is a new member of the 1, 4-phenanthraquinone derived from the ethanolic extract of *Dioscorea membranacea* Pierre (or "Hua-Khao-Yen" in Thai) rhizome that has long been used as a common ingredient in Thai traditional anticancer medicines [1,2]. In this study, we have found that Dioscoreanone mediated strong and selective antiperiferative activity against two human non-small cell lung cancer (NSCLC) cell lines: A549 (adenocarcinoma) and COR-L23 (large cell carcinoma) (IC_{50} 3.03 and 6.19 μM, respectively). This effect occurred in a dose-dependent manner in both cancer cell lines. By contrast, in the human small cell lung cancer (SCLC) cell line NCI-H1688, this compound showed weak cytotoxicity (IC_{50}16.68 μM), indicating that its cytotoxicity was specific to only NSCLC subtype. Similarly, it exerted moderate cytotoxicity against non-tumorigenic human lung fibroblast MRC-5 cells with a significant difference in the IC_{50} of 10.47 μM compared to A549 and COR-L23 cells. Moreover, at doses of 7, 14, 35 and 18 μM, Dioscoreanone caused 100% cell death in COR-L23, A549, NCI-H1688 cancer cells and fibroblast MRC-5 cells, respectively, which suggested its cytotoxic effect. The molecular mechanisms underlying this effect were studied in COR-L23, due to its high sensitivity to Dioscoreanone. DNA fragmentation assay detected ladder pattern characteristic of apoptosis in Dioscoreanone-treated COR-L23 cells in a dose- and time-dependent manner. Taken together, our study showed that Dioscoreanone could exhibit potent as well as selective antiproliferation and cytotoxicity against COR-L23 cells through apoptotic induction. Consequently, its potential as a chemotherapeutic agent for certain cancer types is worthy of further investigation. *Acknowledgements: This work was supported by the National Research Council of Thailand.* **References:** [1] Itharat, A. et al. (2003) Org. Lett. 5:2879 – 2882. [2] Itharat, A. et al. (2004) J. Ethnopharmacol. 90:33 – 38.

PE38 Uptake of xanthohumol in human liver and intestinal cells

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Humulus lupulus L. (hops) has long been used in traditional medicine with sleep disturbances being the main indication. However, scientific evidence has accumulated over the past years pointing to the cancer preventive potential of selected constituents, i.e. xanthohumol (XN), a prenylated chalcone. The mechanisms of protection by XN have been proposed to be inhibition of metabolic activation, induction of detoxifying enzymes and antioxidant activity [1]. Moreover a broad antiinfective potential of XN is described [2]. Despite many advances in understanding the pharmacology of XN, one largely unresolved issue is the low bioavailability of XN in the human organism. Also little is known about actual concentrations and pharmacokinetic of the compound and its metabolites in liver and intestinal cells. We studied the cellular uptake of xanthohumol (XN) in various cell lines, namely hepato cellular carcinoma cells (Huh-7), hepatic stellate cells (HSZ-B) and colorectal adenocarcinoma cells (Caco-2), as well as in primary hepatocytes. Uptake and intracellular distribution of fluorescent XN was analysed using advanced fluorescence imaging techniques. XN concentrations, determined via reversed-phase high-performance liquid chromatography (HPLC), and cell volumes, obtained using 3D-Imaging, were combined for the estimation of inner cell concentrations. We observed a very rapid accumulation of XN, reaching a maximum already after 30 – 60 min. Furthermore we found that HSZ-B and Caco-2 cells showed a much higher XN uptake than Huh-7 cells. In addition different XN metabolites were identified. Our data provide deeper insights into XN biology on the cell level and will contribute to a better understanding of its pharmacology. *Acknowledgements: We thank Dr. Thomas Weiss, Center for Liver Cell Research, Department of Surgery, University of Regensburg, Germany, for providing primary hepatocytes.* **References:** [1] Zanoli, P. et al. (2008) J. Ethnopharmacol. 116:383 – 396. [2] Gerhäuser, C. (2005) Mol. Nutr. Food Res. 49:827 – 831.

PE39 Oleanolic acid rich solubilized triterpene extracts from mistletoe induce apoptotic and necrotic cell death of murine B16. F10 melanoma cells

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Oleanolic acid (OA) is an almost water insoluble (< 0.02 μg/ml) [1], naturally occurring pentacyclic triterpenoid, which has a variety of biological effects (e.g. anti-cancer and anti-inflammatory, reviewed in [2]). OA is the main component (~80%) of triterpene extracts from European mistletoe, which does not contain mistletoe lectins and viscotoxins. The toxic solvent DMSO is normally used for *in vitro* administration of OA. This restricts the use of OA due to limited solubility of OA in DMSO and toxicity of DMSO itself. By using 2-hydroxypropyl-beta-cyclodextrin as complexing agent for triterpene extracts from mistletoe we obtained solubilized triterpene extracts (STE) which allow *in vitro* and prospective *in vivo* administration of OA in a water soluble form with excellent bioavailability without toxic solvents. Apoptosis induction by OA is reported for different non-melanoma cell lines [3]. We show here by annexin-V/PI staining and by an oligonucleosome ELISA that STE and its main component OA (standard) induce apoptosis of B16. F10 mouse melanoma cells. Maximum apoptosis induction was detected with OA concentrations from 15 to 20 μg/ml. Higher OA concentrations (> 30 μg/ml) induce a shift from apoptotic to necrotic cell death. In summary we demonstrate that STE and OA, both solubilized by complexation with cyclodextrins, is able to induce apoptotic and necrotic cell death of B16. F10 mouse melanoma cells. *Acknowledgements: Software AG Stiftung, Darmstadt, Germany; Rudolf Steiner Fonds für Wissenschaftliche Forschung, Nürnberg, Germany.* **References:** [1] Jäger, S. et al. (2007) Planta Med. 73:157 – 162. [2] Liu, J. (1995) J. Ethnopharmacol. 49:57 – 68. [3] Martin, R. et al. (2007) Cancer Res. 67:3741 – 3751.

PE40 Cytotoxic and estrogenic activity of the Thai rejuvenating medicinal plants on MCF-7 mammary cancer cell

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The twenty one extracts of the Thai medicinal plants used in rejuvenating medicine; *Acacia farnesiana, Leucaena leucocephala, Butea superba, Pueraria mirifica, Mucuna collettii, Kaempferia parviflora, Fagraea fragrans, Ziziphus attopensis, Anaxagorea luzonensis, Dracaena conferta, Streblus asper, Gelonum muliflorum, Vitex negundo, Diospyros rhodcalyx, Albizia procera, Tinaspora crispa, Stephania venosa, Stephania erecta, Piper nigrum, Phyllanthus emblica, Melia azedarach, Cyperus rotundus* were evaluated for cytotoxic and estrogenic activity on MCF-7 mammary cancer cells at the concentration of 0, 0.1, 1, 10 100 and 1000 μg/ml for 72 hours. The incubated cell cultures were determined and calculated for the growth percentage compared with the control group (DMSO) by MTT assay. The results showed that plant samples at the concentration of 0.1 – 1 and 10 μg/ml were not influenced to the cell growth rate (106.88 ± 18.53 and 101.60 ± 25.82, respectively). *Melia azedarach* and *Fagraea fragrans* exhibited the highest (416.37%) and lowest (0.00%) cell growth, respectively. All plant extracts showed significantly higher IC_{50} than the control with seven plants exhibited IC_{50} > 1000 μg/ml, and thirteen plants exhibited proliferative effect to MCF-7 in the same pattern as estradiol. The results demonstrated that there are some Thai medicinal plants with estrogenic activity present in the traditional Thai remedies for rejuvenating purposes. *Acknowledgements: The authors*

thank the Office of Research and Academic Service, Suan Sunandha Rajabhat University, and National Research Council of Thailand for a grant support. **References:** [1] Freshney, R.I. (1994) Culture of animal cell: A manual of basic technique. Wiley-Liss, Inc. New York. [2]iMedical Registration Division, Ministry of Public Health. (1998) The Thai Traditional Remedies: Pharmacological Division. Bangkok: Co-operation community of Thailand Printing. [3]iOgawa, S. et al. (1998) Endocrinology 139:5070 – 5081.

PE41 Heterologous expressed human cytochromes are useful tools in synthesis of thymoquinone

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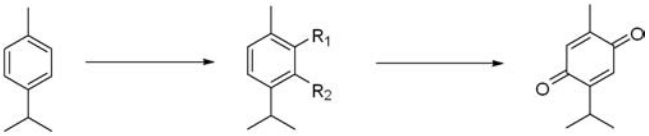
Thymoquinone (**4**) is a phytochemical compound from *Nigella sativa.* Because of its properties like anti-oxidant, anti-cancer and anti-inflammatory effects this compound is interesting for medicinal use. Due to variable contents of thymoquinone in plants the establishment of a standardized medicine is difficult and an alternative synthesis is required. Aim of this project is the biochemical synthesis of the compound of interest directly starting with *p*-cymene (**1**) as a simple and omnipresent terpenoid. Biotransformation of *p*-cymene, thymol (**2**) and carvacrol (**3**) by six human hepatic enzymes, CYP1A1, CYP1A2, CYP2A6, CYP2C9, CYP2D6 and CYP3A4, heterologously expressed in *E. coli* DH5alpha, was investigated. Mixtures of *E. coli* whith expressed enzymes where incubated for 2 hrs at 37 °C and analyzed by HPLC. Human cytochromes CYP1A2 and CYP2A6 are the most promising enzymes in conversion of *p*-cymene to thymoquinone with thymol and carvacrol as intermediates. In case of thymol and Carvacrol as substrates thymoquinone is formed directly, although the transformation of thymol is more efficiently than that of carvacrol. *In-vitro* biotransformation of p-cymene to thymoquinone with heterologous expressed cytochromes could become an interesting alternative to its chemical synthesis.



(1) (2) R1=H, R2=OH (3) R1=OH, R2=H (4)

PE42 Isolation of *Lotus edulis* bioactive flavonoids (Leguminosae) by Centrifugal Partition Chromatography

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Lotus is a genus of *Leguminosae* family which is consist of about 100 species which are native in Mediterranean and N. America countries. The specie *L. edulis* is an annual edible herb which is known in Greece with the common name 'Peratzouni'. The methanolic extract obtained from the aerial part of this plant posses remarkable antioxidant and chemoprotective properties [1]. The photochemical analysis of this extract showed that it is a rich source of Kaempferol glycosides. In order to isolate these flavonoids compounds the Centrifugal Partition Chromatography was used. Twenty one biphasic solvent systems were investigated using TLC and HPLC technique. One of them (Heptane: EtOAc: MeOH: H_2O 1:4:1:4) was proved efficient in the separation of five flavonoids. In addition two more Kaempferol derivatives were separated by with another solvent system (EtOAc:n-BuOH: H_2O 2:1:2). It is important that all the isolated natural compounds are derivatives of 7-O-ramnopyranosyl kaempferol and their purification by conventional chromatographic methods is very difficult. **References:** [1] Spanou, C. et al. (2008) J. Agr. Food Chem. 56:6967 – 6976.

PE43 Synthesis and Cytotoxicity Evaluation of New Aryloxymethylfuranone Derivatives

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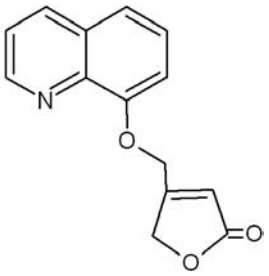
Natural products from plants, traditionally used as therapeutic agents, can serve as templates to synthesize lead compounds to combat disease. Cardiac glycosides such as digitoxin (from *Digitalis*), calotropin (from *Calotropis procera*) and oleanderin (from *Nerium oleander*) have been tapped as a source of anti-tumor drugs [1]. These compounds possess a common butyrolactone (furan(5 H)-2-one) moiety. Such a moiety is considered as the anti-tumor determinant pharmacophore in several cytotoxic and anti-cancer molecules, such as Digitoxin [2], siphonodon [3], and the above cardiac glycosides. In this work several 4-aryloxymethyl furan(5 H)-2-one derivatives have been synthesized and their brine shrimp cytotoxicities were compared to naturally occurring cardiac glycosides. The aryloxymethylfuranones were synthesized from 4-bromomethylfuran(5 H)-2-one and the corresponding substituted phenol under Williamson ether synthesis conditions. The prepared compounds were purified by preparative TLC (EtOAc-hexane, 1:9), and characterized by IR, UV and 1 H-, ^{13}C- NMR. The synthesized compounds exhibited higher brine shrimp activity than their parent phenols, with LC 50 ranging between 40 – 150 ppm. This shows that the activity is partially due to the presence of the furan(5 H)-2-one ring. The highest activity (40 ppm) was shown by 4(quinoline-8-oxymethyl)-furan(5 H)-2-one (figure)



References: [1] Newman, R. et al. (2008) Mol. Interv. 8:36 – 49. [2] Bohlin, L. et al. (2001) Anti-Cancer Drugs 12:475 – 483. [3] Wagner, H. et al. (1981) Planta Med. 43:249 – 252.

PE44 Biological activities of Turkish *Alkanna* species

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Alkanna Tausch is a small genus which comprises of 34 species and 40 taxa in the flora of Turkey [1]. Biological activity studies of naphthoquinone derivatives obtained from *Alkanna* species demonstrated antimicrobial, anti-inflammatory, antithrombotic activity and wound-healing properties as well as DNA-topoisomerase I inhibition [2]. As part of our continuing studies to identify antitumor compounds, we herein report for the first time, a cytotoxic activity study of methanol extracts of 17 *Alkanna* species collected from Turkey. The dried and pulverized roots and aerial parts of plant materials were extracted with methanol, evaporated and freeze-dried. The cytotoxicities of the extracts were evaluated by the MTT assay using six human cancer cell lines (MDA-MB-231, DU145, LNCaP, MCF7, PC-3 and Hep 3B). The extracts obtained from the aerial parts were not active in the dose range of 8 – 32 µG/mL. The root extracts of our endemic species *A. cappadocica, A. pseudotinctoria, A. cordifolia, A. tinctoria* subsp. *subleicarpa, A. tubulosa* showed significant and stronger cytotoxic activities compared to the other extracts and positive control CPT-11 (CAMPTOSTAR®, irinotecan) with IC_{50} values less than 8 µg/mL. Additionally, a bioassay guided fractionation study was performed on *A. cordifolia* for its inhibitory effects on mammalian DNA topoisomerase I. Some of the fractions obtained during RP (C-18) and silica gel fractionations proved to be very potent topoisomerase I inhibitors at nanogram level (~12.5 ng/ml). Further studies are in progress to identify bioactive molecules. *Acknowledgement: This study was sup-*

ported by Turkish Scientific and Technological Research Council of Turkey (Project No: SBAG-3134). **References:** [1] Davis, P.H. (1973) "Flora of Turkey and East Aegean Islands" Vol.3, University Press: Edinburgh. [2] Papageorgiou, V.P. et al. (1999) Angew. Chem. Int. Edit. 38:270 – 300.

PE45 Antiproliferative effects of *Zanthoxilum rhoifolium*

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The present study was designed to evaluate the antiproliferative effects from the stem bark of Zanthoxilum rhoifolium. The basic fractions that were obtained after acid-basic extraction from the methanolic extract, and pure compounds isolated from these fraction, were investigated in vitro toward nine cultured human tumor cell lines, namely, PCO 3 (prostate), UACC62 (melanoma), MCF-7 (breast), NC 460 (lung), K-562 (leukemia), OV-CAR (ovarian), HT-29 (colon), 786 – 0 (renal), NCI-ADR (breast expressing phenotype multiple drugs resistance). From the chlorophorm basic fraction, were isolated eleven compounds, the benzophenantridine alkaloids 6-acetonildihydroavicine (1), 6-acetonyldihydronitidine (2), 6-acetonyldihydrocheleritrine (3), carboximetildihydrocheleritrine (4), dihydrocheleritrine (5), cheleritrine (6), dihydroavicine (7), rhoifoline A (8), rhoifoline B (9), boconnoline (10) and zanthoxyline (11). From the ether acid fraction, the lignanes sesamine (12), gadain (13) and kaerophylin (14) were also isolated. From them, alkaloids 1, 5, 6, 7, 10, 11, and the lignanes 13 and 14 were selected for this study. The Et_2O acid fraction displayed a moderate antiproliferative activities (IC_{50}= 25.0 µg) against the cell lines tested (except for PCO-3, with IC_{50} > 100.0 µg/mL). For MCF7, K-562, OVCAR, PCO-3, and HT29, the $CHCl_3$ basic fraction exhibited the most potent antiproliferative effect, with IC_{50} values of 2.50 µg/mL, and a moderate potency for NCI-460, 786.0 and NCI-ADR, with IC_{50} values of 25 µg/mL. From the isolated metabolites, 6-acetonildiidroavicine (1) diidrocheleritrine (5), and boconnoline (10), were the more promising compounds. 6-acetonildiidroavicine (1), except for K.562, displayed antiproliferative activity against PCO- 3, 786 – 0 and HT 29, with IC_{50} value of 2.5 µg/mL, and against NCI.ADR, UACC.62, MCF7, NCI 460 and OVCAR with IC_{50} value of 25.0 µg/mL. Diidrocheleritrine (5) showed antiprolifetive activity against UACC-62, MCF7, K-562, HT 29 and NCI.ADR with IC_{50} value of 25.0 µg/mL, and cytotoxicity (cell death) against NCI.460, OVCAR, PCO-3 and 786 – 0 at the concentration of 25.0 µg/mL. Bocconoline (10), except for PCO-3 and 786.0 (IC_{50}> 100 µg/mL), showed a moderate antiproliferative activity against K562, NCI.ADR, OVCAR, UACC.62 and NCI 460 with IC_{50} value of 25.0 µg/mL. *Acknowledgement:*The authors thanks CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support. **References:** [1] Gonzaga, W.A. et al. (2003) Planta Med. 69:371.

PE46 Triterpenic structures and polyphenols analysis in birch tree foliar buds

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Foliar buds represent a new category of vegetal products, used in modern phytotherapy as hydro-glycero-ethanolic extracts [1,2]. Foliar buds of birch tree *Betula pendula* Roth were analyzed, regarding polyphenolic structures analysis, qualitative analysis of triterpenic structures and in vitro antitumour activity on cell lines A431 and MCF7. The Folin-Ciocalteu method [3,4] was used to determine total content of polyphenols and a series of 19 standards was used for the HPLC-MS analysis. The TLC chromatography and FT-IR spectroscopy were used for the analysis of triterpenic structures. Birch tree foliar buds contain 545 mg/100 g total polyphenols. TLC and FT-IR analysis emphasized the presence of triterpenic structures in dry extract of foliar buds. In vitro studies on tumour cell lines: skin cancer A431 and breast cancer MCF7 prove that foliar buds extract (5% in DMSO) causes the inhibition of tumoral proliferation. The study showed that HPLC-MS can be successfully used to determine total content in polyphenols and TLC and FT-IR analysis emphasized the presence of triterpenic structures in dry extract of foliar buds. Foliar buds extract in DMSO shows in vitro antineoplasic activity. **References:** [1] Pitera, F. (2000) Compendio di Gemmoterapia Clinica, Editore de Ferrari, Genoa. [2] Hunnius, C. (1998) Hunnius Pharmazeutisches Wörterbuch, Walter de Gruyter, Berlin-New York:. [3] Dehelean, C. et al. (2006) Rev. Chim-Bucharest 57:862 – 865. [4] Peev, C. et al. (2007) Chem. Nat. Compd. 43:259 – 262.

PE47 Modulation of MDR in CEM/ARD 5000 cells by a novel lignan from *Bupleurum marginatum* (Apiaceae)

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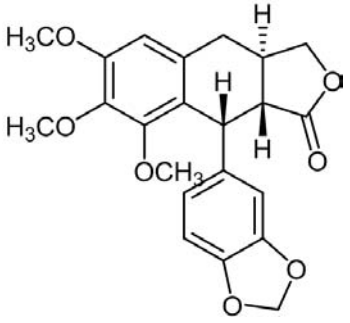
The genus *Bupleurum* (Apiaceae), widely used in Chinese herbal medicine, comprises 200 species that are widely distributed in the northern hemisphere. Based upon traditional Chinese medicine, many *Bupleurum* extracts are used in the treatment of common colds, general inflammations and fever, hepatitis, and different cancers. *Bupleurum marginatum* Wall. ex DC. is one of the popularly used *Bupleurum* species in both TCM and Japanese Kampo. It is a perennial herb with yellow flowers, indigenous to the southern part of China [1]. In continuation of our search for new bioactive secondary metabolites from the plants kingdom which modulate the multidrug resistance in several human cancer cell lines [2,3], we have isolated a novel dibenzylbutyrolactone lignan derivative (BM9) along with isoyatin and other four known flavonoids from *B. marginatum* (Apiaceae) by silica gel column chromatography. The structural elucidation and biological significance of reversing MDR in CEM/ARD 5000 leukaemia cells of the isolated compounds will be discussed.



References: [1] Pan, S.-L., (2006) Bupleurum species: scientific evaluation and clinical applications. Taylor & Francis, Boca Raton. [2] Ma, Y., Wink, M. (2008) Phytomedicine 15:754 – 758. [3] Wink, M. et al. (2006) Planta Med. 72:1121 – 1126.

PE48 Limonin can reverse multidrug resistance (MDR) in human colon and leukaemia cell lines

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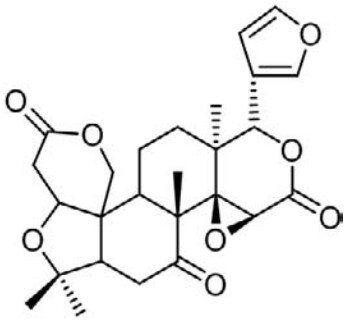
Multidrug resistance (MDR) is a common phenomenon in tumour therapy that is associated with a decreased intracellular drug accumulation because of enhanced drug efflux. It is often related to the over-expression of P-glycoprotein (p-gp) in the cell membrane of tumour cells, thereby reducing the toxicity of cytotoxic drugs [1,2]. In this study, we investigated the effects of naturally occurring limonin from *C. jambhiri* and *C. pyriformis* for its potential to modulate the activity of p-gp in the multidrug-resistant human leukaemia cell line CEM/ADR5000. Limonin inhibited the efflux of the p-gp substrate rhodamine 123 in a concentration-dependent manner. Limonin was more active than verapamil, which was used as a positive control. Treatment of drug-resistant Caco-2 cells with the limonin increased their sensitivity to the cytotoxic doxorubicin and completely reversed doxorubicin resistance, which agrees with a decreased p-gp activity. Limonin was a potent MDR inhibitor, significantly enhancing doxorubicin cytotoxicity to an IC_{50} of 0.395 µM. Limonin and related substances may become candidates for the development of novel MDR reversal agents.



References: [1] Ma, Y. and Wink, M., (2008) Phytomedicine 15:754 – 758. [2] Wink, M. et al. (2006) Planta Med. 72:1121 – 1126.

PE49 *In vivo* toxicological evaluations for betulinic acid and ramified cyclodextrins applied on experimental melanoma models

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The preclinical development of bioactive natural products such as betulinic acid is a major objective of anticancer research programs and their biological applications are very important. Pentacyclic triterpenes with lupan skeleton such as betulinic acid (BA), betulin and lupeol are effective and selective antitumor agents [1,2]. The pharmacokinetic data of BA a structure close to betulin in CD-1 mice had been described by a standard two compartment first-order model applicable also in other in vivo evaluations [1,3]. Branched cyclodextrins are important co-participants to formulations that are increasing the hydrosolubilty [2]. The complexes with oktakis-2,6-di-O-pentyl-gamma cyclodextrin were prepared by kneading procedures in 1:2 ratios. In vivo models were on C57BL/6J mice by a photochemical and inoculation method. The photochemical method used 7,12 dimethyl(a)benzanthracene and TPA as skin promoter and the UVB exposure 5 min/day. The inoculation consists in application of 10^6x0,1 ml A2058 (metastatic melanoma) cells and the same UVB exposure [2]. The skin damages were appreciated by FT-Raman with nanosilver particles and histology techniques. Betulinic acid, an antimelanoma compound lead to important results at 300 mg/bw and increasing of its hydrosolubility accentuate the antitumor activity. The tests were confirmed by vibrational spectroscopy and histopathological evaluation. Skin evolution after the treatment lead to important signal and peak changes and these aspects could be correlated with HE histological evaluation. Betulinic acid is an antimelanoma agent that determines the regression of tumor proliferation in most of cases and malignisation to organs like lungs and changes in the spectral bands for skin between 1100 and 1600 cm^{-1}. *Acknowledgements for financial support to GRANT PN 2-ID 1257/2007.* **References:** [1] Fulda, S. (2008) Int. J. Mol. Sci. 9:1096 – 1107. [2] Dehelean, C. et al. (2008) Rev. Chim-Bucharest 59:887 – 890. [3] Carson, W.E., Walker, M.J. (2002) Tumor models in cancer research, Humana Press, New Jersey.

PE50 Fractionation and determination of the antitumor activity of *Pinus sylvestris* L. bark extracts

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This study was designed to evaluate the antioxidant and antiproliferative effects of pine bark components [1]. Pine bark was extracted with 80% methanol. Liquid-liquid partition of crude extract provided diethyl ether, ethyl acetate, n-butanol and aqueous fractions. The total phenolic content in crude extract and its fractions was determined by the Folin-Ciocalteu method. The polyphenolic profile was analyzed using HPLC-DAD and HPLC-ESI-MS. Screening for antiproliferative effects was investigated on tumour and "normal" cells with the MTT and crystal violet incorporation assays. Antioxidant activity was determined by the DPPH assay [1,2]. The amount of total phenolics ranged from 25.25 ± 0.05 g (diethyl ether fraction) to 69.61 ± 0.22 g (ethyl acetate fraction) gallic acid equivalents per 100 g extract/fraction. The ethyl acetate and the aqueous fractions showed little or no antiproliferative activity. The diethyl ether fraction was the most active as it inhibited MCF-7, PC-3, CaCo-2 and A549 tumour cell proliferation in a dose-depended manner at concentrations ranging from 1 – 100 μg/ml. The n-butanol fraction was less active as it inhibited tumour cell growth at higher concentrations. These fractions had little effect on the growth of "normal" cells (MCF-10A). Both crude extracts and their fractions showed strong antioxidant effects, with the most active being the ethyl acetate fraction (IC_{50}= 7.46 ± 0.03 μg/ml). These studies demonstrate that pine bark is a rich source of compounds with antioxidant and antiproliferative activity and provides leads for their isolation and characterization. *Acknowledgements: This research was supported by a grant from the Cyprus Research Promotion Foundation (KY-ROY/0407/03) and the Romanian Ministry of Education and Research.* **References:** [1] Burdette, J.E. et al. (2002) J. Agric. Food Chem. 50:7022 – 7028. [2] Wangensteen, H. et al. (2004) Food Chem. 88:293 – 297.

PE51 Chemistry and Biological Activities of Naphthoquinones from *Alkanna cappadocica* Boiss.& Bal

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In a continuing program to discover new anticancer agents from plants, especially naphthoquinones from *Alkanna* genus, *Alkanna cappadocica* Boiss.&Bal was investigated [1]. Bioassay-guided fractionation of dichloromethane: methanol (1:1) extract of the roots led to the isolation of four new and four known naphthoquinones. Known compounds *deoxyalkannin* (*1*), β,β-*dimethylacrylalkannin* (*2*), *acetylalkannin* (*3*) and *alkannin* (*4*) [2,3], and new compounds, *5-methoxydeoxyalkannin* (*5*), *8-methoxydeoxyalkannin* (*6*), *5-methoxyacetylalkannin* (*7*), *5-methoxy*-β,β-*dimethylacrylalkannin* (*8*) were fully characterized by spectroscopic analyses (LC-ESI-MS, 1D- and 2D-NMR). Cytotoxicity of the isolated compounds was evaluated versus four human cancer cell lines HT-29, MDA-MB-231, PC-3, LNCaP, together with a normal cell line 3T3 by using MTT assay. Compounds *1, 2, 4, 7* and *8* showed remarkable cytotoxic activity against HT-29, MDA-MB-231 and PC-3 cell lines, comparable or stronger than the other compounds and positive control CPT-11 (CAMPTOSTAR®, irinotecan) with IC_{50} values in between 0.1 μM and 1 μM. In order to confirm mechanism of action through DNA topoisomerase I inhibition which is a common feature for naphthoquinones, the compounds were incubated with topoisomerase I (topo I) and supercoiled DNA. These studies revealed that β,β-*dimethylacrylalkannin* (*2*) and *acetylalkannin* (*3*) have great potential as topo I inhibitors compared to other compounds and CPT-11, with 2 – 3 μM inhibition value. *Acknowledgement: This study was supported by Turkish Scientific and Technological Research Council of Turkey (Project No: SBAG-3134).* **References:** [1] Davis, P.H. (1978) "Flora of Turkey and East Aegean Islands" Vol.3, University Press: Edinburgh. [2] Papageorgiou, V.P. et al. (1999) Angew. Chem. Int. Ed. 38:270 – 300. [3] Kourounakis, A.P. et al. (2002) Arch. Pharm. 335:262 – 266.

PE52 Composition and antimicrobial activity of essential oil from *Dorema ammoniacum*

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The genus *Dorema* is represented in the flora of Iran by six species, two of which are endemic: *D.aucheri* Boiss. and *D. ammoniacum* D. Don [1,2].

The antioxidative activity of the essential oil of *D. ammoniacum*, grown wild in Pakistan has been reported without identifying any compounds [3]. However, the activity of the essential oil of *D. ammoniacum* is less than that of the standard antioxidants like tocopherol, BHA and BHT [3]. The aerial parts of *D. ammoniacum* were collected at the full flowering stage and the essential oil was isolated by hydrodistillation and analyzed by a combination of capillary GC and GC-MS. 29 components were identified, representing 95.17% of the total oil. Z-ocimenone (22.31%) and E-ocimenone (16.13%) were the main components. The *in vitro* antimicrobial activity of the essential oil of *D. ammoniacum* was studied against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The results of antimicrobial testing of the essential oil by the disc diffusion method and MIC values indicated that the oil exhibited moderate to high antimicrobial activity. **Reference:** [1] Rechinger, K.H. (1980) In: Flora Iranica, Umbelliferae, vol 162. 2. Mozaffarian, V.A. (1996) Dictionary of Iranian Plant Names. 3. Rahman, U. et al. (1991) Chem. Soc. Pak. 131:56 – 59.

PE53 Toxicological evaluations for betulinic acid in cyclodextrins complexes on *in vitro* and *in vivo* melanoma models

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Vegetal compounds such as pentacyclic triterpenes with lupan skeleton proved and important antitumor activity. From that group betulinic acid (BA) is an effective antitumor agent with an anti-inflammatory effect [1,2]. These aspect lead to the obtaining of new bioavailable formulations for biological administration that could capacitate their properties and solve their low solubility including cyclodextrin complexation [1,2]. The complexes with gamma cyclodextrins, were prepared by kneading method procedure in 1:2 ratios. In vivo models used C57Bl/6J mice, female, 8 weeks old by inoculation of 1×10^5 B16 cells (mouse melanoma). Cells were suspended in saline solution [3].The cancer promotion was increased by the UVB exposure 5 min/day, 2days/week. The skin damages and metastasis evolution were appreciated by SERS (surface-enhanced Raman scattering) technique and histopathological analysis (HE coloration). Betulinic acid, an important antitumor and selective melanoma compound [1] was tested in vitro on B16 cell culture and on human mesenchimal stem cells from a stock solution, started at 50 µg/ml and in vivo at a dosage of 350 mg/bw. Complexes activity (inhibition of proliferation) was increased *in vitro* with over 10% on both type of tests comparing with the single compound and determine a good answer on in vivo application. The tests were confirmed *in vivo* by vibrational spectroscopy (peak changes) and histology evaluation (metastasis status). Betulinic acid as antimelanoma agent reduces metastasis and tumor dimension if is administrated before the vertical development of the pathology and increasing of its available fraction on biological medium improve the therapeutic answer. *Acknowledgements for financial support to GRANT PN 2-ID 1257/2007* **References:** [1] Fulda, S. (2008) Int. J. Mol. Sci. 9:1096 – 1107. [2] Dehelean, C. et al. (2008) Rev. Chim. Bucarest 59:887-.890. [3] Carson, W.E., Walker, M.J. (2002) Tumor models in cancer research, Humana Press, New Jersey.

PE54 Cytotoxic activity of mammea type coumarins from *Mammea siamensis* flowers

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Mammea siamensis (Miq.) T. Anderson (local name: sarapi) is a Thai medicinal plant in the family Clusiaceae and used in indigenous medicine as a heart tonic. The *n*-hexane fraction from *M. siamensis* flowers CH_2Cl_2-CH_3OH (1:1) crude extract yielded coumarins of the mammea type: mammea A/AA, mammea A/AA cyclo D, mammea A/AB cyclo D, mammea A/AC cyclo D, deacetylmammea E/BA, and deacetylmammea E/BB. The isolated compounds were examined in an *in-vitro* XTT assay against the human MDA-MB-231 (breast adenocarcinoma), U-251 (central nervous system), HCT-116 (colon cancer), as well as the CCRF-CEM (leukemia) cancer cell lines. Only mammea A/AA and the mixture of deacetylmammea E/BA and deacetylmammea E/BB were found to possess significant cytotoxic activities, at 10 µg/mL in CCRF-CEM with values of 78.1 ± 0.8% and 95.9 ± 1.1%; in MDA-MB-231 with values of 58.9 ± 0.8% and 82.4 ± 0.9%; in U-251 with values of 27.7 ± 3.2% and 78.8 ± 1.6%; and in HCT-116 with inhibition values of 73.5 ± 4.9% and 97.6 ± 0.6% in the four human cancer cell lines, respectively, comparable to vinblastine at 0.01 µg/mL, with values of 44.2 ± 8.5%, 51.5 ± 12.9%, 71.0 ± 2.5%, and 54.8 ± 9.4%, respectively. Mammea A/AA and the mixture of deacetylmammea E/BA and deacetylmammea E/BB showed significant cytotoxic activities against the human MDA-MB-231, U-251, and HCT-116 as well as the CCRF-CEM cancer cell lines with IC_{50} values of 7.2 ± 1.0, 5.2 ± 1.0; *nd*, 19.1 ± 1.0; 16.6 ± 1.1, 6.5 ± 1.0; and 20.9 ± 1.4, 4.9 ± 1.0 µM, respectively.

PE55 Protein Kinase Inhibitors from the Endophytic Fungus *Alternaria* sp. isolated from *Polygonum senegalense* Growing in Egypt

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Protein kinases, which function as components of signal transduction pathways, play a central role in diverse biological processes, such as control of cell growth, metabolism, differentiation, and apoptosis [1]. Identification of the key roles of protein kinases in cancer has led to extensive efforts to develop kinase inhibitors for the treatment of a wide range of cancers [2]. In continuation of our efforts to discover natural protein kinase inhibitors we studied extracts of liquid and rice cultures of the fungal endophyte *Alternaria* sp. isolated from the Egyptian medicinal plant *Polygonum senegalense*. Chromatographic separation of the extracts yielded the known compounds alternariol (1), alternariol 5-*O*-methyl ether (2), altenusin (3), 2,5-dimethyl-7-hydroxychromone (4), tenuazonic acid (5), altertoxin I (6), talaroflavone (7), and altenuene (8), in addition to seven new metabolites (9 – 15). The structures of the compounds were unambiguously established on the basis of NMR spectroscopic and mass spectrometric data. Compounds 1 – 3, 9, and 12 showed cytotoxic activity toward L5178Y mouse lymphoma cell line with EC_{50} values ranging from 1.7 to 7.8 µg/mL. When analyzed *in vitro* for their inhibitory potential against 24 different protein kinases, compounds 1 – 3, 6, 9, and 11 – 13 inhibited several of these enzymes (IC_{50}

values 0.22 – 9.8 μg/mL). *Acknowledgements: DAAD (Germany) for a scholarship, Prof. W.E.G. Müller, Institut für Physiologische Chemie und Pathobiochemie, Johannes-Gutenberg-Universität, Mainz, Germany, for MTT assay.* **References:** [1] Fabbro, D. et al. (2002) Pharmacol. Ther. 93:79 – 98. [2] Dancey, J., Sausville, E.A. (2003) Nat. Rev. Drug Discov. 2:296 – 313.

PE56 A new phenolic glycoside from the stems of *Clematis parviloba*

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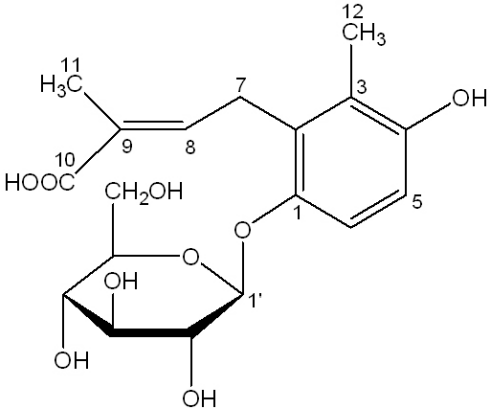
A new phenolic glycoside, clemaparviloside A (**1**) together with three known megastigmane glycosides, linarionoside A (**2**), linarionoside C (**3**) and staphylionoside K (**4**) were isolated from the stems of *Clematis parviloba* [1,2]. Their structures were determined on the basis of spectroscopic analysis and chemical evidence. The megastigmane glycoside compounds are reported for the first time to be obtained from *Clematis* genus. In addition, compound **1** was examined its inhibitory activity against murine fibrosarcoma L929 cells *in vitro*.



References: [1] Hideaki, O. (1994) Phytochemistry 37:461 – 465. [2] Qian, Y. et al. (2005) Chem. Pharm. Bull. 53:800 – 807.

PE57 The extent of risk of using natural antioxidants from region with ecological load in East Slovakia

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Many studies highlighted the high concentration of beta caroten and vitamin C in carrot root which is a reach source of antioxidants and other substances (iron, calcium, potassium, sodium vitamin C). To measure the mercury concentration in particular parts of the carrot (*Daucus carota*) in some areas of Slovakia was the main aim. We monitored mercury existence in carrot (n = 20) and the soil. The results were evaluated according to existing domestic norms [1]. To find the concentration of mercury in some commodities the analytic method for stating mercury by method of fireless atomic absorption spectrometry AMA 254 [2] was used. The lowest concentration was in root (0.01039 mg.kg^{-1}). The lower one was in samples of leaves (0.11897 mg.kg-^{1}). The highest concentration of Hg was in stalk (1.12143 mg.kg^{-1}). In samples of soils there were in the scale from 190 mg.kg^{-1} up to 420 mg.kg^{-1} Hg. The concentration of Hg in underground parts of carrot was surprisingly low. This fact is in contradiction with the statement that mercury is concentrated in roots. Concentration of Hg is even lower than 0,010 mg.kg $^{-1}$, a value expected by the standard norm. *Aknowledgements: MS SR VEGA project 1/4365/07.* **References:** [1] Codes of the Ministry of Agriculture and Ministry of health of Slovak Republic (2004) N°608/2004 – 100. [2] Handbook – AAS AMA 254 (1998) Prague.

PE58 A review on *Pygeum africanum*

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Introduction: *Pygeum africanum*, a member of rosaceae family, is an ever green tree native to Africa and western Indian Ocean lands. It grows about 900 – 3400 meters of altitude. The mature tree is 10 – 25 meter high, open branched and with a round crown of 10 – 20 meter diameter in grasslands. *Pygeum* bark extract has been used in Europe since the mid-1960 s to treat men suffering from benign prostatic hyperplasia (BPH) the aim of this study was to assess the plants treatment potentials. Material and **Methods:** this data was collected by searching in medical databases such as: British medical journal, Pubmed, etc. and published books such as USP, JP, etc. **Results:** Chemicals: Active constituents of *Pygeum* include phytosterols (e.g. β-sitosterol), pentacyclic triterpenes (ursolic and oleanic acids) and feroic acid esters (n-docosanol and tetracosanol). Specifications: Identification of sterols is done by gas chromatography. The derivatizing solution is a mixture of bis(trimethylsilyl)acetamide and trimethylchlorosilane (9:1). The internal standard solution contains 2 mg per ml of 5α-cholestane in chloroform. Identification of docosyl ferulate is done by liquid chromatography. Solution A is a mixture of methanol and water (95:5) and solution B contains filtered and degassed acetonitrile. The mobile phase is a mixture of solution A and solution B (85:15). Bioactive chemicals: Estrogenic activity: β-sitosterol, stigmasterol. Androgenic activity: β-sitosterol. Analgesic and protisticide activity: ursolic acid. Antispasmodic activity: daucosterol. Conclusion: The name of the remedy, *Pygeum*, comes from the name of the plant, which was discovered to botany by Gustav Mann during his now famous first European exploration of the Cameroon range. Indications: *Pygeum africanum* is mostly used for BPH and prostatic adenoma (nacturia, dysuria, pollakiuria, micturitional disorders, and/or bladder fullness). It can also be used for chronic prostatitis and obstruction-induced contractile dysfunction. Mechanism: Its exact mechanism of action is still unclear, in animal model *Pygeum* has been shown to modulate bladder contractility by reducing the sensitivity of the bladder to electrical stimulation, phenylephrine, adenosine triphosphate and carbachol. *Pygeum* also can decrease production of leukotriens and other 5-lipoxygenase metabolites. Fibroblast production and adrenal androgen secretion can be affected by *Pygeum africanum* extract. **References:** [1] Monograph *Pygeum africanum* (2002) Altern. Med. Rev. 7:71 – 74. [2] United States pharmacopeail convention. United states pharmacopeia (2007) 31st ed.- The national formulary, 26th ed. vol.1. - Washington: Rockville. [3] Monograph of *Pygeum africanum* (2008) available at: http://pygeum.net/Monograph_pygeum_africanum1.htm.accesssed [4] *Prunus africana* (Hook.F) Kalkman (2008) available at: http://www.ars-grin.gov.accessed

PE59 *Rosmarinus officinalis* L. extract inhibits human melanoma cell growth

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Products able to inhibit reactive oxygen species (ROS) and reactive nitrogen species (RNS) production by UV-R could be used in the prevention of skin cancer [1]. *Rosmarinus officinalis* L. (rosemary) is used as a folk medicine around the world, as well as in cosmetics. In medicine, the extract is receiving increasing attention due to its anti-inflammatory and antioxidative constituents [2]. The antioxidant properties of rosem-

ary have been well documented, and there are several reports that have established carnosic acid as the major phenolic diterpenoid present in rosemary leaves with antioxidant activity [2]. Recently, this phenolic compound has attracted wide interest as a potential therapeutic agent against several diseases, and researches showed that it has chemopreventive, anti-neoplastic and antimutagenic effects [2]. Our recent studies evidenced that *R. officinalis* extract, containing 31.7% of carnosic acid, was able to contrast deleterious effects of UV-R, protecting plasmid DNA by hydroxyl radical generated by UV-A [2]. In this work, we evaluated the effect of this extract on pBR322 DNA cleavage induced by nitric oxide, and the growth inhibitory activity against two human melanoma cell lines (M14 and A375). The results obtained indicate that our sample at 200 – 800 µg/ml concentrations, like carboxy-PTIO (1 mM), an NO trapping agent, was able to reduce the NO-induced plasmid DNA damage, and at non toxic concentrations (20 – 80 µg/ml) for normal human fibroblast cells, was able to reduce significantly ($p < 0.001$) the growth (MTT assay) of both melanoma cell lines. In addition, our results seem to indicate that apoptotic cell demise appears to be induced in M14 and A375 cells. In fact, no statistically significant increase in LDH release was observed in melanoma cells, correlated to a fragmentation of genomic DNA, determined by COMET assay. **References:** [1] Russo, P.A.J., Halliday, G.M. (2006) Photobiol. 155:408 – 415. [2] Garbarino, J. et al. (2006) Nat. Prod. Commun. 1:1123 – 1128.

PE60 Clove extracts prepared by supercritical CO_2 and their anti-cancer activity

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In recent years, significant attention has been directed to natural extracts of herbs due to their antioxidants, and other nutrition and health promoting properties. Natural compounds (i.e. rhizoma zedoariae, astragali radix, paeoniae radix, aloe vera, garlic, red clover, barberry, fenugreek, black seed, Jujube, fig, calotropis, and chamomile) may block, reverse, or prevent the development of invasive cancers; therefore, they offer protective and therapeutic actions to cancer cells with low cytotoxicity to normal cells [1 – 12]. Supercritical CO_2 was used to obtain high quality extracts from clove buds. The effect of CO_2 temperature (40 – 60 °C) and pressure (80 – 300 bar) on the extraction process was investigated. The highest extraction yield was obtained under the Supercritical Fluid Extraction (SFE) condition (50 °C, 300 bar, and 200 ml), whereas SFE condition (50 °C, 80 bar, 200 ml) gave the lowest yield. Selected SFE extracts were tested for their anti-cancer activity. Results of MTT cytotoxicity assay show that the clove extract obtained at SFE condition (50 °C, 300 bar, and 200 ml) has promising anti-cancer activity on various cancer cell lines: the human cervical carcinoma (Hela), the T-cell lymphoma (Jurkat), the human leukemia (HL-60), and the human neuroblastoma (SH-SY5Y). The clove extract showed over 80% reduction in cell viability. Moreover, FACS analysis of the treated cells confirmed these observations and revealed that the cytotoxic effect of clove is due to the induction of apoptosis. In addition, the clove extract was shown to inhibit histone deacetylation as measured by a reduction in the expression of certain HDACs. **References:** [1] Zheng, S. et al. (1997) J. Cell Biochem. Suppl. 27:106 – 112. [2] Lin, J. et al. (2003) World J. Gastroenterol. 9(4):670 – 673. [3] Lee, S.M. et al. (2002) Life Sci. 71(19):2267 – 2277. [4] Harris, C. et al. (1991) Mol. Biother. 3(4):207 – 213. [5] Takeyama, H. et al. (1993) Oncology 50(1):63 – 69. [6] Jarred, R.A. et al. (2002) Cancer Epidemiol. Biomarkers Prev. 11(12):1689 – 1696. [7] Iizuka, N. et al. (2002) Int J Cancer 99(2):286 – 291. [8] Hibasami, H. et al. (2003) Int. J. Mol. Med. 11(1):23 – 26. [9] Gali-Muhtasib, H. et al. (2004) Int. J. Oncol. 25(4):857 – 866. [10] Munoz, S.E. et al. (1999) Nutrition 15(3):208 – 212. [11] Hernandez-Ceruelos, A. et al. (2002) Toxicol. Lett. 135(1 – 2):103 – 110. [12] Rubnov, S. et al. (2001) J. Nat. Prod. 64(7):993 – 996.

PE61 Chemical composition of essential oil from *Lippia citriodora* H.B.K. of Iran

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The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees. Most of them are traditionally utilized as remedies for gastrointestinal and respiratory problems. Some species have shown antimalarial, antiviral and cytostatic properties. It is believed that their essential oils and phenolic compounds (flavonoids) are responsible for these properties. One of these species *Lippia citriodora* H.B.K. mainly used as a spice and medicinal plant. It grows spontaneously in South America and is cultivated in different regions of the world [1, 2, 3]. The aerial parts of *L. citriodora* grown at Karaj in the north-west part of Iran were hydrodistilled for 3 hours, using a Clevenger-type apparatus to yield 0.8% (w/w) of orange yellow oil. The essential oil was dried over anhydrous sodium sulphate and stored in a sealed vial at +4 °C until analysis. The oil was analyzed by GC and GC-MS. The constituents of the essential oil were identified by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [4]. Forty compounds were characterized in the essential oil of *L. citriodora*, representing 96.17% of the oil, of which caryophyllene oxide (13.6),*1,8-cineole* (12.5%), nerol (5.54) were found to be the major components. *Acknowledgement: The authors acknowledge the financial contribution from the Research and Technology Deputy of ACECR (Academic Centre for Education Culture & Research) for supporting this research.* **References:** [1] Argyropoulou, C., et al. (2007) Biochem. Syst. Ecol. 35:831 – 837. [2] Valentão, P. et al. (2001) J. Agric. Food Chem. 47:4579 – 4582. [3] Pascual, E. et al. (1999). J. Ethnopharmacol. 76:201 – 214. [4] Adams, R.P. (2001) Identification of Essential oil Components by Gas Chromatography/Quadrupole Mass spectroscopy. Allured Publishing Crop. Carol stream, IL.

PE62 Anti-inflammatory, antioxidant, anti-tumor and physiological studies on *Levisticum officinale*-Koch plant

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Levisticum officinale-Koch (lovage) plant is a member of family Abiaceae" Umbellifera" used as expectorant and stomach stimulant. Essential oil of control plants was extracted by hydrodistillation. Anti-oxidant and anti-tumor activities of essential oil were studied. The essential oil showed antioxidant activity using DPPH method [1] (IC50, 65 µg/ml). The essential oil has anti-tumor activity against HepG2 and MCF7 by 98% and 95% at 100 µg/ml, respectively and less activity against HT29 at the same concentration while the essential oil showed weak activity at 50 µg/ml (65% inhibition) and no activities at lower concentrations. Petroleum ether and chloroform extracts of plant have anti-inflammatory activity after 4hrs in carrageenan-induced oedema in rats [2]at dose of 200 mg/kg b.wt. Lovage plant seeds were cultivated in loam soil in two successive seasons (October 2007& 2008) at different distances (20, 40 and 60 cm in between plants) and fertilized using compost (25, 37.5, 50 m^3/Hectar). The best cultivation distance was 40 cm; it produced 3150 g fresh herb/m^2 while the best compost level was 50 m^3/Hectar (750 g fresh herb/m^2/harvest) as compared to control plant (330 g fresh herb/m^2/harvest) Key words: Anti-inflammatory, antioxidant, anti-tumor, compost, *Levisticum officinale-koch* 1. Hsu, C.L. et al. (2003) Food Chem. 83(1):85 – 92 2. Winter, C.A. etal. 91962) Proc. Soc. Exp. Biol. 111:544

PE63 Investigation on Cytotoxic activity of *Centaurea bruguierana* ssp. *belangerana*

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Total 80% MeOH extract and petroleum ether, $CHCl_3$, EtOAc, *n*-BuOH, and remaining MeOH fractions obtained by solvent-solvent fractionation of the whole fruiting samples of *Centaurea bruguierana* ssp. *belangerana* (Asteraceae) collected from the south of Iran were investigated for cytotoxicity against HT-29 (colon carcinoma), Caco-2 (colon adenocarcinoma), T47D (breast ductal carcinoma) and NIH-3T3 (Swiss embryo fibroblast) cell lines by MTT cytotoxicity assay [1]. The $CHCl_3$ and EtOAc fractions showed significant cytotoxic activity on T47D and Caco-2 cell lines (IC_{50}< 100 μg/ml), which $CHCl_3$ fraction exhibited the most potent in vitro cytotoxic activity against Caco-2 cell line with an IC_{50} value of < 10 μg/ml, and therefore, can be considered to have active compound(s) against the Caco-2 colon cancer cell line that may be due to the presence of the sesquiterpene lactones [2,3]. Although, the IC_{50} values of these two fractions are much higher than IC_{50} of methotrexate as an anticancer drug, but this may be due to the impurity of fractions consisting of so many constituents other than active compounds. Interestingly, the IC_{50} values of these two fractions on normal NIH-3T3 cell line is higher than that of T47D and Caco-2 cell lines, which can be considered as inactive on normal cells while active on cancer cells. Finally, the isolation and structure elucidation of the active compounds of $CHCl_3$ and EtOAc fractions as well as determination of IC_{50} values and understanding the mechanism of inhibition would be of interest. *Acknowledgements: This research has been supported by Tehran University of Medical Sciences & health Services grant No. 6091 – 33 – 03 – 86 on November 12, 2007.* **References:** [1] Mossman, T. (1983) J. Immunolog. Methods 65:55 – 63. [2] Yesilada, E. et al. (2004) J. Ethnopharmacol. 95:213 – 215. [3] Özcelik, B. et al. (2007) Microbiol. Res. doi: 10.1016/j.micres.2007.05.006.

PE64 Study of the anti-inflammatory and antitumour effects of a hydroethanolic extract of the plant *Piper marginatum*

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In Brazilian folk medicine, the aqueous extract of *Piper marginatum* Jacq. (Piperaceae) has been reported to have anti-inflammatory and wound-healing properties [1]. Screening of hydroethanolic Venezuelan plant extracts for both anti-inflammatory and antitumour activities indicated that this plant merited further study [2], especially in the light of the proven role of inflammation in cancer growth and metastasis [3]. In this study, a hydroethanolic extract of the leaves of *Piper marginatum* (PM) was tested for both cytotoxicity and anti-inflammatory activity *in vitro* and against primary tumour growth and metastasis in a mouse model. PM was cytostatic for three tumour cell lines at relatively high concentrations (GI50 = 80 – 230 μg/ml) in a 48 h sulphorhodamine assay but showed no cytotoxic effect even at the highest dose tested (300 μg/ml). In different experiments, PM inhibited by approximately 50% tumour necrosis factor production by LPS-stimulated RAW 264.7 cells and NF-κB activation in an NF-κB-dependent luciferase reporter system using stably transfected HeLa cells, but only at higher doses (up to 300 μg/ml). However, in repeated experiments this extract significantly reduced the primary tumour growth in C57BL/6 mice inoculated with B16/BL6 melanoma cells (up to 80% inhibition, P< 0.05). The inhibition was comparable to that obtained with cisplatin as the control drug. In 3 separate experiments, PM also inhibited metastasis to lung by 40 – 60%. These results suggest that PM does not inhibit tumour growth through a direct cytotoxic effect, but possibly via another mechanism which requires further investigation. *Acknowledgements:* Project Misión Ciencia # 2007000881, MPPCT, Venezuela. **References:** [1] D'Angelo, L. et al. (1997) Phytomedicine 4:33 – 40. [2] Villasmil, J. et al. (2006) Pharmacologyonline 3:808 – 816. [3] Coussens, L.M. et al. (2002) Nature 420:860 – 867.

PE65 Phytochemical characterization and cytotoxic activity of *Iranecio elbursensis* Bioss.

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Iranecio elbursensis Bioss. (Asteraceae), an endemic plant of Iran, aerial parts total extract and dichloromethane, ethyl acetate and methanolic fractions were investigated for their *in vitro* cytotoxic activity as well as their phytochemical constituents. The total extract was prepared by cool percolation method using hydro-alcoholic solution [1]. The fractions were obtained by using Soxhlet apparatus [2]. In phytochemical screening, total extract was tested for alkaloids, saponins, tannins, anthraquinones, flavonoids, cardiac glycosides and cyanogenic glycosides [3]. The results revealed the presence of flavonoids and alkaloids in total extract. Human breast carcinoma cell line, T47D, was used for evaluating cytotoxic activity by MTT assay method and doxorubicin was used as positive control [4]. The total extract exhibited marked cytotoxic activity against human breast carcinoma cell line with IC50 of 1 mg/ml. In comparing all fractions at the concentration of IC50, the dichloromethane fraction was the most effective one which shows the presence of some cytotoxic components that are almost nonpolar and can be extracted by nonpolar solvents. **References:** [1] Fazeli, M.R. et al (2007) Food Cont. 18:646 – 649. [2] Francois, G. et al (2004) Phytother. Res. 18:184 – 186. [3] Evans, W.C. (2002) Trease and Evans Pharmacognosy. W.B. Saunders. London. [4] Kaabinejadian, S. et al (2008) J. Biol. Sci. 8:380 – 385.

PE66 Anti-proliferative and pro-apoptotic mechanisms of Iberogast®

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STW 5 (Iberogast®) is widely used in the treatment of gastrointestinal disorders, including functional dyspepsia and colon irritabile. Our objective was to determine anti-proliferative and pro-apoptotic effects of this combination of nine plant extracts on colon adenocarcinoma cells (HT-29) in comparison with non steroidal anti inflammatory drugs (NSAIDs) like aspirin (ASA) or diclofenac (Diclo), substances, for which a reduction of colon carcinoma risk is known from clinical and epidemiological data. HT-29 cells were treated with Diclo (0.025 – 0.1 mM), ASA (0.2 – 2.5 mM), STW 5 (3 – 300 μg/ml) or its components STW 6 (*Iberis amara* totalis; 12.5 μg/ml), STW 5-K II (peppermint leaves; 50 μg/ml), STW 5-K VII (milk thistle fruit; 50 μg/ml) or STW 5-K VIII (lemon balm leaves; 25 μg/ml). The anti-proliferative effects were measured with Sulforhodamine. Apoptosis was identified by YO-PRO-1® staining. Apoptosis relevant Bcl2, BAX and Caspase-3 mRNA expression were quantified by Real-Time PCR. Treatment with either Diclo (0.1 mM), ASA (2.5 mM), STW 5 (100 μg/ml) or its components STW 6 (12.5 μg/ml), STW 5-K II (50 μg/ml), STW 5-K VII (100 μg/ml) or STW 5-K VIII (25 μg/ml) inhibited proliferation by ca. 50 – 60% (ASA or Diclofenac 45 – 50%) in comparison with untreated cells (control). STW 5 (as well as ASA or Diclo) induced a 3 to 4-fold increase in apoptosis. Moreover, 100 μg/ml STW 5 showed a 20% or 30% induction of Caspase-3 or BAX expression, whereas ASA or Diclo revealed inhibitory effects. Furthermore, 100 μg/ml STW 5 inhibited the Bcl2 mRNA expression compared to 25 μg/ml. Our data suggest that STW 5 and some of its components show anti-proliferative and pro-apoptotic effects on HT-29 cells *in vitro,* possibly due to an activation of the caspase cascade. Active concentrations of STW 5 are, in relation to therapeutic doses, comparable to those of ASA and Diclo, suggesting a similar favourable effect on colon carcinoma risk.

PE67 Antiglioma action of *Sideritis scardica* extracts

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Sideritis scardica Griseb., Lamiaceae (mountain tea), an endemic plant of Balcan peninsula, traditionally has been known for its anti-inflammatory and gastroprotective properties [1]. The dried aerial parts of the mountain tea were extracted using ethanol, diethyl ether, ethyl acetate, and *n*-butanol. The total phenolics content was determined by the Folin-Ciocalteu method. The extracts were tested for their antioxidant activity measuring the reduction of DPPH absorption to indicate the capacity to scavenge free radicals. HPLC method was developed for qualitative fingerprint analysis of flavonoid and phelocarbonic acids in investigated extracts (results presented in the Table). Investigation of the extracts influence on viability of C6 rat glioma cells and rat primary astrocytes demonstrated that the extracts decreased the viability of treated C6 rat glioma cells. The most potent was DE extract where in concentration of 50 μg/ml viability decreased to 59.4 ± 3.3% (compared to untreated cells). Contrary, the viability of rat primary astrocytes did not change in presence of investigated extracts in the same concentrations. However, both in C6 rat glioma cells and rat primary astrocytes, extract treatement resulted in changes in cellular morphology and actin distribution in the cells. All investigated extracts increased the production of reactive oxygen species in both, C6 rat glioma cells and rat primary astrocytes, as well as the caspase activation and subsequent apoptotic cell death [2].

Extracts	Total phenolics ±σ (mg GA/g)	DPPH activity EC_{50} ± σ (μg/ml)	Flavonoids and phenolcarbonic acids content (%)					
			Apigenin	Apigenin -7-O-glucoside	Luteolin	Chlorogenic acid	Caffeic acid	Protokatechin
Ethanoli	188.45 ± 12.91	31.50 ± 0.36	-	-	-	-	-	-
Diethyl ether	84.20 ± 7.29	147.27 ± 1.76	0.38	-	-	0.52	0.03	0.05
Ethyl acetate	345.61 ± 21.70	20.07 ± 0.41	-	0.05	-	0.79	-	0.05
n-Butanolic	300.25 ± 13.39	5.67 ± 0.37	-	0.03	0.01	1.65	-	-

Acknowledgements: The authors wish to thank Serbian Ministry of Science for financial support project number TR 20137 **References:** [1] Gabrieli, C.N. et al. (2005) J. Ethnopharmacol. 96:423 – 428. [2] Isakovic, A. et al. (2007) Cell. Mol. Life Sci. 64:1290 – 1300.

PE68 Determination of cytotoxic compounds by HPLC and stability studied of Thai Traditional preparation called Benjakul for cancer treatment

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Benjakul is a Thai Traditional medicine preparation, used for balanced health. From selective interviews of folk doctors in southern Thailand, it was found that Benjakul was used as the adaptogen drug for cancer patients [1]. In our previous study, the ethanolic extract of Benjakul preparation exhibited high cytotoxic activity against lung cancer cell lines (COR-L23). Piperine has been identified as main compound and plumbagin as the most cytotoxic compound [2]. In this study, we developed a reversed-phase high-performance liquid chromatography (HPLC) method for quality control such as chemical fingerprint, quantification and stability of the ethanolic extract of Benjakul preparation. Reversed-phase HPLC was performed with a gradient mobile phase composed of water and acetronitrile, and peaks were detected at 256 nm. Based on validation results, this analytical method is a precise, accurate and stable method to quantify determination of piperine and plumbagin which cytotoxic compounds isolated from the ethanolic extract of Benjakul preparation. The stability of the ethanolic extract of Benjakul preparation was evaluated under the accelerated conditions (45 ± 2 °C with 75 ± 5% RH for 4 months). The results exhibited that plumbagin is unstable but piperine exhibit as a stable compound in accelerated condition. *Acknowledgement Thailand research fund and Faculty of Medicine, Thammasart University for the financial support.* **References:** [1] Itharat, A. et al. (1998) Wisdom of Southern Thai Traditional Doctors. Prince of Songkla University, Songkla. [2] Pimonwan. et al. (2007) Planta Med. 73:1005 – 1006.

PE69 Stability under heat accelerated condition of Dioscorealide B from the ethanolic extract of *Dioscorea membranacea* for Cancer Treatment

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Hua-Khao-Yen-Tai (*Dioscorea membranacea* Pierre) have been commonly used among ingredients in Thai traditional anticancer preparations. The rhizome of *D. membranacea* was found potently cytotoxic and possibly contributed to such a therapeutic effect [1]. Bioassay-guided isolation was used for discovery a selective novel cytotoxic compound, dioscorealides B [2]. In this study, we aimed to determine the stability of dioscorealide B content [3] by HPLC method from ethanol extract under accelerated condition 45, 60, 70 and 80 °C, 75% RH and also tested cytotoxic activity of the ethanolic extract of *Dioscorea membranacea* Pierre against breast human cancer cell line [MCF-7] was also determined. The results of stability of dioscorealide B from the ethanolic extract under heat-accelerated conditions, 45, 60, 70, 80 °C, 75% RH for 1 month, also caused dioscorealide B remaining in the end of the exposure time decrease significant, 84.87 ± 1.89, 61.16 ± 3.72, 42.72 ± 0.92 and 22.97 ± 2.35%, respectively. Even though, the cytotoxic activity against MCF-7 of all samples of every condition were not changed significantly. *Acknowledgements: Faculty of Medicine, Thammasart University for the financial support.* **References:** [1] Itharat, A. et al. (2004) J. Ethnopharmacol. 90:33 – 38. [2] Itharat, A. et al. (2003) Org. Lett. 5:2879 – 2882. [3] Sirikatitham, A. et al. (2007) Songklanakarin J. Sci. Technol. 29(Suppl. 1):101 – 107.

PE70 Anti-proliferative effects by *Sabal serrulata* (Prostasan®) on prostatic cell lines

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Benign Prostatic Hyperplasia (BHP) is one of the most common low urinary tract disorders in males and prostate cancer the most diagnosed cancer in men in Western Countries. Testosterone plays a key role in the enlargement and proliferation of prostatic cells as well as in the development of cancer. In our experiments we looked at the effects of Prostasan® (*Sabal serrulata* extr. spiss. (96% EtOH, DER 9 – 12:1)) to interfere with testosterone-mediated proliferation, with invasion and migration of prostatic cells. *Sabal serrulata* extract effectively inhibited the proliferation of LNCaP cells – as described earlier. Also Prostasan® inhibited the androgen receptor-dependent transcription of a androgen dependent luciferase reporter gene in a dose-dependent manner after induction by testosterone (R1881). The extract alone had no significant steroid effect in this assay. As an indicator for tumorigenesis, the anchorage-independent growth of prostatic cell was investigated. In the presence of testosterone, the addition of Prostasan® reduced the growth of colonies. However our data implicate the independency of STAT-5 phosphorylation that is related to the JAK/STAT signalling cascade in the pharmacological action by Prostasan®. Receptor-binding studies revealed possible involvement of epidermal growth factor (EGF), muscarinic receptors, as well as chemokines receptors in the pharmacodynamic action by Prostasan®, but further studies need to differentiate agonistic from antagonistic effects. Conclusively, Prostasan® interferes with several testosterone-dependent adverse reactions. Via inhibition of proliferation and of anchorage-independent migration of prostatic cells Prostasan® might interfere with the symptoms of BHP and possibly the genesis of prostate cancer. *Acknowledgements: Funding from Bioforce AG Switzerland.*

PE71 Radical scavenging and anti angiogenic potential of an African recipe: *Hymenocardia acida Tul.*

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The pathogenesis of many chronic diseases have been associated with the presence of free radicals and the control of angiogenesis [1,2]. Con-

sequently, the search for radical scavengers and inhibitors of angiogenesis is very active and African rain forest abounds of important therapeutic recipes such as *Hymenocardia acida* of the family Euphorbiaceae [3]. The plant is used against dysentery, inflammations, rheumatic pains, diarrhea, sickle cell anemia and tumours [4,5]. The methanol extract of the different organs were assayed for their radical scavenging (DPPH) and anti angiogenic potential (CAM) using modified methods [6,7] and the most active organ (Root-bark) fractionated and the ethyl acetate fraction was the most active on both assays with IC_{50} 1.49 (DPPH) and 1.1 ± 0.20 score (CAM) at 250 µg/pellet. From our studies, flavonoids found in the ethyl acetate might be responsible for such activity. These findings have validated and authenticated some traditional uses of the plant. *Acknowledgement: The author wishes to acknowledge the material support from the Faculty of Pharmacy, University of Uyo, Nigeria* **References:** [1] Ellnaim, W.M. et al. (2003) Fitoterapia 74:1 – 6. [2] Kashbauer, C.M. et al. (2001) Carb. Res. 330:427 – 430. [3] Dalziel, J.M. (1937) Cr. Ag. Ov. Gov. Adm. Pp. 153, 249, 303. [4] Irvine, F.R. (1961). Oxf. Univ. Pr. 231. [5] Muanza, D.N. et al. (1995) Int. J. Pharm. 33(2):98 – 106. [6] Cuendet, M. et al. (1997) Helv. Chim. Acta 80:1144 – 1152. [7] Marchesan, M. et al. (1998) Phytother. Res. 12:33 – 34.

PE72 Cytotoxic effects of ethyl acetate extract of *Sambucus ebulus* compared with Etoposide on normal and cancer cell lines

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Sambucus ebulus is a native botany and exists in large amount in Iran and consists of anti-cancer substances such as ebulin (RIP, ribosome inactivated protein), flavonoids, etc. Isolation and identification of some potent anti-tumor compounds from medicinal plants, has motivated researchers to screen different parts of plant species for anti-tumor effects. In previous studies, anti-inflammatory effects of n-hexane and methanolic extracts of *S. ebulus*, nephro- and hepato-toxic effects of ethyl acetate extract of this plant and evaluation of role of vitamins C and E on prevention of cellular and pathological disorders induced by the ethyl acetate extract was performed and reported [1,2]. So, cytotoxic activity and IC_{50} of specific concentrations of ethyl acetate extract of fruits of *S.ebulus* on 4 normal and cancer cell lines was studied. Also, Etoposide, a chemotherapeutic drug was selected as control positive group. The normal cell lines were CHO and rat fibroblast and cancer cell lines were HepG2 and CT26. The ethyl acetate extract was prepared by perculation. The cytotoxic effects and IC 50 of the extract on the cell lines were studied followed by MTT assay after 72 hours incubation. The results showed that the ethyl acetate extract of *Sambucus ebulus* posses lower IC_{50} in the cancer cell lines in comparison with the normal cell lines. On the other hand, the extract posses higher IC_{50} in comparison with Etoposide on all 4 normal and cancer cell lines ($P < 0.05$), but it manifested a good cytotoxic compound which can introduce as an anticancer compound. **References:** [1] Ebrahimzadeh, M.A. et al. (2007) Pakistan. J. Biol. Sci. 10(22):4171 – 4173. [2] Saeedi Saravi, S.S. et al. (2008) Toxicol. Letters 45th Congress of EUROTOX 180S:S 57.

PE73 Phycochemical and Cytotoxic Activity of Some Marine Algae

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Discovery of drugs from marine, as they are considered a good natural source of many biologically active constituents, has received our increasing attention [1]. These constituents may be highly effective as anticancer natural agents [2,3]. Various marine Algae brown (*Zostera marina, Sargassum latifolium*), red (*Liagoria rugosa, Grateloupia filicina, Galaxaura oblongata*) and green (*Halimeda tuna, Udotea petiolata, Cladophora albida*) have been collected from Red Sea at Hurghada for evaluation of their anti-carcinoma against different human cell lines *in vitro*. Alcoholic extracts from the collected marine algae have been prepared and phytochemical, LD_{50} and *in vitro* anti-carcinoma screening [4] have been evaluated. Furthermore, carbohydrates have been isolated by cold and hot water extracts and also tested against different human carcinoma cell lines. The obtained results proved that the investigated algae have various cytotoxic activities. **Reference:** [1] Awad N.E. (2000) Phytother. Res. 14:641. 2. Awad, N.E. (2004) Phytother. Res. 18:275. 3. Awad, N.E. et al. (2008) Phytother. Res. 22:1613. 4. Skehan, P. et al. (1990) J. Nat. Cancer Inst. 82:1107 – 1112.

PE74 Determination of some properties of yoghurt made by using some fruit juice concentrate

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Free radicals and other reactive oxygen species play a crucial role in a variety of human physiological functions. However, excess generation of reactive oxygen species can often give rise to oxidative stress that results from the imbalance in the human antioxidant/oxidant status. It has now been recognized that prolonging this imbalance is implicated in a number of human diseases. Recently, the incorporation of plant phenolic into fat-containing foods has received considerable attention with regard to providing functional foods for antioxidant sources. The objective was to study the effect of pomegranate and sour cherry fruit juice concentrate that is dark red with a high antioxidant and phenolic content on the physico-chemical, microbiological and sensory characteristics of yogurt for four weeks storage. The changes in antioxidant and phenolics contents of yogurts supplemanted with pomegranate and sour cherry fruit juice concentrate were examined and pH, titratable acidity, total solids content, ash, fat, water activity, water holding capacity and colour of samples were also determined during four week storage period. Furthermore, in order to determine total bacteria, yeast-mold and total thermophilic lactic acid bacteria microbiologic analyses were also applied to the samples. In preparation process of fruit yogurts, pomegranate and sour cherry juice concentrate were prefered due to their high antioxidant capacity. It was determined that antioxidant capacity and phenolic contents of yoghurts prepared with pomegranate juice concentrate was higher than that of Sour cherry juice concentrate. Antioxidant and phenolic contents were decrased in both samples during storage.

PE75 Cytotoxic effects of hydroalcoholic extract of *Juniperus sabina* compared with hydroalcoholic extract of *Taxus baccata* and Cisplatin on normal and cancer cell lines

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Isolation and identification of some potent anti-tumor compounds from medicinal plants, has motivated researchers to screen different parts of plant species for anti-tumor effects. It has been reported that several conifers posse cytotoxic activities on some human tumor cell lines. Previous investigations revealed that different parts of some species of Iranian *Juniperus sabina* possess cytotoxic effects on some human cancer cell lines. The potent compound of the species of this plant is podophyllotoxin, but active ingredients of other species are lignan, silicicolin called desoxypodophyllotoxin. In this study cytotoxic effects and IC_{50} of specific concentrations of hydroalcoholic extract of fruits of *Juniperus sabina* were compared with hydroalcoholic extract of bark of *Taxus baccata* and Cisplatin, as well known anticancer compounds on normal cell lines (CHO and mice fibroblast) and cancer cell lines (HepG2 and SKOV3). Hydroalcoholic extract of the plant were prepared by perculation. The cytotoxic effects and IC_{50} of the extract on the cell lines were

studied followed by clonogenic assay after 72 h incubation. The results showed that IC_{50} of *Juniperus sabina* extract was significantly higher than the extract of *Taxus baccata* and cisplatin on all 4 normal and cancer cell lines ($P < 0.05$). As a result, It is concluded that the extract of *J. sabina* have almost similar cytotoxic effects with the extract of *Taxus bacata* on cancer cells.

PE76 Investigating the in vitro cytotoxic effects of *Cynara scolymus* L.

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Although medicinal plants are widely used in the world, several studies showed that these plants could have toxic effects in human [1]. In this study, the *in vitro* cytotoxicity of the extracts from *Cynara scolymus L.* was studied using human lymphocyte and cancerous human bone marrow endothelial cells (HBMEC). This plant is traditionally used in Iran as a treatment for diseases such as diabetes and asthma. The extract was obtained in the traditional way by boiling 20 g of dried leaves powder in 900 ml of water until the volume reached to 450 ml. Also, a 10 times higher concentration extract (made using 200 g of dried leaves) was used to compare the toxicity of *C. scolymus L.* in different concentrations. The cell lines, cultured in 96 well plates in their related medium and at 37 ºC and 5% CO_2 condition, were treated with serial dilutions of the plant extracts (1:30, 1:62.5, 1:125, 1:250, 1:500 and 1:1000) for 24 hours. Then, the cell viability was measured using trypan blue and lactate dehydrogenase (LDH) assays, for the lymphocytes and HBMEC, respectively. The results showed that all dilutions obtained from the 10 times extract caused death for 100% of the lymphocytes, however, for the normal extract, only the two first dilutions killed 100% of the cells. LDH assay showed that only the highest subjected dilution (1:30) from the 10 times concentration extracts of *C. Scolymus* had 70% cytotoxic effects on HBMEC and the cytotoxicity of the other dilutions were less than 50%. Based on the findings, we conclude that even the extraction made in the traditional method (lower concentration) is toxic to the lymphocytes and further studies are required to obtain the safe dosage and the proper method of usage for *C. scolymus L.* Although the toxic effect of the extract on cancer cells is desirable, the dilutions which were toxic for cancer cells are also toxic for the normal human lympocytes and further optimizations for the use of this plant for cancer treatment is required. **References:** [1] Souza, A. et al. (2006) J. Genet. Mol. Biol. 29:380 – 383

PE77 Some medicinal plants used in Bangladesh in traditional medicinal treatment of various forms of cancer

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Traditional medicinal practitioners or Kavirajes form the primary health-care providers to the majority of rural population of Bangladesh. Each Kaviraj possess extensive knowledge of medicinal plants and usually has his own formulations for treatment of various ailments. Since cancer affects a huge number of people worldwide including Bangladesh, we conducted an ethnobotanical survey amongst Kavirajes of various regions of the country to learn more about medicinal plants used to treat various forms of cancer. Interviews were conducted with the help of a semi-structured questionnaire and plant specimens as pointed out by the Kavirajes were collected and identified at the Bangladesh National Herbarium. Some of the plant names obtained in our survey included *Abrus precatorius*, *Acacia arabica*, *Acacia nilotica*, *Agave americana*, *Aloe vera*, *Alpinia galanga*, *Amorphophallus konjac*, *Andrographis paniculata*, *Areca catechu*, *Artemisia absinthium*, *Arundo donax*, *Asparagus racemosus*, *Azadirachta indica*, *Barringtonia cutangula*, *Boerhavia repens*, *Borassus flabellifer*, *Brassica oleracea*, *Calendula officinalis*, *Calophyllum inophyllum*, *Camellia sinensis*, *Cassia angustifolia*, *Cassia fistula*, *Catharanthus roseus*, *Celosia argentea*, *Cinnamomum camphora*, *Citrullus colocynthis*, *Citrus aurantium*, *Citrus sinensis*, *Coix lacryma-jobi*, *Colocasia esculenta*, *Cucurbita maxima*, *Curculigo orchioides*, *Curcuma aromatica*, *Curcuma longa*, *Curcuma zedoaria*, *Cuscuta reflexa*, *Cynodon dactylon*, *Daucus carota*, *Dioscorea bulbifera*, *Ehretia microphylla*, *Elettaria cardamomum*, *Eriobotrya japonica*, *Erythrina variegata*, *Euphorbia antiquorum*, *Euphorbia ingens*, *Ficus pumila*, *Gloriosa superba*, *Gnaphalium luteo album*, *Helianthus annuus*, *Hibiscus mutabilis*, *Hibiscus rosa-sinensis*, *Hydrangea japonica*, *Hyptis suaveolens*, *Ichnocarpus frutescens*, *Impatiens balsamina*, *Indigofera tinctoria*, and *Ixora coccinea*. It is expected that scientific studies on these plants shall lead to discovery of novel anticancer compounds.

PE78 Genotoxic effects of aquatic extract of medicinal plants *Symphytum officinale* L. (Boraginaceae)

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The species *Symphytum officinale* contain pyrrolizidine alkaloids mostly alanthoine [1] and use in treatment of It is used in fracture bones, neglected wounds, skin diseases, and some respiratory disorders in some regions of W. Balkan [2]. Because of the content of carbohydrates used in the diet of people [3]. Goal of this study is to research genotoxic effects of over ground and underground parts of these species in vitro conditions. Herbal material for this research was gathered during September of 2007 in area of Bosnia (W. Balkan). Plant samples were dried and exposed to double mazzeration in accordance with Ph.Yug. IV in order to receive extract that was used in making 0.05% and 0.10% solution. Evaluation of geno-toxic effect was conducted by using *Allium*-test, along with observation of chromosomes abnormalities (partition spindle, irregular phases, multi-polarity, stagnating chromosomes, C-mitosis, and others). Effects were observed after 4, 8, 12 and 24-hours treatments. Testing of differences between determined (experimental group) and expected (control group) was conducted by using X^2 test. Extracts of both concentrations, both over and under ground parts, are causing geno-toxic effects in mitosis at meristems cells of *Allium cepa*. Genotoxic effect is in co-relation with length of treatment and solution concentration. Aquatic extract of root showed distinguished geno-toxic effect after 4-hours treatment (mitotic index was 3, 55%, and in control was 10, 15%). Determined was also statistically significant difference for 0, 10% extract of over ground part ($p \leq 0.05$). Lower degree of geno-toxic effect was determined for aerial part. Similar genotoxic effects have been identified and some related species such as *Onosma stellulata*. **References:** [1] El-Shazly, A. et al. (2003) Biochemical Systematics and Ecology, 31(5):477 – 485. [2] Redzic, S.S. (2007) Coll. Antropol. 31:869 – 890. [3] Redzic, S. (2007) Planta Med. 73:1013. [4] Redzic, A. et al. (2008) AJATM (in press).

PE79 Additional new 5,6-dihydroflavanones and cytotoxic constituents from the leaves of *Cryptocarya chinensis*

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Cryptocarya chinensis (Hance) Hemsl. (Lauraceae) is a medium-sized evergreen tree, distributed in southern China, Japan, and Taiwan [1]. Approximately 1400 species of Formosan plants have been screened for cytotoxicity, and *C. chinensis* was shown to be one of the active species. Pavine alkaloids and their derivatives have been extensively studied from the basic fraction of this species [2 – 6]. However, the neutral-$CHCl_3$ soluble fraction of this plant has not been studied. Investigation of the neutral-$CHCl_3$ soluble fraction of the leaves of *C. chinensis* has led to the isolation of additional four new 5,6-dihydroflavanone skeleton, cryptochinenone A (**1**), B (**2**), C (**3**) and D (**4**), together with ten known compounds. Among the all isolates, infectocaryone, crytocaryone, 4'-dihydroxy-2',6'-dimethoxychalcone, cryptocaryanone A and B showed cytotoxic activities (≤ 7.25 μg/mL) against MCF-7, NCI-H460 and SF-268 cell lines, respectively. The structures of these new compounds were determined through spectroscopic analyses including extensive 2D-NMR, X-ray, and CD-ORD data. This poster describes the structural elucidation of these new compounds and the cytotoxic activities. *Acknowledgements: This work was supported by a grant from the National Science Council of the Republic of China.* **References:** [1] Liao,

J.C. (1993) Lauraceae in Flora of Taiwan; 2nd Edn. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan. [2] Lin, F.W. et al. (2002) Chem. Pharm. Bull. 50:157 – 159. [3] Wu, T.S., Lin, F.W. (2001) J. Nat. Prod. 64:1404 – 1407. [4] Lin, F.W. et al. (2001) Chem. Pharm. Bull. 49:1292 – 1294. [5] Chang. W.T. et al. (1998) Phytochemistry 48:119 – 124. [6] Chen, C.C. et al. (1979) J. Nat. Prod. 42:163 – 167.

PE80 Biodiversity of endemic plants as a source of new medicins (W. Balkan, SE Europe)

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Ethnobotanical experience undoubtedly shows that the use of medicinal plants and played a game more and more important role in prevention and treatment of various diseases. So biodiverstiy plants give increasing attention, particularly from the kinds of difficult accessible areas and endemic species that are hidden medical and pharmacological solutions to many diseases. One such area is the Western Balkans [1].The biodiversity of W Balkan includes about 9,000 vascular plants. In ethnobotany of this region people use about 1000 plants in traditional human and veterinarian phytotherapy and nutrition [2, 3]. Wester Balkan area are so rich in endemic species. In our investigation more than 700 plant species have been found on the Dinaric Mts. only [4]. In order to achieve all planned aims, it has been applied adequate methodology: intensive field research on different vertical profiles, including ethnobotanical interviews, followed at the end by comparative taxonomic-biochemical method. Among plants that could be potentially significant in terms of the pharmacology and pharmacy it was detected 500 endemic species of W Balkan. The most significant new resources are contained within families and endemic genera: *Pinaceae* (*Pinus* and *Picea*), *Caryophyllaceae (Drypis, Dianthus, Minuartia, Saponaria, Silene*), *Ranunculaceae (Ranunculus, Anemone, Pulsatilla, Aquilegia, Helleborus, Aconitum, Cruciferae (Aubretia, Malcolmia, Alyssum, Cardamine, Barbaraea, Lunaria), Rosaceae (Potentilla, Sibireja, Alchemilla, Geum, Dryas), Leguminosae (Astragalus, Genista, Oxytropis, Anthyllis), Umbelliferae (Athamanta, Eryngium, Pancicia, Seseli, Bunium), Labiatae (Acinos, Micromeria, Salvia, Satureja, Stachys, Teucrium, Thymus), Scrophulariaceae (Euphrasia, Pedicularis, Scrophularia), Compositae (Achillea, Amphoricarpos, Centaurea, Crepis, Senecio, Doronicum), Liliaceae (Lilium, Chouardia, Allium)* and others. Those plants are potentiall sources of new metabolites, such as alcaloids, heterozides, saponins, essential oils, tannins, carbohydrates as well as other secondary and primary metabolic compounds. **References:** [1] Redzic, S. (2007) Planta Medica 73(9):1013 – 1013. [2] Redzic, S.S. (2007) Collegium Antropologicum 31:869 – 90. [3] Redzic, SJ. (2006) Ecol. Food & Nutr. 45(3):189 – 232. [4] Redzic, S. (2008) Planta Med. 74(9):1143 – 1144.

PE81 Inhibitory activity of *Murraya koenigii* (L.) on Tumor Take in mice

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The methanolic extract of *Murraya koenigii* (L.) leaves was investigated for the tumor take inhibitory activity. The aim of the present study was to asses the tumor take inhibition of the leaves of *Murraya koenigii*. Tumor regression studies showed a regression response for tumor growth in vivo of a murine mouse melanoma. Preventive group animals were injected daily with the extract at dose 100 mg/kg body weight i. p. for 10 consecutive days. The control group animals were injected with double distilled water. The animals were observed for the growth of tumor after injection of B16F10 melanoma cells, three weeks after the last dose of the *Murraya koenigii* extract, into the dorsal skin of mice. In tumor regression studies, the Volume Doubling Time (VDT) and Growth Delay (GD) were calculated from the growth curves of individual tumor bearing mice. Pretreatment with the drug showed delay tumor growth by increasing the VDT and GD. The leaves had shown better mean survival time. **References:** 1. Abraham, A. (1997) Indian J. Exp. Biol. 35:148 – 150. 2. Medicinal Plants of India (1987) Indian council of medicinal research, Cambridge printing works, New Delhi.

Topic F: HIV and viral diseases

PF1 Antiviral activity of EPs® 7630 as assessed in a fibroblast-virus protection assay

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EPs® 7630, a special aqueous ethanolic root extract of *Perlagonium sidoides*, has been shown to be an efficient herbal medicine for the treatment of upper respiratory tract infections [1]. The destabilised immune system resulting from a viral infection (frequently *Piconaviridae)* can clear the way for a secondary bacterial infection. Control of viruses is achieved through IFNs that are produced by host dells. Accordingly, attention was given to IFN-like activities of EPs® 7630 induced in BMMΦ. For this, supernatants of EPs® 7630-activated BMMΦ were analysed for their capacity to protect L929 fibroblasts from the cytopathic effect (CPE) of encephalomyocarditis virus (EMCV). The relative number of protected, i. e. viable cells was determined spectrophotometrically using crystal violet as staining reagent. Appropriate controls were performed using vehicles alone as negative and an rIFN-γ standard (100 U/ml) as positive control for cytoprotection. Prominent cytoprotective effects were observed at 30 μg/ml after only 6 h of incubation, as evident from similar IC_{50} values of sample and IFN standard. After 24 h of incubation, an even nine fold higher inhibition of CPEs was noted. The extract reduced the CPE of EMCV on L929 cells in a concentration-dependent manner (30 – 0.5 μg/ml). In contrast, EPs® 7630 did not inhibit the CPE when L929 cells were either co-incubated with the sample and the virus or pre-treated with EPs® 7630 for 20 h. In order to define the antiviral principle, ELISA was used to measure IFN proteins, showing that the culture medium did not contain any detectable IFN-γ or IFN-α proteins, while similar determination of IFN-ß is in progress. Subsequent fractionation and treatment of EPs® 7630 with skin powder produced samples with different capabilities to inhibit the CPE, suggesting that polyphenols play a role in activating antiviral mechanisms. **Reference:** [1] Kolodziej, H. and Kiderlen, A.F. (2007) Phytomedicine 14 (Suppl. VI):18 – 26.

PF2 An aqueous extract of fragrant sumac (*Rhus aromatica* Aiton), a classic uro-therapeutic phyto-medicine, showed dual mode of action against herpes simplex virus type 1 (HSV-1) and a high safety profile

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Despite the clinical proven efficacy of fragrant sumac extracts for the treatment of urological diseases like overactive bladder and bacterial infection associated symptoms, little is found in literature about the mode of antiviral action and safety of this active pharmaceutical ingredient. For cytotoxicity assay and mechanistic investigation we used RC-37 cells (African green monkey kidney cells). The fragrant sumac extract demonstrated a very low cytotoxic activity in vitro with a CC_{50} (50% cytotoxic concentration) value of 2.7%. Using a plaque reduction assay, the extract showed a high level of anti-HSV-1 activity with an IC_{50} (50% inhibitory concentration) value of 0.0005%. In addition, at maximum noncytotoxic concentration (0.25%) plaque formation was significantly reduced by more than 99.9% when HSV-1 was pretreated with the fragrant sumac extract for 1 h prior to cell infection. Surprisingly, when host cells were treated with the extract for 1 h prior to virus infection, the infectivity of HSV-1 was reduced by 50%. These results suggest that the fragrant sumac extract reveals a dual mode of antiviral action. Some compounds of the fragrant sumac extract may interact not only with the viruses but also with the surface of host cells impairing the ability to adsorb to and penetrate into the host cells. These findings are consistent with the very few reports of minor undesirable side effects from clinical studies with fragrant sumac extract and support also its use for microbiological associated diseases of urinary tract.

PF3 Anti-herpes simplex virus type 1 activity of *Rhododendron ferrugineum* L. extracts

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An aqueous extract and a polysaccharide-enriched extract from *Rhododendron ferrugineum* L. (Ericaceae) were tested for their inhibitory effect on herpes simplex virus type 1 (HSV-1) using MTT assay and plaque reduction assay. In a standard assay HSV-1 was preincubated with the extracts for 1 h, followed by addition of the virus suspension plus the extract to Vero cells. Total incubation time of the assay mixture was 48 h. This test protocol investigated the influence of the extracts on the complete viral replication cycle. IC_{50} values were calculated to be 2.4 μg/mL for the aqueous extract and 1.9 μg/mL for the polysaccharide-enriched extract, respectively. The 50% cytotoxic concentration for host cell growth (CC_{50}) were 288 μg/mL for the aqueous and 263 μg/mL for the polysaccharide-enriched extract. Thus, the selectivity index (ratio of CC_{50} to IC_{50}) was 135 and 120. The antiviral activity was confirmed by both assay systems, plaque reduction and MTT assay. In order to determine the mode of antiviral action, both extracts were added at different time points to the cells or viruses during the infection cycle (pre-, co-posttreatment). No anti-herpetic effect was achieved when cells were preincubated with extracts prior to addition of virus, however, a strong antiviral activity in MTT assay was observed when extracts were added to virus before the attachment of HSV-1 to Vero cells. In a specific adsorption assay, both *R. ferrugineum* extracts were shown to prevent the attachment of HSV-1 to cells. This mechanism was additionally shown by immunostaining of HSV-1 and host cells, indicating that the test extracts are potent inhibitors of virus attachment.

PF4 The inhibitory effect of monoterpene, phenylpropanoid and sesquiterpene components of essential oils against herpes simplex virus

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Herpes simplex virus type 1 (HSV-1) is an important pathogen for humans causing labial herpetic infections and serious disease in immunosuppressed patients. The development of resistant strains of HSV to the available drugs, especially acyclovir, has further attempted to identify and develop new alternative agents for management of HSV-1 infections. Essential oils and their components are potential antiviral agents. 11 monoterpenes e.g. α-terpinene, β-pinene and thymol, 2 phenylpropanoids, e.g. trans-anethol and 6 sesquiterpenes, e.g. β-caryophyllene, caryophyllene oxide and farnesol from essential oils were screened to evaluate their inhibitory effect against HSV-1 *in vitro*. All components were tested for cytotoxicity in a standard neutral red assay. These components from essential oils exhibited a high and concentration dependent activity against herpes simplex virus in RC-37 cells. The potential inhibitory effect against HSV-1 of monoterpenes, phenylpropanoids and sesquiterpenes was analysed by plaque reduction assays and the 50% inhibitory concentrations (IC_{50}) were determined in dose response studies. β-pinene, β-caryophyllene and caryophellene oxide showed potent selectivity indices of 24, 25 and 140, respectively. At maximum noncytotoxic concentration, herpes virus infectivity was reduced by 100% for β-pinene, by 98% for β-caryophyllene and by 84% for caryophyllene oxide. Most components revealed high antiviral activity against HSV due to direct inactivation of viral particles. We conclude that components of essential oils are highly effective antiherpetic agents.

PF5 *In vitro* evaluation of EPs® 7630 for its ability to inhibit neuraminidase using sodium (4-methyl-umbelliferyl-α -D-N-acetylneuraminate) as substrate

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The claimed antiviral activity of a special extract of the roots of *Pelargonium sidoides*, EPs® 7630 [1], prompted the present investigation in terms of its neuraminidase (sialidase 1) inhibiting potential, a key enzyme for the release of influenza virus progeny from host cells. To evaluate the effects of EPs® 7630 on the viral associated neuraminidase, we used a fluorometric-based assay for neuraminidase (EC 3.2.1.18) from *Vibrio cholerae* with 2"-(4-methylumbelliferyl)-α -D-N-acetylneuraminic acid [2]. The liberated coumarin derivative was determined in a spectrofluorometer using excitation light at 365 nm and fluorescence emission at 465 nm. The therapeutically used inhibitor zanamivir served as positive control in our assays. Compared to zanamivir (IC_{50} of 71 μg/ml), EPs® 7630 exhibited pronounced in vitro neuraminidase inhibiting activity with IC_{50} of 0.9 μg/ml. To provide a chemical rationale for the inhibiting potential, EPs® 7630 was fractionated into a MeOH soluble and a MeOH insoluble portion. Again, both subfractions showed prominent inhibitory activities as reflected by IC_{50} 0f 1.8 μg/ml and 1.3 μg/ml, respectively. In contrast, treatment of the extracts with skin powder produced inactive fractions in concentrations up to 100 μg/ml. This finding suggests that polyphenols apparently represent the underlying active principle. For further information, some phenolic constituents including caffeic acid, a series of flavan-3-ols, an enriched proanthocyanidin dimeric fraction as well as the coumarin umckalin were tested, providing support for this conjecture. **References:** [1] Kolodziej, H., Kiderlen, A.F. (2007) Phytomedicine 14 (Suppl. VI):18 – 26. [2] Potier, M. et al. (1979) Anal. Biochem. 94:287 – 296.

PF6 New phorbol analogues from *Euphorbia grandicornis*

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Phorbol esters are well known as activators of protein kinase C (PKC), which regulates different signal transduction pathways and other cellular metabolic activities. Recently, phorbol diterpenes, e.g. prostratin and phorbol-13-monoesters command special interest due to their HIV-1 latency reactivating effect by PKC-dependent NF-κB activation. This effect makes phorbol derivatives promising candidates of drug development for the HIV therapy [1,2,3,4]. We report herein the isolation and structure determination of three diterpenes from *Euphorbia grandicornis* Goebel (Euphorbiaceae), a succulent cactiform South African plant whose phytochemical investigation has not been reported previously. The methanol extract of the fresh aerial parts of *E. grandicornis* was subjected to solvent partitioning to furnish chloroform- and water-soluble fractions. The organic phase was fractionated by column chromatography on polyamide, then by vacuum liquid chromatography on silica gel. Selected fractions from these separations were further purified by CPC, preparative TLC and HPLC to yield three pure compounds, including two new natural products. The structure elucidation was carried out by HRESIMS and extensive NMR studies using advanced experiments (^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, HSQC and HMBC). Two compounds were identified as 12-deoxyphorbol di- and triesters, acylated with acetic, angelic and isobutyric acids. The third compound was found to have an unusual parent alcohol substituted with angeloyl, isobutyroyl and two keto groups. The isolated compounds are structurally similar to PKC-activating phorbol esters, therefore they are worthy for pharmacological studies. *Acknowledgements: This work was supported by Hungarian Scientific Research Fund (OTKA) (PD 78145)* **References:** [1] Fraser, C. et al. (2000) AIDS 14:659 – 669. [2] Bocklandt, S. et al. (2003) Antivir. Res. 59:89 – 98. [3] Trushin, S.A. et al. (2005) J. Virol. 79:9821 – 9830. [4] Marquez, N. et al. (2008) Biochem. Pharmacol. 75:1370 – 1380.

PF7

In vitro study of the antiviral activity of *Zingiber officinale*

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Aims: In the past, herbs were the only source of most drugs; ethnopharmacological research may represent a crucial step in the development of drugs from natural sources. Trial and error have led to the correlation of a particular herb with the amelioration and/or complete curing of certain diseases. Ginger (*Zingiber officinale*, Zingiberaceae) has been widely used as a dietary spice, and as a traditional oriental drug. The rhizome of ginger contains pungent vanillyl ketones, including [6]-gingerol and [6]-paradol, which have been credited with therapeutic and preventive health benefits, including anti-cancer activity. The current work seeks to identify novel lead compounds with antiviral effect on hepatitis C virus (HCV). A lyophilized juice extract from *Zingiber officinale* at different concentrations (5, 25, 50, 75, 100, 150 and 200 µg/ml) were tested *in vitro* as anti-HCV using the hepatocellular carcinoma HepG2 cell line infected with HCV. Inhibition of viral replication was detected by amplification of viral RNA segments using the reverse transcriptase (RT)-RNA technique. The test compound was *Zingiber officinale* considered to be active by inhibiting the viral replication inside the HCV-infected HepG2 cells, as evidenced by the disappearance of the (+) and/or (-) strands of viral RNA- amplified products detected by RT-RNA (compared with the positive control). **Results:** The inhibitory dose was found to be effective at 100 µg/ml. Newer insight into molecular basis of the efficacy of *Zingiber officinale* as anti-HCV will help us to formulate an alternative cheap natural drug to avoid the high cost and adverse effect of synthetic drugs. **References:** [1] Eddouks, M. et al. (2002) J. Ethnopharmacol. 82:97 – 103. [2] Habib S.H. et al. (2008) Clinics 63:807 – 813.

PF8

Antiviral activity and mode of action of a peptide isolated from *Helianthus annus*

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HSV-1 is generally associated with primary and recurrent mucocutaneous facial, ophthalmic or genital lesions, but under certain conditions can produce serious infections of the central nervous system, causing acute necrotizing encephalitis and meningitis in patients with immune deficiencies [1,2]. Most of the treatments for HSV-1 are based on acyclovir (ACV) and ACV-like nucleoside analogues, but these are toxic, and some immunocompromised patients with recurrent HSV lesions develop resistance to ACV [3]. Antimicrobial proteins have been discovered in plants, insects and animals as important components of the innate defense system [4]. We have investigated the activity of crude extracts, a fraction, and an isolated peptide from seeds of *Helianthus annuus* against HSV-1. The plaque reduction assay showed a dose-dependent effect against HSV-1 with EC_{50} values 21.5 µg/mL for crude extracts, 15.9 µg/mL for the fraction and 4.8 µg/mL for the isolated peptide. In an evaluation of the antiviral mode of action, the isolated peptide showed EC_{50} values of 5.3 µg/mL before the infection, 4.5 µg/mL during the infection and 78.6 µg/mL for a direct viricidal effect. The cellular toxicity of the peptide showed a CC_{50} value of 3.278 µg/mL, thus exceeding the EC_{50} value by 683 times. Based on the results of this study, the isolated peptide appears to be an alternative for the development of new antiviral drugs. *Acknowledgements: CNPq, CAPES, PRONEX/Fundação Araucária.* **References:** [1] Jenssen, H. et al. (2004) Antiviral Res. 64:119 – 126. [2] Ammendolia, M.G. et al. (2007) Antiviral Res. 76:252 – 262. [3] Zhu, W. et al. (2006) Phytomedicine 13:695 – 701. [4]Camargo Filho, I. et al. (2008) Phytomedicine 15:202 – 208.

PF9

Bioactivity-guided separation of anti HSV-2 and antioxidant metabolites from the plant *Phyllanthus orbicularis*

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The *Phyllanthus* genus includes nearly forty Cuban-endemic species, which have been widely used in traditional medicine for the treatment of jaundice, dysentery, urinary disorders, chicken pox and diabetes [1]. The anti herpetic properties of *P. orbicularis* (H.B.K.) was first reported by our group [2,3] and neither previous phytochemical characterization nor elucidation of antiviral metabolites and their modes of action have ever been conducted for this species. In this work, an antiviral-guided separation protocol was followed in order to isolate compounds or families of compounds responsible for HSV-2 inhibition. HPLC and MS analyses revealed the presence of flavones, flavan-3-ols, procyanidins, hydrolysable tannins, flavanols and flavonol glycosides. Fractions containing flavan-3-ol gallates, procyanidin B1 and B2, procyanidin dimer gallates, procyanidin trimers and procyanidin trimer gallates exerted the strongest anti HSV-2 activity. However, the higher antiviral selectivity index was recorded for crude methanol extract, suggesting that some synergistic effects contributing to antiviral activity could be lost with separation. Antioxidant power of *P. orbicularis* fractions was assessed in parallel, and a strong positive correlation between both antioxidant and antiviral activities was observed. These results highlight the potential of *P. orbicularis* to be included in topic formulations for the management of herpes-derived skin lesions, and also as an antioxidant dietary supplement. **References:** [1] Calixto, B.J. et al. (1998) Med. Res. Rev. 18:225 – 258. [2] Del Barrio, G. and Parra, F. (2000) J. Ethnopharmacol. 72:317 – 322. [3] Fernández-Romero, J.A. et al. (2003) Phytother. Res. 17:980 – 982.

PF10

Antimicrobial activity of *Jatropha multifida* L. against bacteria and fungi s.t.d. organisms

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Jatropha multifida L. (Euphorbiaceae) is a multipurpose shrub commonly planted as an ornamental but often exploited as a medicinal plant in many parts of Africa. Many *Jatropha* plants have toxic and irritant properties and are used in folklore medicines to cure various diseases in Africa, Asia and Latin America [1]. As part of a continuing investigation of the biological activity of *Jatropha* species [2,3], this study was carried out to investigate the antimicrobial activity of this plant against different microorganisms especially those responsible for sexually transmitted infections and isolate the bioactive constituents. Hexane, ethyl acetate and methanol extracts of the plants were obtained and subjected to phytochemical and antimicrobial analysis. The extracts and purified fractions were screened against many pathogenic microorganisms comprising gram positive and gram negative bacteria and fungi. The extracts and fractions displayed potent antimicrobial activity against many of the organisms including *Gardnerella vaginalis, Neisseria gonorrhoea* and *Candida albicans* giving Minimum Inhibitory Concentration (MIC) as low as 12.5 µg/mL. Further phytochemical investigation resulted in the isolation of different compounds including a coumarin, 8-hydroxy-6,7-dimethoxy coumarin. The structures of the compounds were determined by MS, 1D and 2D NMR experiments. The results confirmed the potency of this plant in treating different diseases including sexually transmitted infections. **References:** [1] Burkill, H.M. (1994) The useful plants of West Tropical Africa. Vol. 2, Royal Botanical Gardens, Kew. [2] Aiyelaagbe, O.O. et al. (2000) Phytother. Res. 14:60 – 62. [3] Aiyelaagbe, O.O. et al. (2007) Int. J. Pharmacology 3:106 – 110.

PF11 Phytochemical study of some medicinal plants used by tradipraticiens in Nde division (Cameroon)

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The accelerated destruction of the flora and fauna in Cameroon on a daily basis has rendered their exploration and exploitation for scientific purposes more difficult. Ethnobotanical and phytochemical studies have been carried out in Nde division (Cameroon) to identify species drugs as source of anti HIV/aids, cancer, malaria and for other diseases that affect the population. Plant material for screening was botanically identified and the organs used, harvested, (leaves, roots, barks, etc.) treated and subjected to a rapid search for broad categories of chemical compounds and active ingredients (alkaloids, saponins, tannins, flavonoids, terpenes, sterols, quinones, coumarins, etc.) as a preliminary assessment. This initial assessment gave some indications and served as a pointer to select species for extractive research intended for commercial pharmaceutical development. The analysis and selection of plant species abundantly rich in alkaloids showed strong pharmacological activity that justified their use by traditional practitioners. Thus, out of the 129 species catalogued, 29 plants, belonging to 20 families proved active and have been recommended for further investigations that may find solutions to hitherto incurable diseases. Among these plants, we recommend the study of secondary metabolites of species whose bibliography confirms the pharmacological properties as indicated in the table below:

Plant	Families	Parts used	Indication
Aspilia africana	Asteraceae	leaves	antimalarial
Bidens pilosa	Asteraceae	leaves	antimalarial antidiabetic hepatoprotector
Maytenus senegalensis	Celastraceae	leaves	cancer
Picralima	Apocynaceae	fruits	antimalarial

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PF12 Synergistic immunopharmacological effects of N-alkylamides in *Echinacea purpurea* herbal extracts

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Echinacea purpurea (L.) Moench extracts are used in the production of standardized herbal medicines for the prevention and treatment of upper respiratory infections. Unsaturated N-alkylamide lipids, the main constituents of *E. purpurea* and *E. angustifolia* preparations capable of activating the cannabinoid receptor type-2 (CB_2) have been suggested to play a role as potential anti-inflammatory and immune-modulatory principles. Here we show that ethanolic *E. purpurea radix* and *herba* extracts produce synergistic pharmacological effects on the endocannabinoid system in vitro. Superadditive action of N-alkylamide combinations were seen at the level of intracellular calcium release as a function of CB_2 receptor activation. Likewise, synergism of the *radix* and *herba* tinctures was observed in experiments measuring LPS-stimulated cytokine expression from human PBMCs. While the expression of the anti-inflammatory cytokine IL-10 was significantly superstimulated, the expression of the pro-inflammatory TNF-alpha protein was inhibited more strongly upon combination of the extracts. We show that N-alkylamides act in concert and exert pleiotropic effects modulating the endocannabinoid system by simultaneously targeting the CB_2 receptor, endocannabinoid transport and degradation.

Topic G: Quality control and safety assessments of phytomedicines

PG1 Standardization of *Garcinia mangostana* fruit rind extract

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The extract of *Garcinia mangostana* fruit rind has been popularly used in food supplement and herbal cosmetics. Therefore, it is necessary to establish the specification for quality assessment of the extract. The 95% ethanolic extracts of *G. mangostana* fruit rind collected from 15 different locations in Thailand were prepared by Soxhlet extraction. The content of α-mangostin, which is a major component, was analysed by HPLC method [1]. Loss on drying, solubility, TLC fingerprint and chemical identification of each extract were determined. The heavy metal, pesticide and microbial contamination were also determined. The extract ratio (crude drug:extract) was 3 – 5:1, while the contents of α-mangostin in dried powder and in the extract were in the range of 1.71 – 2.70 and 5.79 – 11.12, respectively. The extracts were yellow-brown powder with characteristic odor, while loss on drying was in the range of 4.52 – 9.27%w/w. All extracts were soluble in ethanol, propylene glycol and polyethylene glycol but insoluble in water. The extracts gave a positive result with Shinoda's test, gelatin salt test and promote green-color with ferric chloride solution. TLC-fingerprints of all extracts showed similar pattern with band of a major α-mangostin. The extract contained arsenic < 0.05 ppm, lead < 0.25 ppm, cadmium < 0.02 ppm and pesticide contamination was not detected. The extracts contained total aerobic bacteria count and total yeast and mold count not more than 5×10^5 and 5×10^3 cfu/gram, respectively and no pathogenic bacteria was found. This information will be useful for quality assessment of *G. mangostana* fruit rind extract used as raw material in pharmaceutical and herbal cosmetic products. *Acknowledgements: This study is a part of Ph.D. thesis of Mahidol University and was granted by the University Research Fund.* **Reference:** [1] Pothitirat, W. and Gritsanapan, W. (2007) Planta Med. 73:892 – 893.

PG2 Validation of GC-MS method for determination of varroacide residues in propolis

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Propolis is a resinous material collected by honeybees (*Apis mellifera* L.) from various plants and enriched with bee salivary gland secretions and wax. Number of factors, such as beekeeping practice, can affect the quality and consequently the therapeutic value of propolis and its preparations. The most important source of propolis contamination is improper administration of varroacides that have to be used for long-term control of honeybee mite *Varroa destructor*. Therefore, the analysis of these noxious substances is essential in quality control of propolis. In this study the method based on the extraction with *n*-hexane followed by purifying of extract on florisil column was used prior to sample analysis. Purified extracts were analyzed by gas chromatography-mass spectrometry hyphenated technique. The procedure was optimized and validated for the determination of bromopropylate, amitraz and coumaphos residues in propolis. Investigated validation parameters of applied procedure were selectivity, linearity, precision, accuracy, robustness and limits of detection (LOD) and quantification (LOQ). All these parameters satisfied the ICH criteria [1] in the test samples of standards mixture for all three examined compounds. The applied method for varroacide analysis in validation sample of propolis was suitable for determination of bromopropylate and coumaphos. Recoveries were 100% for bromopropylate and 63% for coumaphos with LOQ of 0.08 and 1.5 μg/g, respectively. On the other hand, the procedure was inappropriate for determination of amitraz, probably due to its instability in complex matrix of propolis as was previously described for honey and beeswax [2]. **Refer-**

ences: [1] International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use (2005) Validation of Analytical Procedures: Text and Methodology Q2(R1). [2] Korta, E. et al. (2001) J. Agric. Food Chem. 49:5835 – 5842.

PG3 Chemical composition of water extract of *Gnaphalium uliginosum*. Part 2

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Water extract of *Gnaphalium uliginosum* is used in Russia as hypotensic. The analysis of the dried water extract by RP-HPLC (Fig. 1) shows that there are two groups of peaks for compounds differing in lipophylicity: 1 – 6 and 7 – 10. In recent study [1] we reported the composition of the first part (**1 – 6**) of extract. It was established that all substances belong to a general class of hydroxycinnamic acids. Current work is devoted to the structure estimation for main components of the second (more hydrophobic) part of this extract. Using SPE, analytical and preparative HPLC, UV and high resolution NMR spectroscopy, we found that the more hydrophobic group of compounds contains: 1,3,4,5-tetra-hydroxy-cyclohexanecarboxylic acid-bis-4,5-(3,4-dihydroxycinnamate) (**7**); 1,3,4,5-tetra-hydroxycyclohexanecarboxylic acid-bis-3,5-(3,4-dihydroxycinnamate) (**8**); 1,3,4,5-tetrahydroxy-cyclohexanecarboxylic acid-bis-3,4-(3,4-dihydroxycinnamate) (**9**) and 1,3,4,5-tetra-hydroxycyclohexanecarboxylic acid-tris-1,3,5-(3,4-dihydroxycinnamate) (**10**).

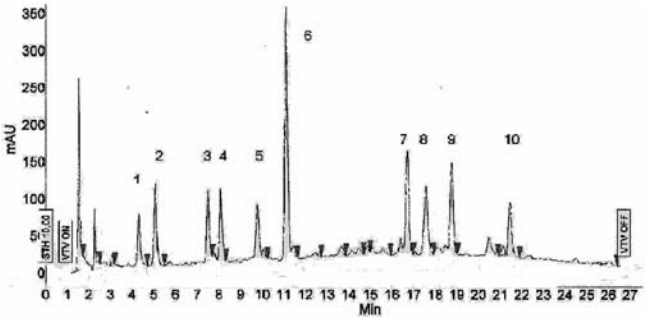

Fig. 1. RP-HPLC of dried water extract of *Gnaphalium uliginosum*.

Reference: [1] Kolesnik, Y. et al. (2008) Planta Med. 74:1095.

PG4 Absence of mutagenic effects of a particular *Symphytum officinale* L. liquid extract in the bacterial reverse mutation assay

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Comfrey (*Symphytum officinale* L.) root preparations are traditionally used for the topical treatment of contusions, strains and sprains. Besides allantoin and phenolcarbonic acids (e.g. rosmarinic acid) which are discussed as pharmacologically active principles, the drug contains pyrrolizidine alkaloids (PAs) (e.g. intermedine, lycopsamine, symphytine, echimidine) and their N-oxides which are known for their hepatotoxic, carcinogenic and mutagenic properties [1]. The commercially available herbal medicinal products Kytta-Salbe® f (ointment) and Kytta-Plasma® f (paste for cataplasms) contain a liquid extract from comfrey root (DER 1:2; extraction solvent: ethanol 60% v/v) as active substance. The aim of this study was to demonstrate the absence of genotoxic effects of this particular extract in the bacterial reverse mutation assay (Ames test). Briefly, comfrey root liquid extract was investigated for its ability to induce gene mutations in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535 and TA 1537 with and without metabolic activation using the mammalian microsomal fraction S9 mix. Reference mutagens were used to check the validity of the experiments [2]. The investigated comfrey root fluid extract showed no biologically relevant increases in revertant colony numbers of any of the five tester strains, neither in the presence nor in the absence of metabolic activation. In conclusion, the comfrey root fluid extract contained in Kytta-Salbe® f and Kytta-Plasma® f was not mutagenic in the bacterial reverse mutation assay. References: [1] Wichtl, M. (2009) Teedrogen und Phytopharmaka. 5th ed., Wiss. Verlagsges. mbH Stuttgart. [2] Ames, B.N. et al. (1973) Proc. Natl. Acad. Sci. 70:2281 – 2285.

PG5 Herbal medicines prepared by traditional and contemporary methods – a comparative study

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The traditional use of herbal medicines is often cited as justification for their efficacy and safety. This argument, however, fails to take into account the potential differences between preparations made using traditional aqueous extraction techniques compared with more contemporary methods using ethanol. The aim was to compare the amount of specific active constituents found in a typical daily dose of extracts prepared using different extraction methods and different herb-to-extract ratios. Three widely used medicinal plants, *Matricaria recutita*, *Glycyrrhiza uralensis* and *Withania somnifera* were prepared in a variety of ways reflecting different traditional methods (infusions and decoctions) and more contemporary methods (hydroethanolic macerations and percolations). The resulting extracts were analysed using HPLC and/or GC-MS. Specific constituents (3 – 6 for each species) were quantified using calibration curves of pure reference compounds to enable comparisons between the different extraction methods and herb-to-extract ratios. Traditional, aqueous extraction methods were less efficient at extracting the constituents under investigation. However, because traditionally prepared infusions and decoctions are made from a greater amount of raw material, the total amount of the examined constituents delivered per daily dose was higher for these methods. Further, the concentration of constituents in macerations and percolations did not show a linear relationship with the herb-to-extract ratio. This may be due to saturation effects. Traditional aqueous extracts such as infusions and decoctions are not necessarily less potent than ethanol based extracts, although practitioners often assume so. These findings have implications for the safe and efficacious prescribing of herbal medicines.

PG6 Method validation for the determination of toxic pyrrolizidine alkaloids and their N-oxides in *Symphytum officinale* L. using GC-MS

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Pyrrolizidine alkaloids (PA) are constituents of various species of the Asteraceae, Boraginaceae and Fabaceae. PA with a 1,2-unsaturated necine structure show an acute toxic, carcinogenic, mutagenic and teratogenic effect. Based on this fact a maximum daily doses of these substances applied as the respective herbal drugs or drug preparations was established [1]. To control this regulation an appropriate analytical method was developed which is able to detect all PA and their N-oxides in total. An analytical concept was developed to determine the retronecine type PA occurring in comfrey calculated as the reference substance senecionine by GC-MS via typical mass fragments of the retronecine structure: 93, 120 und 136. These mass numbers are recorded as Total Ion Chromatogram (TIC). Consequently it is guaranteed that all relevant toxic 1,2-unsaturated PA are detected using this GC-MS-method. Validity of the assay is determined in the lower concentration range for a purified extract up to higher concentrations occurring in the original comfrey macerate. According to the ICH guideline for method validation the repeatability and intermediate precision, the accuracy (determination of recovery rate) and the linearity of the measuring range for the reference substance senecionine and the comfrey PA are proven. The main focus of the method validation is set to the robustness of the method as well as the validation of the reduction step for the alkaloid N-Oxides. As a result a precise and robust method with a variation coefficient of 7% for the higher concentration range and 9% for the lower

concentration range, a recovery rate between 80 and 105% over the examined range and a limit of quantification of 0.1 mg/kg is presented. **References:** [1] Bekanntmachung zur Abwehr von Arzneimittelrisiken – Stufe II, June 5th 1992 (BAnz p 4805, June 17th 1992).

PG7 Validation of an HPLC-method for an antiplasmodially active stem bark extract of *Nauclea pobeguinii*

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Stem bark extracts of *Nauclea pobeguinii* (Rubiaceae) are widely used in African traditional medicine against malaria. Alkaloids, such as the major compound strictosamide, may be responsible for this activity [1,2]. An HPLC-method for the quantification of strictosamide in the stem bark extract of *N. pobeguinii* was developed. The method was validated according to the ICH guidelines. The response function of ajmalicine HCl, used as a secondary standard, was linear in a range from 4.2 to 21.2 µg/mL. The method was shown to be precise in respect to the time (RSD of 2.2%, 3 days, n = 6) and with respect to the concentration (RSD of 2.6%, 3 levels, n = 6). The accuracy of the method was investigated by means of a recovery experiment (mean recovery of 92.2% and RSD of 9.4%). A crude ethanolic extract of the bark, containing 5.6% (w/w) strictosamide, was evaluated *in vivo* in the *Plasmodium berghei* mouse model (PO at 300 mg/kg for two times 5 daily doses). Chloroquine was used as positive control at 10 mg/kg. Treatment with the crude extract resulted in moderate depression of parasitaemia during dosing, however quickly followed by a full relapse (mean survival time = about 13 days). One group received the treatment by intraperitoneal (IP) route at the same dosing regimen and showed the same results. At termination of the experiment at day 21, a single survivor in the PO group, was apparently cured (no parasitaemia). The single survivor in the IP group showed high parasitaemia and was in a moribund state. It can be concluded that the crude extract of *N. pobeguinii* has slight antimalarial potential when administered orally in a suppressive dosing regimen of two times 5 days at 300 mg/kg. Its action is likely to be static since full relapse occurs quickly after ending the daily dosing. **References:** [1] Zeches, M. et al. (1985) J. Nat. Prod. 48:42 – 46. [2] Abreu, P. et al. (2001) Nat. Prod. Lett. 15:43 – 48.

PG8 The structure of main component of *Hoodia Gordonii* extract

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A plant *Hoodia Gordonii (Asclepladaceae)* is nowadays regarded as a source of biologically active compounds of natural origin with appetite-suppressant effect. There are few medicinal drugs based on extract of this plant in the pharmacy market [1]. In a series of first fundamental publications interdependency of the biological activity of the extracts with the presence of a steroid-based compound of the pregnane series was postulated. Structure of the compound entitled as P57 was elucidated by a variety of physical-chemistry methods [2]. In latest publications [3] it was shown, that extracts of *Hoodia Gordonii* contain also other steroid-based compounds of the pregnane series entitled as Hoodiigenin A and Calogenin. In our HPLC-analysis of alcohol extracts of different *Hoodia Gordonii* samples it was shown that all these samples contain major peak with the same retention time. We isolated this compound from extract by SPE and preparative HPLC. Its structure was elucidated using the whole set of 1D and 2D NMR techniques: (3β,20*S*)-20-{[6-*O*-(6-*O*–D-*glycero*-hexopyranosyl-β-D-*glycero*-hexopyranosyl)-β-D-*glycero*-hexopyranosyl]oxy}-14-hydroxypregn-5-en-3-yl 2,6-dideoxy-4-*O*-{6-deoxy-2-*O*-methyl-4-*O*-[(2*E*)-2-methyl-2-butenoyl]-β-L-*erythro*-hexopyranosyl}-3-*O*-methyl-β-L-*erythro*-hexopyranoside This information can be used for authentication of plant raw materials of *Hoodia Gordonii*, extracts and preparations made from it. **References:** [1] Saklani, A., Kutty, S.K. (2008) Drug Discov. Today 13:161 – 171. [2] van Heerden F.R. et al. (2007) Phytochemistry 68:2545 – 2553. [3] Pawar, R.S. et al. (2007) Steroids 72:881 – 891.

PG9 Proficiency testing in the phytochemical industry – development of the PHYTAS scheme

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The quality of botanical products is a great uncertainty faced by many consumers, analysts and regulators. In many sectors proficiency testing (PT) provides an independent quality assurance tool, enabling laboratories to assess the quality of their analytical measurements. Regular PT participation facilitates the implementation of improvement measures, thereby improving the quality of analytical measurements made and reducing the risks associated with poor measurement results. Despite the increasing use of botanical materials in health care products and food supplements, and the number of analytical measurements made on such materials, PT is not well established in the phytochemical industry. A new phytochemical PT scheme, PHYTAS, was recently launched with a trial round. This consisted of two test materials, one for confirmatory analysis of an extract of milk thistle, and one for the quantification of total Silybin A & B, Taxifolin and Silychristin in an extract from an unknown material. Participation in the PT was international with results submitted from laboratories in eleven different countries. Laboratory performance for the confirmatory analysis was excellent with most participants positively confirming the material to be an extract of milk thistle. A range of different methods was employed by the laboratories. Performance for the quantitative analysis was varied, both in terms of laboratories and the specific analyte. Overall performance scores were similar for Taxifolin and total Silybin A & B, but those for Silychristin were poorer. This trial round of PHYTAS has provided important feedback on their performance to the participating laboratories. However, this only provides a single indication and ongoing participation is required for laboratories to monitor their performance over time. Based on these results, and to facilitate ongoing and regular monitoring of performance, the future direction of PHYTAS is discussed.

PG10 Capillary electrophoresis of phenolic compounds from *Eupatorium perfoliatum* L. (Asteraceae)

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E. perfoliatum is a medicinal herb from the North American continent, also known as boneset or thoroughwort. Nowadays it is mainly used as immunostimulating remedy. Recently six caffeic acid derivatives (including 3 hitherto unknown depsides of caffeic acid with glucaric acid) have been isolated and identified from the ethyl acetate soluble fraction of a methanol/water extract of *Eupatorium perfoliatum* (Asteraceae) [1]. Analyzing the extract by means of HPLC showed a satisfying separation within 40 min [1,2].

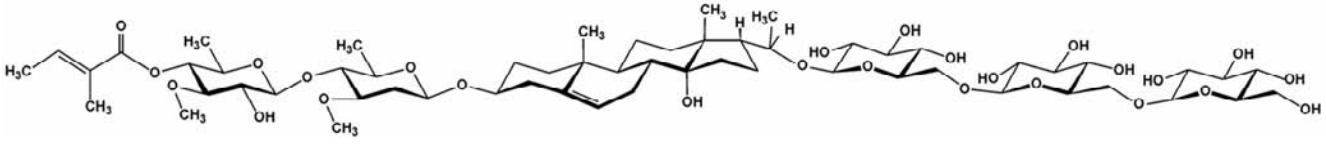
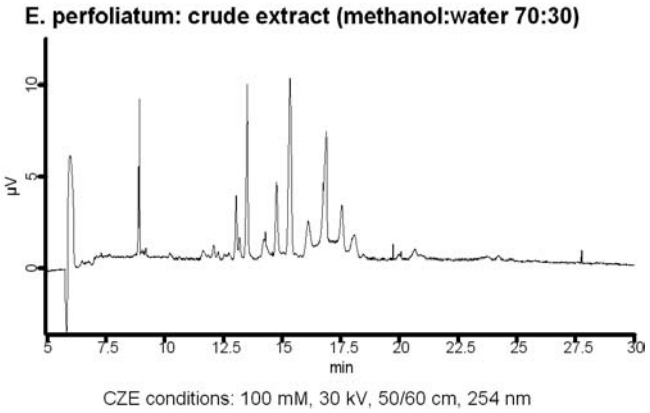


Fig. PG8



CZE conditions: 100 mM, 30 kV, 50/60 cm, 254 nm

Due to the increasing costs for purchasing and waste disposal of solvents we developed an alternative method characterised by simple sample preparation ("dilute and shoot") and low solvent consumption. Capillary zone electrophoresis (CZE) with a simple borate buffer and PDA-detection meets these demands. The electropherogram shows a fast separation of the main phenolic compounds after simple dilution of the extract. In further experiments assignment of the peaks was done by spiking experiments and comparison of UV-spectra with reference compounds. Optimization and validation experiments indicated effects of pH, buffer concentration, voltage, length of capillary and buffer additives (e.g. cyclodextrins: β-CD, HP-γ-CD, 6-O-Maltosyl-β-CD, HS-β-CD, HDAS-β-CD) on migration times and quality of separation. Quantification was done using an internal standard method. **References:** [1] Maas, M. et al. (2009) Molecules 14:36 – 45. [2] Maas, M. et al. (2009) Validation of a HPLC-Determination of Caffeic Acid Derivatives and Flavonoids from *Eupatorium perfoliatium* L., Poster Presentation at Young Researcher Meeting, 13.-14.3.2009, University of Muenster.

PG11 Determination of anthraquinone contents in *Cassia fistula* leaves for alternative source of laxative drugs

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Cassia fistula L. (Fabaceae) is locally called Khun in Thailand [1]. The ripe pods have been popularly used as a laxative drug of which active components are anthraquinones [2]. The leaves also contain anthraquinones while rhein is a major component. The leaves of *C. fistula* collected from 10 provinces in Thailand were extracted by decoction and the aqueous extracts were analyzed for the contents of total anthraquinones and total anthraquinone glycosides, calculated as rhein, by UV-vis spectrophotometric method. The contents of rhein and aloe-emodin were also determined by HPLC. The leaf extracts contained total anthraquinones 1.13 – 7.96% w/w (average 4.55% w/w), total anthraquinone glycosides 0.62 – 2.01% w/w (average 1.52% w/w), rhein 0.76 – 3.37% w/w (average 1.71% w/w) and aloe-emodin 0.01 – 0.15% w/w (average 0.07% w/w). In dried leaves, total anthraquinones, total anthraquinone glycosides, rhein, and aloe-emodin contents were found to be 0.16 – 2.12% w/w (average1.12% w/w), 0.09 – 0.63% w/w (average 0.36% w/w), 0.16 – 1.06% w/w (average 0.41% w/w), and 0.01 – 0.05% w/w (average 0.02% w/w), respectively. Compared with *C. angustifolia* leaf officially in European Pharmacopoeia, which contains not less than 2.5% w/w of total hydroxyanthracene glycosides [3], anthraquinone glycosides in the leaves of *C. fistula* are about 7 times less than in the leaves of *C. angustifolia*, while the *C. fistula* leaf extract contained less hydroxyanthracene glycosides than the leaf extract of *C. angustifolia* about 3.5 times. Thus, in case that *C. fistula* leaf extract would be used as an alternative source of laxative drugs, it has to be used for about 3 – 4 times more than the amount of *C. angustifolia* leaf extract. *Acknowledgements: This work was granted by Thailand Research Fund (TRF) with Office of Small and Medium Enterprises Promotion (OSMEP).* **References:** [1] The Forest Herbarium, Royal Florest Department. Flora of Thailand vol. 4, part I Leguminosae-Caesalpinioideae. Bangkok. [2] Pongbunrond, S. et al. Kasembannakit. Bangkok. [3] Council of Europe. European Pharmacopoeia-Supplement. Council of Europe. Strasbourg.

PG12 Morphoanatomy and histochemistry of *Maytenus heterophylla* leaf, an African medicine

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Leaves of *Maytenus heterophylla* (Eckl & Zeyh.) Robson (Celastraceae) are used in East Africa to treat different diseases such as infections, respiratory diseases and sores [1]. Despite some chemical studies have already been reported for this part of the plant, respective biological studies are scarce and there is a lack of studies aiming at its' botanical characterization. Alkaloids, triterpenes and tannins have previously been identified in the leaves [2]. Hereby we present results concerning the macroscopic and microscopic identification of *M. heterophylla* leaf as an herbal drug. Methodology includes the analysis of the whole, fragmentized and powdered plant material by light and scanning electron microscopy techniques. Some histochemistry and quantitative microscopy studies were also performed. Among the identified characters the most useful for leaf identification includes the typical leaf bilateral organization; the presence of anomocytic stomata, more frequent in lower epidermis, and surrounded by a ring of four to six subsidiary cells appear with an irregular distribution; papillate cells on the surface of epidermal cells; multicelled uniseriate covering trichomes (rare). Calcium oxalate cluster crystals are present frequently in the palisade parenchyma, near the phloem cells of the midrib and occasionally occur on epidermis. Histochemical results confirm the presence of the major chemical classes previously reported, and allowed to know its' distribution: lipids on the surface epidermis and cuticle; alkaloids, tannins, terpenoids and starch in the mesophyll, and of some terpenoids on the collenchyma cells near the midribs. Obtained results can be included in an herbal drug quality monograph of *M. heterophylla*. **References:** [1] Kokwaro, J. O. (1976) Medicinal Plants of East Africa. East African Literature Bureau. Nairobi. [2] Orabi, K. et al. (2001) Phytochemistry, 58:475 – 480.

PG13 Assessment of genotoxicity of herbal medicinal preparations according to the guideline EMEA/HMPC/107079/2007: A model project of Kooperation Phytopharmaka, Bonn, Germany

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Guidance for the assessment of genotoxicity of herbal medicinal substances (preparations) is given by a recent guideline of the Committee on Herbal Medicinal Products (HMPC) of the European regulatory agency EMEA [1]. A draft concept paper of the HMPC [2] recommends a bracketing and matrixing approach, thus offering an alternative to testing each individual preparation (extract) from a certain herbal drug by a joint conduction of tests. In accordance to these documents, Kooperation Phytopharmaka, a German scientific organisation in the field of HMPs, has started a model project for the screening of herbal preparations, including extracts produced with polar to unpolar extraction solvents to cover the whole spectrum of constituents of the herbal drug. This even allows an assessment for powdered herbal drugs otherwise not accessible to *in vitro* methods. Until now the project has produced data on 24 of the most important herbal drugs used in Europe, including e.g. St. John's wort, caraway, lemon balm, garlic, ginkgo and hawthorn. The project was conducted in accordance with all modern guidelines including those of OECD, ICH and EMEA in cooperation with renowned laboratories, starting with the first step of the test strategy, the Ames test. The project has not only broadened the knowledge about the safety of important herbal drugs used in Europe and beyond and allowed to meet current regulatory requirements, but has turned out to be an important step in the continuous process of updating the safety profile of modern phytotherapy, which is already now documented excellently. For expanding the project to further herbal drugs, cooperation partners are welcome. **References:** [1] Guideline on the assessment of genotoxicity of herbal medicinal substances/preparations, Doc. Ref. EMEA/HMPC/107079/2007, in effect since 1 December 2008. [2] Draft concept paper on selection of test materials for genotoxicity testing for traditional herbal medicinal products/herbal medicinal products, Doc. Ref. EMEA/HMPC/315413/2008.

PG14 Dietary supplements and herbal medicinal products – for a clear differentiation – Statement of the Society for Phytotherapy (GPT) to the "Article 13 Health Claim list" of the EFSA

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Herbal medicinal products are trusted by the public. Their therapeutic indications are validated in established authorization procedures by regulatory authorities. Regulation (EC) 1924/2006, in effect since 19 January 2007, is dedicated to meet the expectations of the consumers in correctness and scientific validity of health claims for dietary supplements, as claims will have to be authorized by the European Food Safety Authority EFSA in the future. The EC "Consolidated List of Article 13 Health claims" [1] published by the EFSA seems to contradict these expectations. In this list, provided by the EFSA, the claim proposals of manufacturers of dietary supplements from all over Europe are presented. This list contains, besides many other substances, almost all herbal drugs in "well established use" or "traditional use" as herbal medicinal products with therapeutic indications authorized by European drug regulatory agencies, as e.g. the German drug regulatory authority BfArM. This fact has raised doubts, whether it will be possible also in future to distinguish dietary supplements with scientifically unfounded claims from herbal medicinal products authorized for the treatment of patients and therefore meeting high quality standards. The German Society for Phytotherapy therefore states, that a clear distinction of dietary supplements from herbal medicinal products is necessary and has to be possible also in the future. Herbal medicinal products are dedicated to the treatment of diseases and have pharmacological actions, whereas dietary supplements are food, for use in healthy consumers, having health-related physiological effects only. On the background of the well established quality, efficacy and safety of herbal medicinal preparations, EFSA should conduct the evaluation for dietary supplements of herbal origin based on a well-founded differentiation from the herbal medicinal preparations, in tight cooperation with EMEA. This is a necessary precondition for providing efficacious and safe products to patients and consumers also in the future. **Reference:** [1] EFSA, Consolidated list of Article 13 Health claims, URL: http://www.efsa.europa.eu (16 January 2009).

PG15 Authentication of the traditional Chinese medicinal plant *Saussurea involucrate* using enzyme-linked immunosorbent assay (ELISA)

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Saussurea involucrate (Kar. et Kir.) Sch.-Bip. (Asteraceae) has been used as a traditional Chinese medicine for the treatment of rheumatic arthritis and lower abdominal pain [1]. Some other *Saussurea* species, in particular, *S. eriocephala*, *S. hypsipeta*, *S. medusa*, *S. obvallata* and *S. tangutica* are often marketed as *S. involucrate*, and thus, the therapeutic effects of *S. involucrate* are not achieved. In an attempt to develop an immunoanalytical method for discriminating among these species, peptides from the six *Saussurea* species were analyzed and isolated by SDS-PAGE. Antisera were developed in mice against the protein pool of the species and tested by Western blot analysis. Using these antisera, some *S. involucrate*-specific peptides were detected. One of the antibodies in the antiserum developed against *S. involucrate* proteins recognized a single *S. involucrate*-specific peptide with a molecular mass of 28 kDa in 10000-fold dilution. It did not give any positive reactions against the sample prepared from adulterants, so that they could be easily distinguished at the molecular level. Finally, an enzyme-linked immunosorbent assay has been developed to authenticate *S. involucrate* in commercially prepared crude drugs. This method provides effective and accurate identification of *S. involucrate* *Acknowledgements*: *This research was supported by the Natural Science Foundation of China (NSFC 30770153).* **References:** [1] The state Pharmacopoeia Commission of the PRC. (2005) Pharmacopoeia of the People's Republic of China. Chemical Industry Press. Beijing.

PG16 Variability of flavonoids contents in young flowers of Siamese neem tree

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Siamese neem tree (*Azadirachta indica* A. Juss. var. *siamensis* Valeton) of the family Meliaceae is a medicinal plant found in every part of Thailand. Young leaves and flowers of this plant are commonly consumed as a bitter tonic vegetable [1]. The flower extract has been reported to exhibit *in vitro* free radical scavenging activity and can inhibit lipid peroxidation of bronchogenic cancer cell line [2]. Active compounds in the flowers are flavonoids such as rutin and quercetin [3]. Decoction extract of the flowers of Siamese neem tree gave the most effective DPPH scavenging activity [4]. In this experiment, the decoction extracts of the young flowers collected from 14 different locations in Thailand were quantitative analyzed for the contents of active components rutin and quercetin. By validated HPLC, the aqueous flower extracts contained rutin, and quercetin in the ranges from 429.81 ± 0.18 to 1081.77 ± 0.68 mg %w/w (average 757.74 ± 251.60 mg %w/w), and 3.12 ± 0.02 to 19.62 ± 1.06 mg %w/w (average 9.84 ± 6.27 mg % w/w), respectively. HPLC chromatograms of all extracts showed similar pattern which rutin is a major active constituent. The ranges of flavonoids contents will be useful as a guidance for standardization of the flower extracts of this plant for pharmaceutical purposes. *Acknowledgements: This project was granted by The Thailand Research Fund (TRF) with Office of Small and Medium Enterprises Promotion (OSMEP).* **References:** [1] Clayton, T. et al. (1996) Medicinal plants in Thailand, Amarin Printing, Bangkok, Thailand. [2] Sithisarn, P., Gritsanapan, W. (2005) Mahidol J. Pharm. Sci. 32:31 – 35. [3] Sithisarn, P. et al. (2005) J. Ethnopharmacol. 99:109 – 112. [4] Chaisawangwong, W., Gritsanapan, W. (2007) Proceedings of Pharma Indochina V, Bangkok, Thailand.

PG17 Composition of the essential oil of the leaves of *Cordia verbenacea*

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Cordia verbenacea DC (Borraginaceae) is a native plant of the Brazilian coasts, used in folk medicine to treat arthritis, wounds and contusions.The essential oil of the leaves has shown anti-inflammatory and anti-allergic activities, and it is used in a topically applied herbal medicinal product (Acheflan®) for the treatment of chronic tendonitis, myofascial pain, muscular traumas and injuries. The anti-inflammatory activity has been related mainly to a decrease of TNFα, and α-humulene and *trans*-caryophyllene have been identified as active constituents of the oil [1]. The aim of the present work was to gain a better knowledge of the composition of this active essential oil. The oil was analysed by GC-FID and GC-MS using two columns of different stationary phases (methylsilicone and Supelcowax® 10). The identification of the constituents was achieved from their GC linear retention indices (both, relative to alkanes and to fatty acid methyl esters) in the two columns, by comparison of their MS fragmentation patterns with those stored in our own library, in the GC-MS mass spectra library (Wiley 6) and with literature data. In some cases, chromatographic comparison with authentic reference compounds was also used. More than 91% (46 constituents) of the essential oil was identified. The oil was mainly composed by terpene hydrocarbons, both of monoterpene (41.5%) and sesquiterpene (42.7%) types. Oxygen-containing monoterpenes and sesquiterpenes represented only 3.7% and 3.4% of the oil, respectively. The major constituents were α-pinene (36.5%), β-caryophyllene (11.7%) and α-santalene (8.6%). The oil contained also significant amounts of *allo*-aromadendrene (4.3%) and α-humulene (3.0%). Additionally, GC separation methods were optimised for application to routine quality control of the oil. *Acknowledgements: Enric Gibert, for technical assistance.* **References:** [1] Passos, G.F. et al. (2007) J. Ethnopharmacol. 110:323 – 332.

PG18 Multivariate analysis of the effects of soil parameters and environmental factors on the flavonoid content of leaves of *Passiflora incarnata* L.

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Passiflora incarnata L. (Passifloraceae) leaves are relevant raw materials for phytomedicines in Brazil and this species is also described in several European pharmacopoeias. The *Passiflora* flavonoids are associated with their pharmacological properties, and therefore the total flavonoid content is one important parameter with respect to the quality assessment of *Passiflora* phytomedicines. In order to assess the feasibility of producing raw material for the phytopharmaceutical industry through commercial cultivation of *P. incarnata* in subtropical climate (Brazil, SP state), the effect of soil characteristics (pH, macro- and micro-nutrients), environmental factors (temperature, humidity, period of the year and time of day of collection) and meteorological conditions (rain, sun, cloud and cloud/rain) on the flavonoid content of leaves of *Passiflora incarnata* L. were evaluated. Samples of leaves of mature plants were harvested and the environmental factors and meteorological conditions during each collection of the material were monitored. The total flavonoid contents of leaf samples harvested were quantified by HPLC-UV/PAD, according to a method developed in our laboratory [1], and the chromatography data acquired were submitted to chemometric analysis. Chemometric treatment of the data by PCA (principal component analysis) and HCA (hierarchic cluster analyses) showed that the samples do not have a specific classification in relation to the environmental and soil variables studied, and that the environmental variables were not significant for describing the data set. On the other hand, the levels of Fe, B and Cu in the soil showed an inverse correlation with the total flavonoid content of the leaves of *P. incarnata*. To the best of our knowledge the present study is the first relating to the application of chemometric methods to data derived from the HPLC analysis of flavonoids from a species of *Passiflora*. *Acknowledgements: FAPESP, CNPq, Anidro do Brasil Extrações Ltda.* **Reference:** [1] Pereira, C.A.M. et al. (2004) Phytochem. Anal. 15:241 – 248.

PG19 Near infrared spectroscopy supported by multivariate data analysis and GC-MS for discrimination and classification of different species in *Achillea* genus

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This study evaluated the use of near infrared spectroscopy (NIRS) for discriminating and classifying traditional medicinal plants. *Achillea millefolium* and three of its related species, namely, *A. clypeolata*, *A. collina* and *A. nobilis* were chosen as sample material because they are well known in the field of traditional medicine. The present study was subdivided into following sections: 1.) Discrimination of *A. millefolium* flowers and leaves by using NIRS and gas chromatography hyphenated to mass spectrometry (GC-MS) as reference method. 2.) Classification of differently treated *A. millefolium* samples by principal component analysis (PCA). 3.) Classification of four *Achillea* species by PCA. The results showed that NIRS is suitable to discriminate between different *A. millefolium* parts (e.g., flowers and leaves), as well as between different sample preparation techniques (e.g. air-dried, oven-dried). Furthermore, the established NIRS method proved great potential for classification of related *Achillea* species. This approach allowed the clustering by NIRS according to the individual ingredient patterns, applying GC-MS as a reference method for calibration. This developed NIRS method proved to be rapid and nondestructive technique for identification, discrimination and classification of traditional medicinal materials.

PG20 Rapid dereplication of secondary metabolites from *Thymus vulgaris* L. using LC-SPE-NMR as discriminators identification tool in NMR based metabolic profiling

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NMR based metabolic profiling techniques are rapidly emerging as promising tools for the quality control of medicinal plants and plant derived products [1]. However, their use is often limited by sample complexity, which makes the identification of discriminating constituents a challenging task [2]. The use of hyphenated techniques such as LC-SPE-NMR has been proven to facilitate this process by separating selected analytes from the matrix and providing valuable ^{1}H and ^{13}C NMR data, which aids the identification process. In a previous work, we described the use of NMR based metabolic profiling for the differentiation of 15 different *T. vulgaris* lots [3]. In this study, we present the application of LC-SPE-NMR for the rapid dereplication of known secondary metabolites in *T. vulgaris* extracts and fractions obtained by ASE (Accelerated Solvent Extraction), liquid-liquid extraction and HSCCC (High Speed Counter Current Chromatography). Multiple peak trapping allowed the acquisition of 1D (^{1}H) and 2D NMR spectra (COSY, ROESY, HSQC, HMBC) with excellent signal-to-noise ratio. This approach enabled the unambiguous identification of seven compounds, namely thymol, cirsimaritin, cirsilineol, xanthomicrol, 8-methoxycirsilineol, 3,4,3',4'-tetrahydroxy-5,5'-diisopropyl-2,2'-dimethylbiphenyl and rosmarinic acid. Based on the outcome of the previously reported PCA (Principal Component Analysis) [3], it was possible to identify thymol and rosmarinic acid as discriminating constituents. The identification process was carried out within three weeks using 8 g of starting plant material. Thus, this report underlines the importance of LC-SPE-NMR as a fast and effective dereplication tool for the identification of discriminators in NMR based metabolic profiling. *Acknowledgement: This work was financially supported by Bionorica research GmbH, 6020 Innsbruck, Austria.* **References:** [1] Holmes, E. et al. (2006) Planta Med. 72:771 – 785. [2] Seger, C. et al. (2007) J. Proteome Res. 6:480 – 497. [3] Pieri, V. et al. (2008) Planta Med. 74:1093.

PG21 Determination of primary and secondary metabolites in *Matricaria chamomilla*

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Compounds of *Matricaria chamomilla* (common name: chamomile, family: Asteraceae) are of considerable interest because of their potential pharmacological activities [1]. Modern phyto-medicine is aiming to produce phyto-pharmaceuticals of high quality, high pharmacological efficacy and innocuousness for which analytical characterization of pharmaceutical drugs is of utmost importance. A comprehensive approach was adopted in order to determine the primary and secondary metabolites in flowers of *M. Chamomilla*. Focus within the study was placed on the qualitative and quantitative analysis of flavonoids, amino acids and carbohydrates. Flavonoids were determined in the methanolic extract of plant using HPLC-PDA. The extract was subjected to acid hydrolysis with 6 M HCl in order to release aglycons from the glycosidic forms. For the qualtitative and quantitative analysis of primary metabolites – amino acids and carbohydrates in aqueous extract of chamomile flowers, thin layer chromatography (TLC) [2,3,4,5], amino acid analyser [6], gas chromatography-mass spectrometry (GC-MS) [7,8] and a newly developed mass spectrometric method, i.e. matrix free material enhanced laser desorption ionization time of flight mass spectrometry (mf-MELDI-MS) [9,10] was used. Among the flavonoids luteolin, quercetin, apigenin and isorhamnetin were quantified, yielding highest amounts for apigenin. TLC analysis proved the presence of various amino acids and carbohydrates (mono-, and disaccharides) in the extracts. The application of mf-MELDI-MS further confirmed the presence of amino acids and carbohydrates. For quantification of carbohydrates, samples were derivatised prior to GC-MS analysis with BSTFA solution in pyridine as derivatising reagent employing microwave radiation for 4 min at 180 Watts. Glucose, fructose and sucrose were quantified. Fructose gave highest amount

than other carbohydrates. Proline proved to be in appreciable amount among the amino acids quantified. Finally work performed for the exploitation of chamomile flowers proves the performance of mf-MELDI-MS, as it allows to screen plant extracts in a very short time. In this way an analytical profile of the plant is gained, necessary for the quality control in phyto-pharmaceutical industries. **References:** [1] Maday, E. et al. (1999) Eur. J. Drug Metab. Pharmacokinet. 24:303 – 308. [2] Hodisan, T. et al. (1998) J. Pharm. Biomed. Anal.18:319 – 323. [3] Randic, Z.G. et al. (2004) Farmaceutski Glasnik 60:1 – 6. [4] Mitsuhiro, Y. et al. (1987) Kanzei Chuo Bunsekishoho 27:147 – 157. [5] York, H. et al. (1990) Thin layer chromatography. VCH, Weinheim. [6] Pharmacopoeae Europa 4.06, chapter 2.2.56. [7]Silva, F.O. et al. (2004) Food Chem. 88: 609 – 612. [8] Rojas-Escudero, E. et al. (2004) J. Chromatogr. A 1027:117 – 120. [9] Ahsan Hashir M. et al. (2009) Int. J.Mass Spectrom. 279:15 – 24. [10] Ahsan Hashir, M. et al. (2007) Rapid Commun. Mass Spec. 21:2759 – 2769.

PG22 Toxicological screening of methanolic extract of *Gmelina arborea* in experimental animals

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The present investigation was carried out to evaluate the safety of methanolic extract of *Gmelina arborea* bark (ME) by determining its potential toxicity after acute and repeated dose administration in rodents. In the acute toxicity study, the methanolic extract was administered orally to Swiss albino mice in single doses of 0, 300, 2000 and 5000 mg/kg. General behavior and mortality was noted up to 14 days. For the repeated dose toxicity study, the extract was administered orally at doses of 0, 300, 1000 and 2000 mg/kg for 28 days to Wistar rats. The effects on body weight, food and water consumption, organ weight, hematological parameters, biochemical parameters as well as histology of important organs were studied. In acute toxicity study, administration of methanolic extract not showed any general behavioral adverse effects and mortality at all selected doses. The no-observed adverse effect level (NOAEL) of methanolic extract was 5000 mg/kg. In repeated dose toxicity study, no mortality was observed when different doses of extract were administered daily for a period of 28 days. There were no significant differences in the body weight, organ weights and feeding habits between control and treated animals of both sexes. Repeated administration of methanolic extract did not caused any changes in hematological and biochemical parameters as compared with control. Histopathological examination of important organs at the end of study showed normal architecture indicating no morphological disturbances. The high NOAEL value in acute toxicity study and lack of significant effect on hematological parameters, biochemical parameters and histopathology in repeated dose toxicity study indicates that the methanolic extract of *Gmelina arborea* does not appear to have significant toxicity. Thus the methanolic extract of *Gmelina arborea* was found safe in acute and repeated dose toxicity studies. **Reference:** [1] Rhiouani, H. et al. (2008). J. Ethnopharmacol. 118:378 – 386.

PG23 Reduction of safrole and methyleugenol in *Asari radix* and *rhizoma* by decoction

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Asari radix and *rhizoma* (Xixin, Manchurian Wildginger, *Asarum* spp) is a herbal drug commonly used as an ingredient in Traditional Chinese Medicine (TCM). Many species of *Asarum* contain safrole and methyleugenol as the main components of their volatile oils [1,2,3]. However, toxicological studies have shown that safrole may be a hepatocarcinogen and genotoxic leading to concerns regarding the habitual consumption of this herbal drug [4,5]. An HPLC method was established to assess the levels of safrole and methyleugenol in five batches of *Asari radix* and *rhizoma* and two TCM formulae containing this herbal drug as an ingredient. Analysis showed that the content of safrole in the dried herbal drugs tested ranged from 0.14-2.78 mg/g whilst the content of methyleugenol ranged from 1.94-16.04 mg/g. The present study demonstrated that following a 1 hour decoction, the amount of safrole was decreased by more than 92% resulting in the equivalent of no more than 0.20 mg/g safrole remaining in the aqueous extract. Such a reduction in the content of safrole is regarded as acceptable for therapeutic use. Similarly, the content of methyleugenol was decreased to the equivalent of 0.30-2.70 mg/g. Furthermore, both TCM formulae, after decoction, showed negligible amounts of safrole (maximum, the equivalent of 0.06 mg/g), and only 1.38-2.71 mg/g of methyleugenol. Therefore, the present study shows that a decoction procedure, traditionally used for Chinese herbal preparations, is able to reduce the amount of safrole and methyleugenol effectively. *Acknowledgments: We thank Lian ChinaHerb, Switzerland, and Mr Stöger, Austria, for the supply of herbal drug material as well as SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for the financial support.* **References:** [1] The State Pharmacopoeia Commission of China (2005) Pharmacopoeia of the People's Republic of China, Vol. 1. Chemical Industry Press. Beijing. [2] Xiao, P.G. et al. (2002) Modern Chinese Materia Medica, Vol. 3. Chemical Industry Press. Beijing. [3] Cai, S.Q. et al. (2008) Fitoterapia 79:293 – 297. [4] Scientific Committee on Food, European Commission (2001) Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties. http://ec.europa.eu/food/fs/sc/scf/out116_en.pdf. [5] Scientific Committee on Food, European Commission (2001) Opinion of the Scientific Committee on Food on Methyleugenol (4-Allyl-1,2-dimethoxybenzene). http://ec.europa.eu/food/fs/sc/scf/out102_en.pdf.

PG24 HPTLC of *Citrus* fruit peels

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Several *Citrus* fruit peels are described in different pharmacopoeia monographs, i.e. European Pharmacopoeia [1], Swiss Pharmacopoeia [2] and Chinese Pharmacopoeia [3]. Tinctures or syrups are monographed as well. Up to now there is no TLC identification test for sweet orange (*Citrus sinensis* Osbeck) and lemon (*Citrus limon* (L.) Burm. fil.) in the pharmacopoeias. Therefore, the aim of the study was to establish a suitable HPTLC test to identify the citrus peel drugs as well as preparations in order to revise the pharmacopoeia monograph. Moreover, for aged tangerine peel (*Citrus reticulata* Blanco), a drug traditionally used in Chinese medicine and called Chenpi, a test to discern it from bitter-orange epicarp and mesocarp was established. The spraying of an aluminium chloride solution (UV 366 nm) was found to be a suitable HPTLC detection mode to visualize some typical citrus flavanones, e.g. hesperidin or naringin. The resulting fingerprint allows distinguishing orange, lemon and bitter-orange. In order to discern between aged tangerine and bitter-orange, a subsequent derivatization by natural products/polyethylene glycol 400 (NP/PEG) solutions and evaluation in visible light was found to be effective. Whereas bitter-orange shows a prominent red zone of neoeriocitrin, in tangerine this compound is nonexistent. The results show that an identification of different *Citrus* species by HPTLC fingerprint is possible. However, since orange and tangerine have similar flavanone contents [4], the identification solely by HPTLC remains insufficient and has to be complemented by macroscopic and microscopic examination. *Acknowledgments: We thank Dixa AG and Hänseler AG, Switzerland, for the supply of herbal drug material and herbal preparations, as well as SWISSMEDIC, Swiss Agency for Therapeutic Products, Pharmacopoeia division, for the financial support.* **References:** [1] European Directorate for the Quality of Medicines (2009) European Pharmacopoeia 6th Edition (6.5). Online-Edition. [2] Swissmedic (2006) Pharmacopoeia Helvetica 10th Edition. BBL, Vertrieb Publikationen. Berne. [3] The State Pharmacopoeia Commission of China (2005) Pharmacopoeia of the People's Republic of China, Vol. 1. Chemical Industry Press. Beijing. [4] Peterson, J.J. et al. (2006) J. Food Compos. Anal. 19:S66-S73.

PG25 Standardization of two different varieties of *Capsicum* obtained from North East India

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Capsicum annuum L. var. *grossum* (family: Solanaceae) is widely grown in India with different varieties and pungency. They differ in capsaicin content depending on the source of the raw material [1]. In the present study, amount of capsaicin was determined in the two different varieties of capsicum collected from Manipur and Nagaland by HPTLC densitometry [2]. Fruits were extracted with hydroalcoholic mixture (50:50) and the % yield of the extracts of the two varieties was found to be 25.08 and 31.91 respectively. The standard capsaicin and test samples

were applied on precoated silica gel GF_{254} plates and developed in a solvent system comprising benzene, ethyl acetate and methanol (75:20:5). The plate was scanned at 283 nm using HPTLC scanner 3, CAMAG. Quantities of capsaicin in extracts were determined as 12.2% in the Manipur variety and 8.8% in Nagaland variety using calibration curve. For visualization of the standard capsaicin, plate was sprayed with 1% methanolic solution of 2,6-dichloroquinone chloroimide and immediately exposed to ammonia vapor to get bluish black colored spots. R_f of capsaicin was found to be 0.44. The method was validated in terms of accuracy, precision, specificity etc. [3] The calibration curve was found to be linear between 300 – 900 ng of capsaicin per spot. Regression via area was best described by Y = 315.494 + 2.638*X with a correlation coefficient of 0.99952 and standard deviation of 1.26%.

Method validation data	
Correlation coefficient	0.99952
Standard deviation	1.26%
Linearity range	300 – 900 ng/spot
Precision	%RSD < 2
Accuracy	98.54 – 100.97%
Limit of detection	52 ng/spot
Limit of quantification	157 ng/spot

Acknowledgements: State Government, Govt. of West Bengal, India; Mr. R.K. Sinhal **References:** [1] Sanatombi, K., Sharma, G. J. (2008) Not. Bot. Hort. Agrobot. Cluj 36:89 – 90. [2] Mukherjee, P.K. (2002) Quality Control of Herbal Drugs. Eastern Publishers (Business Horizons Ltd.). New Delhi, India. [3] Kumar, V. et al. (2008) Phytochem. Anal. 19:244 – 250.

PG26 Recent study on safety monitoring herbal medicines of Thai National Essential Drug List (NEDL)

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To promote the use of herbal medicines in hospitals, 8 single formulations were added into the Thai NEDL. There are *Curcuma longa, Zingiber officinale, Cassia alata, Andrographis paniculata, Centella asiatica, Clinacanthus nutans, Capsicum frutescens* and *Zingiber purpureum.* As with other modern medicines, herbal medicines should be governed by standards of safety monitoring that are equivalent to those required for modern medicines. Thai FDA issued the notification on safety monitoring herbal medicines within 2 years. In the meantime, the HVC has conducted a project: Intensive safety monitoring on herbal medicines of Thai NEDL. Objective: To investigate and categorize adverse events (AEs) of herbal medicines mentioned above. Duration of this project is two years, from 2007 – 2009. Method: Prospective study by intensive monitoring all patients who took herbal medicines in hospitals which were enrolled in the study. The patients were interviewed by pharmacists and structured questions form was given to them asked for followed up 2 times by telephone or come back. All reports were analyzed. **Results:** A herbal using reports were 2,335 times during the study period (Oct 1, 2007-Jan 31, 2009). Of these, 59% were *Curcuma longa,* 30% were *Andrographis paniculata* and 11% were the rest of 8 formulations. All 54 AEs occurred and they were assessed by WHO algorithm. Thirty six (67%) reports of *Curcuma longa* AEs were collected. Of these, 61% were gastrointestinal system disorders: nausea, abdominal pain, flatulence, anorexia, diarrhea, etc. Thirty three percents were general disorders including chest discomfort, headache, dizziness etc. Seventeen percents of AEs reports were *Andrographis paniculata*: abdominal pain, anorexia, inflammations. Conclusion: Almost all AEs occurred from using herbal medicines were known but for using among large populations, we should be continuing efforts to monitor safety, to assure and promote using them based on scientific evidenced.

PG27 The effects of *Echinacea* and its alkylamides on CYP3A4 transcriptional activity

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An important safety concern with herbal medicinal products (HMP) is interactions with conventional medicines, particularly ones mediated by the Cytochrome P450 enzyme system (CYP). Previously, we established that *Echinacea* and its immunomodulatory alkylamides [1] can weakly inhibit the activity of the main drug metabolising CYP isoforms [2]. However, the ability of *Echinacea* to alter CYP expression levels is not well characterized. In this study we investigate whether exposure of HepG2 cells to a commercial *Echinacea* extract (Echinaforce®) and four alkylamides (dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetranoic acid isobutylamide (**1**), dodeca-2*E*,4*E*-dieonoic acid isobutylamide (**2**), undec-2*E*/*Z*-ene-8,10-diyonic acid isobutylamides (**3**) and dodec-2-ene-8,10-diyonic acid isobutylamide (**4**)) can promote the transcription of CYP3A4 (the most important drug metabolizing CYP). HepG2 cells were treated for 96 hours with clinically relevant concentrations of either Echinaforce® (22 μg/ml or 11.6 μg/ml or 1.16 μg/ml) or the alkylamides (1.62 nM or 44 nM). Real-time RT-PCR analysis demonstrated that there were no statistically significant changes in the steady state mRNA levels of CYP3A4 compared to the vehicle control (medium with 0.1% ethanol). In contrast, treatment with 50 μM rifampicin resulted in a 3.8-fold upregulation of the steady state mRNA level of CYP3A4. Observations of β-actin upregulation by Echinaforce® in human monocytes/macrophages [1] do not appear to be relevant to HepG2 cells. Using GAPDH as a reference gene we found that β-actin was not upregulated by Echinaforce® or its alkylamides in HepG2 cells. Our data suggest *Echinacea* is unlikely to affect the transcription of CYP3A4, at concentrations previously shown to induce mild CYP 3A4 inhibition. *Acknowledgements: Bioforce and the Mapplethorpe Trust Fund, University of London, for funding this project.* **References:** [1] Gertsch, J. et al. (2004) FEBS letters 577:563 – 569. [2] Modarai, M. et al. (2007) J. Pharm. Pharmacol. 59:567 – 573.

PG28 Toxicity studies for antidiabetic herbal formulation: a crude mixture (1:1:1) of *Stevia rebaudiana*, *Andographis paniculata*, and *Tinospora cordifolia*

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Present study, evaluates "Acute toxicity" and "Genotoxicity" [1] of polyherbal antidiabetic formulation [2]. A "14 Days Dose Range Finding Study" and "*in vivo* Micronucleus Test" was also carried out, in rats. For "Acute toxicity study", formulation was administered to the rats, orally (1000 and 2000 mg/kg b.w), observed as per OECD guidelines, no toxic symptoms were observed. In a "14 Days Dose Range Finding Study"; the drug was administered orally at three different doses: 250, 500 and 750 mg/kg b.w, once daily for 14 days. Various hematological and biochemical parameters: Glucose, Urea, *etc,* were measured. Animals were sacrificed on 15th day. Different organs (liver, kidney and heart, *etc.*) were processed for histopathological study. The bone marrow smears were also evaluated for micronucleus induction potential. Test substance did not produce any adverse pathological effect or its related changes in either sex, at the high dose (750 mg/kg) level. Maximum dose of 750 mg/kg b.w was well tolerated, after 14 days continues administration. Bone marrow smear evaluation reveals that, MicroNucleated PolyChromatic Erythrocyte, MicroNucleated NormoChromatic Erythrocyte and PolyChromatic Erythrocyte/NormoChromatic Erythrocyte in high dose group animals, were comparable to control group animals. Results were analyzed, using Student's 't' test and one way ANOVA .This formulation was found to be non toxic (at acute and genotoxic level) up to 750 mg/kg b.w dose level. **References:** [1] Matsui, M. et al. (1996) Mutagenesis 11:573 – 579. [2] Chandra, S.J. et al. (2007) J. Health Sci. 53:245 – 249.

PG29 Constituents and quality control parameters of the vegetable oil from *Cucurbita moschata*, Duchesne, cultivated in Cuba

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Vegetable oils are essential in global nutrition. Depending on the used plant species and on regional conditions, a variety of oils with different qualities is produced. The pumpkin seed oils are produced from the seeds of pumpkins (*Cucurbita* species) and they have been utilized in pharmaceutical industry, first as a teniacid, then to relieve from disorders of the prostate gland and urinary bladder caused by benign prostate hyperplasia (BHP). The goal of this work is the study of the physicochemical properties and the fatty acid composition of pumpkin seed oil obtained from the variety *Cucurbita moschata* Duchesne, cultivated in Cuba. The virgin oil was obtained from raw materials of special quality by mechanical procedures (high-pressure screw-pressing) and evaluated for its physicochemical characteristics. The fatty acid profile analysis of the oil was also carried out by gas liquid chromatography. The oil obtained from the pumpkin seed had a relative density of 0.9, a refractive index of 1.4 and an optical rotation of 0.34. Its saponification, acid, peroxide, and iodine values were 221 (mg KOH/g oil), 1.7(mg KOH/g oil), 15 (meq peroxide/kg oil) and 144 (g I_2/100 g oil), respectively. The unsaturated fatty acids content was 77% and comprises 27% oleic acid and 49.9% linoleic acid. The saturated fatty acids concentration was 22.8% and is consisting of 16.4% palmitic acid and 6.5% stearic acid. These results are confirmed by the findings of Al-Khalifa [1] and Younis et al. [2] for the variety *C. pepo*, and by the analytical monographs of the European Pharmacopoeia, 5th ed. [3] for other vegetable oils. The oil studied had high iodine values, thus reflecting a high degree of unsaturation, whereas the presence of high amounts of the essential linoleic acid suggests that the pumpkin seed oil is highly nutritious. The parameters evaluated could be useful, as quality criteria, to the seed oil obtained from *Cucurbita moschata* Duchesne, cultivated in Cuba. **References:** [1] Al-Khalifa, *A.* (1996) J. Agric. Food Chem. 44:964 – 966. [2] Younis, Y. et al. (2000) Phytochemistry 54:71 – 75. [3] European Pharmacopeia. (2005) 5th ed. Council of Europe, Strasbourg, France.

PG30 Investigations of the underground parts of medicinally used plants and possible adulterations of various *Cardueae* and *Cichorieae*

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Asteraceae is the taxonomically most diverse family of plants comprising of more than 23,000 species and about 1,600 genera. Numerous representatives such as *Taraxacum officinale*, *Cichorium intybus, Silybum marianum,* and *Hieracium pilosella* to mention only few thereby have a long history in both traditional and western medicine. The anatomy of rhizomes and roots of a representative number of species from the tribes *Cardueae* and *Cichorieae* was analysed in detail as respective comparative studies on underground parts of medicinally used drugs and possible adulterations are missing yet for these diverse taxa. Until now, 28 genera and 37 species have been collected and examined by means of light microscopy and a database of typical anatomical characters created. In addition, some of the studied species were cultivated to follow the ontogenetic development of the underground organs at different states of growth. Particular attention was thereby spent to the secretory system: Endodermal resin ducts are characteristic to the *Cardueae*, whereas, according to literature data, these anatomical elements are restricted within the tribe *Cichorieae* to *Scorzonera hispanica*, *Tragopogon porrifolius*, and the genus *Scolymus*. *Cichorium intybus* and *Lapsana communis* were reported to exhibit the "doubling" of the endodermis, but with ducts missing [1]. Based on anatomical structure, we could distinguish different types of resin ducts. These various forms of resin ducts and the structural context of their occurrence, particularly with respect to tissue of origin and their position relative to prominent anatomical elements such as vascular bundles (e.g., a centrifugal position in *Centaurea scabiosa* versus an interfascicular position in *Carlina acaulis*) provided valuable characters to discriminate among the species studied. **Reference:** [1] Van Tieghem, M. Bull. Soc. Bot. Fr. 1884:112 – 116.

PG31 *In Vitro* genotoxic activities of the aqueous extract from Thai Noni's Leaves (ANL) in human lymphocytes

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Noni (*Morinda citrifolia* L.; Rubiaceae) is an evergreen tree that their roots, fruits and leaves have been traditionally used to treat various diseases such as cancer, malaria and arthritis [1]. Our earlier study reported that Thai Noni fruit juice is not genotoxic against human lymphocytes in spite of showing cytotoxicity at high doses (≥100 mg/ml) [2]. Aims of this study were to investigate the genotoxic activities of the aqueous extract from Thai Noni's leaf (ANL) in human lymphocytes. Chromosome aberration assay and sister chromatid exchange (SCE) assay *in vitro* were performed. Treatment of ANL (0.8 – 25 mg/ml) alone for 3 h did not significantly induce chromosomal aberration nor SCE ($p < 0.05$). Nevertheless, they could temporary arrest cell cycle as shown by lowering mitotic index measured after the first cell cycle. While proliferation index measured after the second cell cycle were getting higher value as compared to the positive control. Cytotoxicity was shown at higher doses (≥50 mg/ml). Therefore, concentration usage of ANL as food supplement is needed to be considered carefully for human safety. Nevertheless, ANL might be useful for treatment of human hyperproliferative disorder at appropriate dose. Since they interfere with cell cycle without possess genotoxic activities. Further scientific study is needed to verify the usefulness of the aqueous extract of Noni's leaf. *Acknowledgements: This study was supported by Research Fund, Faculty of Medecine, Thammasat University, Thailand.* **References:** [1] McClatchey, W. (2002) Integr. Cancer Ther. 1:110 – 120. [2] Ratanavalachai, T. et al. (2008) Songklanakarin J. Sci. Technol. 30:583 – 589.

PG32 Study of decomposition behaviour of absinthin from *Artemisia absinthium* using LC/MS and LC-SPE-NMR

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Artemisia absinthium L., commonly known as wormwood, is a well-known medicinal plant with monographs in several pharmacopoeas (e.g. Ph.Eur.). The aerial parts are used to treat anorexia and indigestion. Sesquiterpene lactones e.g. absinthin, a dimer guaianolide, and the monomer artabsin account for the bitter taste of this plant. Absinthin is also regarded as marker substance to confirm the authenticity of wormwood. In weak acidic medium absinthin is unstable and isomerizes into anabsinthin [1]. Older literature describes absinthin as a glucoside, being decomposed by hydrolysis into sugar, a liquid and a resinous compound [2]. To determine the stability of the bitter substance, the degradation behaviour of absinthin was investigated under different stress conditions (hydrolytic, oxidative, photolytic and thermal). An aqueous ethanol solution of absinthin was found to be stable for up to 6 months (recovery rate by HPLC analysis ≥95%). This was also the case when the solid compound was kept in the refrigerator at -35 °C. In contrast, the colourless needles, when stored in an exsiccator at 25 °C and exposed to light, turned yellow. In total, 3 decomposition compounds were detected by LC/MS and LC-SPE-NMR, and identified as dimeric sesquiterpene lactones. The major degradation product was anabsinthin, which is not only formed in acidic medium, but also under acid-free conditions [1]. *Acknowledgemenst: This work was financially supported by Bionorica research GmbH, 6020 Innsbruck, Austria* **References:** [1] Hänsel, R. and Sticher, O. (2007) Pharmakognosie – Phytopharmazie, 8. Auflage, Springer Verlag, Heidelberg. [2] Senger, O. (1892) J. Chem. Soc. 62:1240 – 1241.

PG33 Optimization of qualitative determination of Mezereon homeopathic tincture by applying rapid horizontal TLC

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Daphne mezereum L., Thymelaeaceae, Mezereon or February Daphne is a deciduous shrub up to 150 – 200 cm with spicy-fragrant pink flowers native to Europe as far as Siberia, Caucasus, Western Asia and cultivated in North America [1]. In homeopathy, preparations from fresh bark of branch collected at the beginning of blossom, are used in the treatment of respiratory and skin diseases, indigestions, neuralgia, bone pain, and other pain symptoms [2,3]. Mezereon is monographed in the German Homeopathic Pharmacopoeia, the described TLC-analytical conditions are comperative and might be updated. Continuing our work on optimization of TLC-analytical investigations for improved homeopathic pharmacopoeia monographs an easy and time and material saving horizontal TLC for Mezereon homeopathic tincture is proposed. Small amounts (2 – 10 µl) of homeopathic tinctures and references (scopoletin, umbelliferone, mezerein, daphnetoxin) are directly tested by using 5 x 5 cm silica gel plates (Si 60-, HPTLC-, RP-material) and applying 2 – 3 ml of various mobile phases containing toluene, ethyl acetate, and formic acid at different proportions. After eluation (2 – 3 min) and drying the plates are detected by vis, UV_{254}, and UV_{366}. Scopoletin, umbelliferone, and daphnetoxin (in traces) are distinctively identified in the ethanolic tinctures, mezerein was not found in the bark. The applied procedures may be proposed for an up-dated and optimized TLC identification test of the homeopathic monograph of *Daphne mezereum* L. easily being performed as a routine qualitative analytical method. **References:** [1] Brendler, Th. et al. (2003) Herbal Remedies, medpharm, Scientific Publishers, Stuttgart. [2] Hagers Handbuch der Pharmazeutischen Praxis (2006) Springer, Heidelberg. [3] Deutsches Homöopathisches Arzneibuch (HAB 2008), Monograph *Daphne mezereum* (Edition 2000).

PG34 Molecular identification and quantitation of *Hypericum perforatum* in mixed samples

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The requirement for quality assurance in commercial medicinal plant products has been brought into focus as European national regulatory authorities move towards the 2011 deadline to implement the Traditional Herbal Medicines Directive (Directive 2004/24/EC. Medicinal plant products for human use in the European Union). In this context, we report the development of a DNA-based method for the identification and authentication of plant species, based upon the economically important St John's Wort (SJW) (*Hypericum perforatum* L.). The ITS regions of the nuclear-encoded ribosomal RNA genes provided the target for primer design. Sequences from 91 *Hypericum* species were analysed in order to identify the most divergent regions, and four PCR primers were designed to anneal specifically to *H. perforatum* in these regions. All of these were proved empirically to be SJW-specific when compared to DNA from likely contaminant or adulterant species. As herbal medicinal products are often sold as mixtures, a quantitative method of identification is of particular interest. Real-time PCR enables quantitation of template DNA by virtue of monitoring the reaction at every cycle, and this procedure was optimised for quantitation of SJW in a mixed preparation. A generic primer pairing was used to measure all of the amplifiable nuclear DNA within a sample; the specific primers allowed quantitation of SJW DNA. This gives a measure of the specific DNA as a proportion of total DNA, which could be used to calculate the ratio of SJW plant material in a mixed sample, or detect the presence of contaminant or adulterant plant material in pure SJW preparations.

PG35 Determination of best harvest time of German chamomile (*Matricaria chamomilla* L.) flowers based on solid-phase microextraction-GC-MS analysis data

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Solid-phase microextraction (SPME) technique is a relatively simple, rapid, inexpensive and solvent-free sampling technique for the determination of volatiles in plant essential oils when compared to conventional sampling techniques like steam distillation solvent extraction (SDSE). Moreover, newer SPME can be automated and does not lead to thermal degradation of chemical components [1,2]. In order to determine best harvest times in terms of quality of chamomile (*Matricaria chamomilla* L.) grown in Belgium, SPME was applied. On April 15, 2005, 90 day old seedlings were transplanted into the field. At harvest, flowers were divided in two groups on the basis of development stage [3]. Stage I flowers corresponded to initial up to full development of the ligulate flowers, while tubular flowers were closed. Flowers were categorized as stage II when tubular flowers were partially (first circle) up to completely open. After drying, flower heads were analyzed by headspace SPME-GC-MS. Six marker compounds, i.e. (*E*)-β-farnesene, α-bisabolol oxide A and B, α-bisabolone oxide, (*Z*)-spiroether and spathulenol were identified and quantities compared statistically for the two different stages of flower development based on their GC peak area. Results indicate that most of the measured traits were not significantly influenced by the stage of development, except for (*E*)-β-farnesene. The peak area of this compound in stage I was significantly higher than in stage II. These results are in agreement with previous results obtained by SDSE. Therefore, SPME-GC-MS analysis is proposed to be a suitable technique for the differentiation in quality of chamomile flower development stages. **References:** [1] Shen, S. et al. (2005) J. AOAC Int. 88:418 – 423. [2] Rubiolo, P. et al. (2006) Phytochem. Anal. 17:217 – 225. [3] Franz, C. et al. (1978) Acta Hort. (ISHS) 73:229 – 238.

PG36 Phytochemical analysis and biological activity of the flavonoids from the Mongolian medicinal plant *Dianthus versicolor* Fisch.

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Dianthus versicolor Fisch. is used in Traditional Mongolian Medicine against various liver diseases. Until now chrysoeriol-C-glycosides and triterpenoid saponines were found in the plant [1,2]. Aqueous (OWE) and methanolic extracts were tested in the isolated rat liver perfusion model [3] and both types of extracts showed a dose dependent increase of the bile flow. For preparation of the OWE aerial parts were powdered and extracted with water (pH 2, trifluoroacetic acid) for 1 h by shaking gently. This simulates the traditional way of intake where the crude drug is taken together with a certain amount of water. The OWE was further fractionated by SPE yielding four fractions. The 40% methanolic fraction showed a dose-dependent effect on the bile flow which was – even though in higher concentrations – comparable to the liver-affecting cynarin. From this fraction six main flavonoids were isolated using CC, CPC and semi-preparative HPLC. Their structure elucidation (UV, MS, 1D- and 2D-NMR) revealed apigenin, luteolin and chrysoeriol C- and O-, di- and triglycosides. Three further flavonoids were characterized by UV and MS-data, one of them was identified as isovitexin-7-O-glucoside (saponarin). The nine flavonoids were quantified by HPLC on Aquasil C_{18} using a MeCN/H_2O (pH 2.8, trifluoroacetic acid) gradient and quercetin-3-O-rutinoside (rutin) as internal standard. Additionally, the total flavonoid content was determined by establishing a UV-spectrophotometric method following the European Pharmacopoeia. Plant material of two different origins was compared. The total flavonoid content amounted to 0.75% and 1.19% in the crude drug; the two OWEs contained 1.78% and 3.59%, respectively. **References:** [1] Boguslavskaya, L.I.

et al. (1983) Khim. Prir. Soedin. 6:783 – 784. [2] Ma, L. et al. (2009) J. Nat. Prod. 72:640 – 644. [3] Glasl, S. et al. (2007) Planta Med. 73:59 – 66.

PG37 Identification of *Rhei Rhizoma* by DART-TOF-MS: chemotypes discrimination

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Related crude herbal drugs with close morphological characteristics but different origins and chemical principles are commonly misused in local markets and can be significant problems in the quality controls of crude herbal drugs related products such as functional foods and botanical drugs. For example, *Rhei rhizoma* has been commonly used in Korea as a purgative and haemostatic agent. Among *Rheum* genus *R. palmatum*, *R. tanguticum*, and *R. officinale* were specified as official origin of *Rhei rhizoma* in Korean Pharmacopoeia. *Rheum undulatum* belongs to same genus with *R. palmatum* but has different chemical profiles and pharmacological properties. Since these two crude herbal drugs are used as alternatives each other at the market, it is important to develop efficient method to differentiate between these related species. Direct Analysis in Real Time (DART) ion source has developed for the real time measurement of small molecules from any surface in open air. As protonated molecular ions are observed by DART-TOF-MS for most compounds without tandem fragmentations, it produces relatively simple and clear whole mass profiles for the sample with multi-components. In total ion spectra from DART-TOF-MS of both *R. palmatum* and *R. undulatum* were observed free anthraquinones such as chrysophanol, emodin, rhein and physcion. Molecular ion peak of rhaponticigenin, aglycon of rhaponticin was detected only from *R. undulatum*. The existence of rhaponticigenin molecular ion peak can be a characteristic indicator for the discrimination between *R. palmatum* and *R. undulatum*. A few seconds analysis of raw materials of *Rheum* genus with DART-TOF-MS was enough to get the whole mass spectrum from each species. These results showed the unprecedented power of DART-TOF-MS in discrimination of crude herbal drugs by its chemotypes within a few seconds of analysis.

PG38 Detection of characterising compounds on TLC by DART-MS

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The new ion source technique, direct analysis in real time (DART) atmospheric pressure ionization allows high resolution mass measurements of gas, liquid and solid samples. Ionization is proceed through a combination of processes including direct energy transfer from the metastable species and proton transfer after ionization of atmospheric water by the metastables. In positive ionization mode, analytes vaporized from the sample react with charged water clusters, forming protonated adduct ions. Since protonated molecular ions are observed by DART-TOF-MS for most compounds, it produces relatively simple and clear mass spectra for multi-component samples. In order to take advantage of quick and simple detection capacity of DART-mass for the analysis of characterising compounds of crude herbal drugs directly, separation on TLC plate was performed for some medicinal plants. Four medicinal plants were selected for this study. They have been reported to contain various bioactive constituents such as atractylon from *Atractylodis Rhizoma Alba*, paeonol from *Moutan Cortex Radicis*, berberine from *Phellodendri Cortex*, and anethol from *Foeniculi Fructus*. The developed plate was placed between the DART gas beam and the orifice for the real time analysis of each band directly. The bands expected to contain characterising compounds produced exact molecular ion peaks of four compounds within a few seconds of measurement. These results show that the real time analysis of characterising compounds on TLC by DART-TOF-MS could be an efficient tool for natural products analysis.

PG39 Development of HPTLC densitometric method for analysis of lycopsamine in comfrey (*Symphytum officinale* L.) using retrorsine as a reference compound

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Comfrey (*Symphytum officinale* L.) has been used topically for treating inflammatory disorders such as arthritis, gout and thrombophlebitis and internally for treating diarrhoea. Apart of allantoin and other constituents, which are considered to have therapeutic effect, it also contains toxic pyrrolizidine alkaloids (mainly lycopsamine, intermedine, their acetylated derivatives and symphytine). Because of their high toxicity it is of interest to determine these alkaloids in medicinal products even at low concentrations [1,2]. Unfortunately, many reference compounds of pyrrolizidine alkaloids are not commercially available [3]. Commercially available alkaloids may be used as reference compounds, but they must be standardised against marker alkaloids, which are actually present in the plant. Therefore, lycopsamine was isolated from comfrey roots and employed together with commercially available retrorsine for development of a high performance thin layer chromatography (HPTLC) method for quantitative densitometric analysis of pyrrolizidine alkaloids after derivatisation with Dann-Mattocks reagent. The method was validated according to ICH directives. It proved to be linear within 0.7 to 7.0 mg of lycopsamine per application of a sample. The mass factor retrorsine/lycopsamine was 1.18 (calculated with height of peaks) and 0.98 (calculated with area under curve). The method also proved to be specific and to have good repeatability (with RSD 2 – 4% within the plate). **References:** [1] DerMarderosian, A. et al. (2006) The Review of Natural Products: Comfrey. Wolters Kluwer Health. St. Luis, MO. [2] Wichtl, M. (2009) Teedrogen und Phytopharmaka (5th ed.). Wissenschaftliche Verlagsgesellschaft mbH. Stuttgart, Germany. [3] Wuilloud, J.C. et al. (2004) Analyst 129:150 – 156.

PG40 The pharmaceutical quality of 10 commercial samples of *Matricaria chamomilla* L. flowers used for medicinal teas

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This study is part of a larger research for a PhD thesis concerning the quality of commercial *Chamomillae flos* samples. Knowing that natural products do not necessarily mean safe products, we tried to evaluate the pharmaceutical quality of 10 different commercial samples of *Matricaria chamomilla* L. The samples were bought from specialized salespeople and pharmacies. A number of qualitative (macroscopic and microscopic tests, thin layer chromatography – TLC) and quantitative (spectrophotometer, HPLC) methods were used to establish the composition of the plant material. The macroscopic study revealed the presence of major impurities for the majority of the plant material and the microscopy indicated that most of the samples contain true chamomile. The qualitative analysis showed similar compound spectra for the flavones and polyphenolic acids. The flavones (values between 0.4915 and 0.8041 mg/100 g drug) and polyphenolic acids (up to double concentration for the richest sample compared to the poorest) content varied a lot from one sample to the other, confirmed both by spectrophotometric and HPLC analysis. It seems that the ferulic acid is best represented, while the lowest values are found for caffeic acid. All in all, we can state that even if the samples have a similar compound spectra, the extractibility for each active substance is different. Also, the commercial samples can not be considered as equivalent, the role of the pharmacist regarding the right posology is decisive for the expected pharmacological activity. **References:** [1] Franke, R. and Schilcher, H. (2005) Chamomile Industrial Profiles, Taylor and Francis, Boca Raton, USA. [2] Wagner, H. et al. (1983) Drogenanalyse, Dünnschichtchromatographische Analyse von Arzneidrogen, Springer Verlag, Berlin, Germany.

PG41 Analysis of ginseng dietary supplements – content of ginsenosides

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Products based on *Panax ginseng* are the most popular commodity of Chinese traditional herb medicine worldwide. Due to the minimal side effects ginseng is predominantly used as component of various tonic and adaptogenic dietary supplements [1]. However, because dietary supplements, despite of their increasing popularity, are not subject to the same regulations that pharmaceuticals are, there are concerns for their purity and potency [2]. Ginseng saponins (ginsenosides), which are unique for *Panax* species and are associated with their pharmacological activity, appear as suitable marker compounds for quality control [3]. In our work, 11 ginseng products (tablets, capsules, extracts, mixtures) commercially available on Czech market were evaluated for the presence and quantity of 12 ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, Rh_2). Each of them were identified by comparison of retention times with standards and confirmed by LC/MS. The quantification was carried out by external standard method using UV detection (at 203 nm). Ginsenosides were found in 8 products only. Three preparations contained low concentrations of total ginsenosides (from 1.0 to 2.2 mg per recommended daily dose). In three other products no saponins were detected. The results suggest that not all of the products on Czech market where ginseng addition is declared contains sufficient amount of the ginsenosides. *Acknowledgement*: *This work was supported by KJB 400550705 project.* **References:** [1] Cai, Z. et al. (2002) J. Mass. Spectrom. 37:1013 – 1024. [2] Harkey, M.R. et al. (2001) Am. J. Clin. Nutr. 73:1101 – 1106. [3] Li, W.K. and Fitzloff, J.F. (2002) J. Liq. Chromatogr. R T 25:2485 – 2500.

PG42 Metabolic profiling of the Brazilian medicinal plant *Erythrina velutina* (Willd) Fabaceae

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Erythrina velutina (EV) is popularly used in Brazil against central nervous system disorders. Recently, anticholinesterase activity (ACA) of the plant has been detected in mice brain, suggesting a potential therapeutic usage for EV to combat Alzheimer disease (AD) symptoms. To get insight into the variation in metabolite composition between different EV trees and to study the effect of growing area and harvest season, we profiled leaf extracts using an untargeted LC-QTOF-MS metabolomics approach. Multivariate data analyses tools were subsequently applied to identify differences and similarities between samples and to link the variation in their metabolic profiles to variation in bioactivity. Principal component analysis of the LC-MS data showed a clear separation of the extracts, which, however, was independent of growth location or harvest time. As a measure of bioactivity, we assayed *in vitro* both ACA and antioxidant activity, as antioxidant compounds have been shown to exert neuroprotective effects in AD models. Individual antioxidants were subsequently profiled by HPLC with an on-line antioxidant detection system and identified by LC-MS/MS. The antioxidant profiles showed the presence of three main antioxidants present in all leaf material analyzed though at varying levels. Using partial least square regression-discriminant analysis (PLS-DA), the metabolic profiles could be clustered into two groups related to differential bioactivity, for both ACA and antioxidants, and the metabolites most significantly contributing to the clustering were selected. Currently, the structural elucidation of the most active compounds is underway. *Acknowledgments: CAPES, CNPq, PRI.*

PG43 Changes in the content of hyperoside in aerial parts of *Pulmonaria mollis* during the vegetation period

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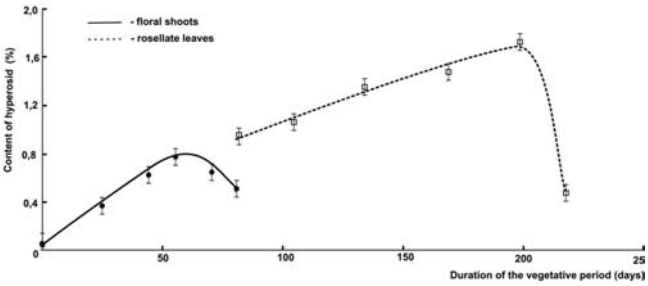
Extracts taken from the aerial parts of *Pulmonaria mollis* Wulf. ex Hornem. (Boraginaceae) have anti-anemic activity. Bioactive compounds having anti-hemorrhagic activity play an important role in the phytotherapy of anemia. Such compounds include flavonoids which can be found in *P.mollis*. The presence of some flavonoids (predominantly hyperoside) in the aerial parts of *P.mollis* was established by TLC (SiO_2). The content of flavonoids was analysed using spectrophotometric methods with $AlCl_3$. The total amount of flavonoids was calculated as the hyperoside equivalent.



There are 2 periods in the development cycle of *P.mollis*. The first one is a short period from the beginning of vegetation to fruiting, in which floral shoots are developing and the second one is a long period of rosellate leaf formation, where leaves are growing from underground resting buds. The pattern of change in the amount of hyperoside in the aerial parts of the plant gathered at different periods is similar – the quantity of hyperoside increases linearly to a maximum and then there is a sharp decrease. For extracts of the buds, there is only one maximum in the UV spectrum (λ_1= 360 nm). This fact confirms the absence of hyperoside at the bud stage. The specimens gathered at later vegetation stages have 2 maxima of absorption (λ_1= 360 nm and λ_2= 408 nm in UV-spectrum), with the older plants having a lower maximum at λ_1= 360 nm. These results show the optimum collection time for the plants, when the content of hyperoside is at a maximum.

PG44 Change in content of trace elements in the aerial parts of *Pulmonaria mollis* in the flowering stage

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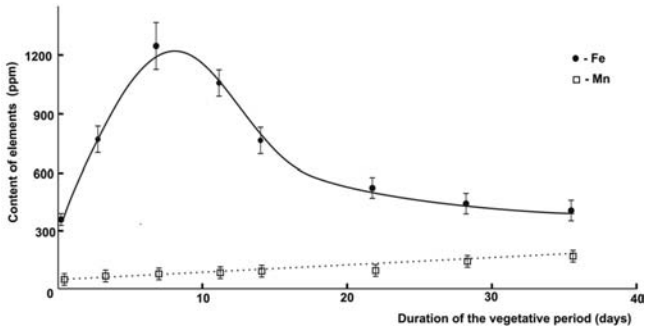
A found antianemic activity of the extract of aerial parts of *Pulmonaria mollis* Wulf ex Hornem of the family Boraginaceae is mainly connected with the significant amount of microelements of a hematopoietic complex Fe, Mn, Cu and Co. The amount of microelement was determined by means of mass-spectroscopy with inductively coupled plasma. It has also been found out that the amount of Fe and Mn is changing significantly in the process of development.



The determined dependence of iron amount on length of vegetation during flowering stage is most probably connected with the peculiarities of a development cycle of *P. mollis*. *P. mollis* is a typical ephemeroid that is why in winter after snow melting floral shoot begins to develop sharply. In connection with this the usage of such a biogenic element as Fe intensifies greatly. But at the same time its absorbtion from the ground is limited by the root system without compensating increased consumption. Sharp increase of the biomass of a plant in the flowering stage on condition of insufficient getting of iron from the root system leads to considerable decrease of Fe concentration. The amount of Mn in flowering stage increases and at some moment it becomes equal to the amount of Fe. In this case the ratio of standard oxidation potentials Fe^{3+}/Fe^{2+} and Mn^{3+}/Mn^{2+} is that *"in vitro"* the oxidation reaction from Fe^{2+} to Fe^{3+} will proceed spontaneously. The presence of Fe^{3+}-ions in the structure of plant extract is very useful for phytotherapy of iron-deficiency anemia.

PG45 Analysis of *Forsythia suspensa*, for the purpose of establishing a monograph draft proposal for the German Pharmacopoeia

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The development of appropriate analytical procedures is proposed for the TCM-herbal drug *Forsythia suspensa*, which is planned for an implementation in the German Pharmacopoeia DAB. The quantification method described by the respective monograph of the Chinese Pharmacopoeia (English Edition) [1], suggesting a HPLC method for the quantification of forsythoside A, was improved by a more simple methanol reflux extraction. As a first step for the implementation of an appropriate quantification with HPLC, the method suggested by Wagner [2] for the fingerprint analysis of *Forsythia* was investigated. We propose the use of a H_2O/acetonitrile/acetic acid gradient on a RP-18 column (Purospher Star, 5 µm, 250 x 4) which allows the quantification of the forsythoside A at a detection wavelength 325 nm within 35 minutes. In a second step we developed a completely new densitometric HPTLC method for the quantification of forsythoside A, using fully wettable RP18 plates and a mixture of H_2O/acetonitrile/acetic acid. For the densitometric TLC and the HPLC quantification method a validation protocol is established. In addition, identification methods for this herbal drug (macroscopic, microscopic, TLC/HPTLC) and appropriate tests for purity are proposed [2]. All the newly developed and controlled methods will be the basis of a monograph draft suitable for the DAB. *Acknowledgements: The BfArM (Kurt-Georg-Kiesinger-Allee3, 53175 Bonn, Germany) is gratefully acknowledged for financial support.* **References:** [1] Anonymous, Pharmacopoeia of the People's Republic of China Engl. Edit. 2005. [2] Wagner, Chinese Drug Monographs and Analysis, Fructus Forsythiae 2004. [3] Anonymous, (2005) European Pharmacopoeia 5th Edit.

PG46 Sitosterol content of some rapeseeds cultivars developed in Romania

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Rapeseeds (*Brassica napus L. ssp. oleifera*) play an important role as a source of oil in human diet. They provide the most concentrated source of energy and also help in absorbtion of fat-soluble vitamins [1]. Sterols are the major constituents of the unsaponifiable fraction from most vegetable oil. Phytosterols are known to inhibit absorbtion of dietary cholesterol [2]. Sitosterol is the major phytosterol identified in rapeseed oil. In this paper, seed samples of 10 rapeseed cultivars developed in Romania (ADER, ATTILA, MILENA, SAVANNAH ONTARIO, COULVERET, POTOMAC, BELINI, TENNESSE, LG) were analysed for sitosterol content. Oil was extracted with solvent and the sitosterol content was analysed using high performance liquid chromatography coupled with mass spectrometry (HPLC/MS) [3]. Analytical column used is Zorbax SB-C 18 100 mm x 3.0 mm i.d., 5 µm, pre-column Zorbax SB-C 18. The detection limits (200 ng/ml), regression coefficients and reproductibility of the method were established. The high sitosterol content was registred to the SAVANNAH cultivar 1375.7 (µg/g) and the small quantities (2.9 µg/g) to COULVERT cultivar. **References:** [1] Mortuza, M.G. (2006), Pakistan J.Biol. Sci. 9:1812 – 1816. [2] Eskin, N.A. and McDonald, B.E. (1996) Baily's Industrial Oil and Fat Products, Hui, Y.H ed., 5th edition, John Wiley & Sons, Inc. New York, USA [3] Sanchez-Machado, D.I. and Lopez-Hernandez, J. (2004) Biomed. Chromatogr. 18:183 – 190.

PG47 Comparative study of HPLC methods for the Analysis of Diterpene Glycosides from *Stevia rebaudiana*

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Extracts of *Stevia rebaudiana* have demonstrated sweetness up to 300 times greater than table sugar, and have recently been granted approval through the GRAS certification process for use in commercial food and beverage products. With the growing commercial use of *Stevia* extracts there is an increased need for testing for the known 'impurities' of *Stevia* extracts. A comparative study is made between the industry accepted JECFA method which utilizes NH_2-columns under isocratic conditions [1] and an improved method, developed by the authors, which utilizes a Phenomenex Synergi Hydro-RP column and a linear gradient [2], for the HPLC analysis of rebaudioside A and related diterpene glycosides found in *Stevia rebaudiana*. The improved method offers greater sensitivity and greater resolution of minor constituents and maintains resolution over extended use of the column. The improved method is also readily compatible with additional detection techniques including mass spectrometry (LC/MS) and Evaporative Light Scatting Detection (ELSD), overcoming a limitation of JECFA method. The two methods have been evaluated using standards of rebaudiosides A, B, C, D and F, dulcoside A, isosteviol, isosteviol monoside, steviol, steviol glucuronide, stevioside and steviolbioside. **References:** [1] Kolb, N. et al. (2001)J. Agric. Food Chem. 49:4538 – 4541. [2] Hoekstra, B., Schaneberg, B. (2007) Graz Poster Presentation.

PG48 Swedish bitter – Total polyphenols and HPLC-MS analysis

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Bitter is a natural traditional tonic initially prepared by Paracelsus and then re-discovered by Swedish doctors in the XVIIIth century [1,2]. Bitter contains volatile oils and bitter principles of carminative, antispastic, cholagogue-choleretic or aperitif action [3]. The present study aims to clarify the types of vegetal products used for the preparation of two Bitter formulas: I and II and also the analysis of polyphenols and flavonoids. The vegetal mixture was purchased from Galke (Germany). An

alcoholic extract was prepared by cold maceration, during 10 days and daily stirring, using a vegetal product-alcohol 70% ratio of 1:10. Total polyphenols were determined using the Folin Ciocalteu method [4] and a series of 19 standards was used for the HPLC-MS analysis. Non-hydrolyzed and hydrolyzed extracts were analyzed. The first bitter formula (I) contains a mixture of 11 vegetal products and 0.62 mg/100 ml total polyphenols and the second formula (II) a mixture of 17 vegetal products and 0.82 mg/100 ml total polyphenols. The HPLC-MS method emphasized 3 types of ploypheno ls in the nonhydrolized sample and 4 types in the hydrolyzed one for Bitter I. For the Bitter II formula 2 polyphenols were found in the nonhydrolized sample and 3 in the hydrolyzed one. **References:** [1] Weiss, R. and Fintelmann, V. (2000) Herbal Medicine Thieme, Stuttgart, New York. [2] Wagner, H. (1999) Arzneidrogen und ihre Inhaltsstoffe, Stuttgart. [3] Istudor, V. (2001) Farmacognozie, fitochimie, fitoterapie, Editura Medicala. [4] Peev C. (2006) Analiza în laborator a produselor naturale medicinale, Ed. Mirton, Timişoara.

PG49 Evaluation of *Lycopus uniflorus* water extract for anti-inflammatory, anti-ulcerogenic and antioxidant activities

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Lycopus uniflorus, commonly known as bugleweed, is usually found near freshwater wetland and has a wide abundance in Lebanon. The plant has some folk use for treatment of gastrointestinal disorders and reduction of inflammation. No studies were conducted on *Lycopus uniflorus*, but other species were reported to possess anti-allergic [1], anti-inflammatory [2], antihyperthyroidism [3], *Yarnell and Abascal, 2006* E. Yarnell and K. Abascal, Botanical medicine for thyroid regulation, *Alternative and Complementary Therapies* (2006), pp. 107 – 112. **Full Text** via CrossRef | View Record in Scopus | Cited By in Scopus (2) antioxidant [4] and anticancer [5] activities. The present study was conducted to investigate the potential role of *L. uniflorus* water extract on inflammation and gastric ulcer in rat model. Pretreatment with the extract (50, 100 and 200 mg/kg BW by gastric gavage) produced a dose dependent protection against gastric ulcer induced by 60% ethanol. Results showed that 50 mg/kg and 100 mg/kg caused 20.87 and 80.10%, respectively, compared to 41.70% for cimetidine at 11.5 mg/kg BW dose. The same doses were used to test for anti-inflammatory activity against edema induced in hind paw by carrageenan. Intra-peritoneal doses of 100 and 200 mg/kg BW of the extract showed significant ($p < 0.05$) anti-inflammatory activity when compared with the untreated control. The highest inhibition of edema development was achieved at 100 mg/kg BW dose and the edema was decreased by 67% compared with diclofenac (60%) at 10 mg/kg BW. No signs of toxicity were observed at used doses. Antioxidant activity was established using DPPH assay and the extract exhibited high DPPH radical scavenging activity (80.4%). The estimated total phenolic content was 228.5 GAE per gram. The results show that *Lycopus uniflorus* possesses a potential anti-inflammatory, anti-ulcerogenic and antioxidant activities. *Acknowledgments: Mr. Jean Karam.* **References:** [1] Shin, T.Y. et al. (2005) Toxicol. Appl. Pharm. 209:255 – 262. [2] Lee, Y.G. et al. (2008) Vasc. Pharmacol. 48:38 – 46. [3] Vonhoff, C. et al. (2006) Life Sci. 78:1063 – 70. [4] Ślusarczyk, S. et al. (2009) Food Chem. 113:134 – 138. [5] Cai, Y. et al. (2004) Life Sci. 74:2157 – 2184.

PG50 Problematics for the validation of analyses of TCM herbal drugs and herbal drug preparations: the case *Fructus Gardeniae* (Zhizi) and its preparata

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Fructus Gardeniae (Zhizi) is the dried ripe fruit of *Gardenia jasminoides* Ellis (Fam. Rubiaceae). According to Traditional Chinese Medicine (TCM) it reduces pathogenic fire, eases the mind, eliminates damp-heat, promotes diuresis and removes heat-toxicity from blood [1]. These preparations are widely used for treatment of many diseases, such as hepatitis, inner fever, hypertension, and diabetes [2]. Zhizi contains a large amount of iridoid glycosides to which can be related the activity of the herbal drug. However, other classes of constituents such as crocins and caffeoyl quinic acids are also present and can contribute in part to the activity [3]. Different preparata represented by the herbal drug dried after steaming are also present on the market and during the heat-treatment some constituents can modify their structures. The aim of this work was the validation of an HPLC/DAD/ESI-MS method to be used for the complete characterization of *Fructus Gardeniae* (Zhizi) and its preparata. The proposed method was validated and because its simplicity, sensitivity, accuracy and reproducibility, can be conveniently used for the analysis of the characteristic iridoids, crocins and quinic acid derivatives. The iridoids identified were scandoside methylester, gardenoside, genipin gentiobioside, geniposide, acetylgeniposide. The crocins were: crocetin, crocin-1, 2 and 3. The quinic acid derivatives identified were 3,4-dicaffeoyl-5-(3-hydroxy-3-methylglutaroyl) quinic acid and 3-caffeoyl-4-sinapoylquinic acid. **References:** [1] Pharmacopoeia of the People's Republic of China (2005) 1:95 – 96. [2] Koo, H.J. et al. (2006) J. Ethnopharmacol. 103:496 – 500. [3] Wagner, H. et al. (2004) Chinese Drug Monograph and Analysis. Fructus Gardeniae – Zhizi 5 (22): ISSN 1430 – 8290.

PG51 Chemotype discrimination of *Curcuma* species by DART-MS

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Ambient ionization mass spectrometry allows for the direct analysis of ordinary compounds in the open atmosphere. DART (Direct analysis in real time) is one of the new generation of ambient desorption ionization techniques [1]. It analyzes samples directly in their native condition, bypassing most elements of the analytical system and transferring ions into the mass spectrometer without any sample preparation steps. Related crude herbal drugs with close morphological characteristics but different origins were subjected to DART-MS analysis for the intraspecies discrimination by their chemotypes. Four groups of samples of rhizomes and leaves from *Curcuma* species were selected for this study. DART-MS spectra profiles showed that four groups were divided into two main species and identified as *Curcuma longa* and *Curcuma phaeocaulis*. In rhizomes samples, molecular ion peaks of curcuminoids were detected only from *C. longa* [2]. In leaves, molecular ion peaks at m/z 285 and 303 were detected only from 1 and 2 groups which were identified as *C. longa*, while the curzerenone, curcumenol and zedoarol ions were detected only from 3 and 4 groups [3]. These sesquiterpenes are main components of *C. phaeocaulis* and easily detected in rhizomes and leaves. A few seconds analysis of small parts of intact *Curcuma* species with DART-MS was enough to get the significant profile of mass spectrum for chemical discrimination. These results showed DART-MS is a fast and easy tool for the determination of the chemical composition and for the chemical discriminations between related species of crude herbal drugs. **References:** [1] Robert, B.C. et al. (2005) J. Anal. Chem. 77:2297 – 2302. [2] Chihiro, T. et al. (2006) Evid.-Based Compl. Alt. 3:255 – 260. [3] Raina, V. K. et al. (2002) Flavour Fragr. J. 17:99 – 102.

PG52 Asbestos fibers in talcum – is this a pharmaceutical problem?

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The inhalation of asbestos fibers can cause different adverse effects, especially to the lung, where the size and diameter of the fibers are critical for their toxicity. Whilst asbestos-induced diseases like lung fibrosis, pleura thickening and mesothelioma are quite common, we report on mostly unknown diseases in humans caused by asbestos. In severe cases, asbestos can cause mesotheliomas, i.e. malignant tumours of the pleura and peritoneum, with a long latency period of about 20 – 40 years. First we report on a veterinary doctor in northern Germany who died from peritoneal mesothelioma within a few months after surgery. His death was most probably provoked by asbestos fibers from powdered medicinal gloves. Talcum, which is used in sports and medicinal gloves, has its natural sources in Russia, Canada and China. Chinese talcum is contaminated with up to 10% asbestos fibers. The widow of the doctor investigated the link from talcum to asbestos fibers to the mesothelioma disease, but the insurance company did not accept this causality. Talcum powder is also found in baby, body and makeup powders and is also part of the ingredients of traditional Chinese medicine (TCM). It is likely that other tumours are also the result of talcum ex-

posure, without this being known. The current situation is that asbestos has been banned in Germany since 1993, in all EU member states since 2005, and also outside Europe in several states like Argentina, Australia, Chile and Saudi Arabia. The U.S.A. has not banned asbestos. This means that asbestos is still used in many products, mainly as an additive to plastics or cement.

PG53 Studies on the effects of *Vernonia amygdalina* leaf extract and fractions on biochemical and hematological parameters in diabetic rats

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Vernonia amygdalina L, Asteraceae (Bitter leaf) is a common traditional anti-diabetic remedy in many parts of Nigeria. Some scientific studies have confirmed its efficacy in both experimental animals and humans. In this study, we investigated the subacute toxicity of the leaf extract and fractions of *Vernonia amygdalina,* as well as their effects on biochemical and hematological parameters in alloxan-induced diabetic rats. Diabetes was induced in rats with a single intravenous injection of alloxan monohydrate (70 mg/kg). The alcohol extract and its chromatographic fractions were orally administered to the diabetic rats once daily for 28 days. The blood sugar lowering effects and the biochemical and heamatological effects of the treatments were determined. The vital organs were also examined for possible abnormality. The results confirmed the anti-diabetic potency of *V. amygdalina* leaf extract. Chromotographic fraction F6 exhibited the most potent anti-diabetic effect at a dose of 160 mg/kg. In addition, it significantly ($P < 0.05$) decreased elevated serum levels of TG, LDL-C, VLDL-C, and increased HDL-C level in diabetic rats. At the doses of 160 and 320 mg/kg, there was a significant ($P < 0.05$) increase in the lymphocyte counts. Urea and creatinine levels were significantly reduced while electrolyte levels were on the average increased. Diabetes associated elevation of hepatic enzymes (AST, ALT and ALP) were significantly reduced. Histological examination revealed no remarkable abnormality in the vital organs of the treated rats. These results suggest that in addition to its hypoglycemic and hypolipidermic effects, V. *amygdalina* is capable of normalizing the biochemical and heamatological abnormalities associated with diabetes mellitus.

PG54 Mycopopulation in five important cultivated medicinal plants in Serbia

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The mycopopulation of medicinal and aromatic plant seeds used as herbal drugs were studied during the last five years to find out the cause of their microbiological unacceptability. Commercial samples (produced by the Institute for Medicinal Plant Research "Dr J. Pančić", Belgrade, Serbia) of caraway (*Carum carvi* L.), coriander (*Coriandrum sativum* L.), flax (*Linum usitatissimum* L.), fenugreek (*Trigonella foenum-graecum*) and fennel (*Foeniculum vulgare* Mill.) were tested. Total of 21 fungal species from 17 genera were isolated: 15 spp. from 11 genera (caraway), 10 spp. from eight genera (coriander), 14 species from nine genera (flax), 17 spp. from 11 genera (fenugreek) and 13 spp. from 11 genera (fennel). The isolated fungi belong to the genera *Fusarium, Alternaria, Phoma, Sclerotinia, Cladosporium, Penicillium, Aspergillus, Mucor, Rhizopus, Cephalosporium, Chaetomium, Verticililum, Epicoccum, Myrothecium* and *Physarum.* The most common fungus on seeds was *Alternaria alternata,* which was isolated from all examinated seeds of medical and aromatic plants. Eleven species belong to the genus *Fusarium: Fusarium oxysporum* (31.4%), *F. verticillioides* (17.7%), *F. solani* (9.7%), *F. equiseti* (5.8%), *F. proliferatum* (4.5%), *F. avenaceum* (3.0%), *F. semitectum* (3.0%), *F. sporotrichioides* (2.2%), *F. tricinctum* (2.2%), *F. graminearum* (2.1%) and *F. acuminatum* (2.0%). The sclerotia of *Sclerotinia sclerotiorum* were found only in one seed lot of caraway. The most of them is known as producer of mycotoxins. On the base of qualitative and quantitative findings on recorded mycoflora of tested seed samples, to assure safety and quality control requirements, results of the analysis of related cytotoxins should be find as detrimental in making final decision on the usability of products. *Acknowledgements: The part of this study was supported by the Serbian Ministry of Science, through the Project TR-2005.*

PG55 Determination of parthenin in *Parthenium hysterophorus* Linn (feverfew) by means of HPLC-UV: method development and validation

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Parthenium hysterophorus Linn, commonly known as feverfew, is generally used by the Cuban population as anti-parasitic (amoebic and antihelmíntic) agent and in the treatment of skin affections. The first step in the development of pharmaceutical solid forms from medicinal plants is the characterization of the starting material, i.e. the plant powder. The main active component present in *Parthenium hysterophorus* is the sesquiterpene lacton parthenin [1,2]. These methods, designed for research purposes, are not suitable for routine quality control due to their complex and laborious sample preparation. For that reasons the objective in this research project was to develop and validate a method suitable for controlling the quality of plant material used in the manufacture of pharmaceutical dosage forms. Because parthenin is not commercially available it had to be isolated from the plant material. A new procedure, non laborious method was developed for the isolation of parthenin reference material. The procedure described in the experimental section is superior to the procedure published in literature [3] in that it is less time consuming, less complex and less expensive (no preparative HPLC). Based on the validation results of all performance criteria, it can be concluded that the method is suitable to determine the parthenin content with acceptable accuracy and precision [4]. The method will facilitate the quality control of the plant material used for preparation of different herbal medicinal products which are in development. **References:** [1] Pickman, A. (1981) J. Chromatogr. 189:187 – 198. [2] Khare, C. (2007) Indian medicinal plants. An illustrated dictionary; New Delhi. Springer. [3] Reinhardt, C. et al. (2004) J. Plant Dis. Protect. 11:253 – 261. [4] ICH. Harmonised Tripartite Guideline. "International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use: Validation of analytical procedures and Methodology, ICH-Q2 A y B. Geneva, 2005.

PG56 Phytomedicine can provide a safe and reversible male contraceptive

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Man used various natural materials since ancient times as a source of medicines. 25% of all prescription drugs of modern pharmacopoeia still contain the drugs derived from plants or compounds isolated from plants [1 - 2]. Literature reveals that plant products have been used for human fertility regulation. Many plants have been screened to find safe and reversible contraceptive agents [3 - 6]. Therefore, *Euphorbia neriifolia*, *Citrullus colocynthis*, *Martynia annua* and *Withania somnifera* were screened with the intention of finding an orally active, cheap, reversible and safe fertility regulating agent for men. 50% ethanolic extracts of these plants were administered orally in male wistar rats according to the WHO guidelines. The weights of testis and accessory reproductive organs were recorded. The hematological and biochemical parameters were investigated for side affects. The sperm motility and density were assessed in the testis and epididymis. Data were analyzed statistically and CPCSEA guidelines were followed. The data shows no change in the final body weights, where as weight of testis and accessory reproductive organs were decreased in extracts treated rats. The decreased protein, glycogen, sialic acid, ascorbic acid and fructose content indicative of reduction of androgens. The mature sperm number in seminiferous tubule, motility and density significantly declined with the treatment reduced fertility of the extracts treated rats. The antispermatogenic effects of the drug reduced the number of spermatocytes and spermatozoa in the lumens of seminiferous tubules and sperm motility. The presence of spermatogonia in the germinal epithelium of the treated rats indicates possible reversibility after discontinuations of the extracts treatment.

References: [1] Waites, G.M.H. (2003) Development of methods of male contraception: impact of the WHO Task Force Fertil Steril 80:1-15. [2] Verma, A.K. et al. (2007) J. Ethnobio. Ethnomed. 3-35. [3] Akerele, O. (1984) WHO chronicle 38:76-81. [4] Mathur, R. (1985) Fertility control through plant products in recent advances in zoology (ed) CBL Srivastava and SC Goel, Rastogi Publication Co. Meerut 107-116. [5] Griffin, P.D. (1996) The annual technical report 1995, WHO, Geneva, 73-87. [6] Vasudeva, N. and Sharma, S.K. (2006) J. Ethnopharmacol. 107:179-181.

PG57 Quality control of cortex *Magnoliae officinalis*

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Cortex Magnoliae officinalis is the dried stem bark, root bark or branch bark of *Magnolia officinalis* Rehd. et Wils. or *Magnolia officinalis* var. *biloba* Rehd. et Wils. (Magnoliaceae). It has been used as traditional Chinese medicine for more than 2000 years for the treatment of epigastric stuffiness, vomiting and diarrhea, abdominal distention and constipation, cough and dyspnea, etc. It will be introduced as a monograph in the future European Pharmacopoeia, so the quality standard of this crude drug has been drafted, including identification, test (loss of drying, total ash, ash insoluble in hydrochloric acid, and aristolochic acid), as well as assay of magnolol and honokiol by HPLC. The TLC identification used toluene:ethyl acetate:methanol (30:2:1 v/v/v) as mobile phase, cineole and honokiol as references, and 1% vanillin sulphuric acid as spray reagent. The different test items were examined according to the corresponding protocols in present European Pharmacopoeia, and their limits were made on the basis of the results of 22 batch samples. However, no aristolochic acid was detected in this plant. The HPLC quantitation of magnolol and honokiol was performed on a reversed-phase C_{18} column with acetronitrile:0.5% acetic acid (60:40) as mobile phase at a flow-rate of 1.2 ml/min, and the detected wavelength was set at 290 nm. This method was validated for its selectivity, linearity, precision and accuracy. The samples from different sources were analyzed, and the contents of magnolol and honokiol were found to be 0.07 – 96.51 mg/g and 0.05 – 91.91 mg/g, respectively. The large quantitative differences observed do justify careful controls to ensure the quality consistency of this crude material.

PG58 Seed germination of medicinal plants as affected by salinity stress

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Salinity stress is a major limiting factor which influences agricultural production worldwide. An in vitro assay was conducted to compare the response of twelve medicinal plants to salinity stress. *Plantago* sp., *Alyssum* sp., *Portulaca oleracea, Sesamum indicum, Origanum majorana, Trigonella foenum, Anethum graveolens, Melilotus officinalis, Trachyspermum ammi, Cuminum cyminum, Lactuca sativa* and *Lallemantia royleana* were used in this study. One-hundred seeds of each species were grown in one petri dish and different concentrations of sodium chloride were applied to the seeds as levels of salinity stress. There was a great difference between species in their tolerance to salinity stress. Elevating concentrations of NaCl resulted in a significant decline of seed germination rate and plant biomass. Species *Portulaca oleracea, Alyssum* sp. and *Trigonella foenum* were tolerant to 450 mM/L of sodium chloride. **References:** [1] Singer, A.C. et al. (2007) Environ. Pollut. 147:74 – 82. [2] Ashraf, M. (2002) Crit. Rev. Plant Sci. 21:1 – 30. [3] Barassi, C.A. et al. (2006) Sci. Hort.109:8 – 14.

PG59 Preliminary assessment of the chemical stability of dried extract from *Guazuma ulmifolia* Lam. (Sterculiaceae)

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Guazuma ulmifolia Lam. (Sterculiaceae), popularly known as "Mutamba", is a tropical-American plant found from Mexico to southern South America. In the popular medicine of several Latin-American countries, it is used for the treatment of burns, diarrhea, inflammations and alopecia. Polysaccharides, epicatechin (EP) and procyanidin oligomers, such as procyanidins B2 (PB2) and B5, three trimers [procyanidin C1; epicatechin-(4β→6)-epicatechin-(4β→8)-epicatechin; epicatechin-(4β→8)-epicatechin-(4β→6)-epicatechin] and one tetramer have been isolated and identified from its extract [1]. The anti-diabetic properties [2], hypotensive and vasorelaxant activity [3], anti-ulcer [4], anti-bacterial activities [5], and antiviral activity [6] from the bark, aerial parts, fruits, crude extract and fractions were attributed to the presence of proanthocyanidins. The preliminary stability of the dried extracts from bark of *G. ulmifolia* containing or not colloidal silicon dioxide (CSD) was evaluated. The physical-chemical properties and compatibility of CSD in the extract were evaluated for 21 days of storage under stress conditions of temperature (45 ± 2 °C) and humidity (75 ± 5%). Thermal analysis (TG) was supplemented using a selective high-performance liquid chromatography (HPLC) for determination of stability of the characteristic constituents (chemical markers), namely PB2 and EP. The results showed that PB2 is an appropriate compound to use as chemical marker in control quality of dried extracts of *G. ulmifolia*. The stress study showed that there was no significant difference between the two extracts. On the other hand, considering the TG data and the high temperatures involved, the results suggest that CSD would increase the stability of dried extracts of *G. ulmifolia*. *Acknowledgements: CNPq, CAPES.* **References:** [1] Hör, M. et al. (1996) Phytochemistry 42:109 – 119. [2] Alonso-Castro, A.J. et al. (2008) J. Ethnopharmacol. 118:252 – 256. [3] Magos, G.A. et al. (2008) J. Ethnopharmcol. 117:58 – 68. [4] Berenguer, B. et al. (2007) J. Ethnopharmacol. 114:153 – 160. [5] Camporese, A. et al. (2003) J. Ethnopharmacol. 87:103 – 107. [6] Felipe, A.M.M. et al. (2006) Biol. Pharm. Bull. 29:1092 – 1095.

PG60 Possibilities of buckwheat *Fagopyrum esculentum* Moench. in modern nutrition and phyto-therapy (Dinarides, W. Balkan)

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Buckwheat *Fagopyrum esculentum* (Polygonaceae) used to be heavily used in nutrition of people in Balkan peninsula. At that time, buckwheat was used for making mashes, pies, bread, and pastry. However, many people were using it as preventive and therapeutic mean in treatment of different diseases [1]. After more than 50 years, buckwheat is becoming a favorite plant again. People started to breed it again in mountain areas of Dinarides. Dried plant (Herba) is used in preparation of infuse and as alco-tincture. Buckwheat honey has distinguished antimicrobial activity [2]. Material was gathered in Sarajevo surrounding at 1300 m of altitude in 2006. By using spectrophotometry, the presence of basic floral pigments was determined: chlorophyll a (8.599 mg/l), chlorophyll b (22.869 mg/l) and carotenoides (0.446 mg/l). Enormously high concentration of chlorophyll b influences distinguished anti-oxidant activity of buckwheat [3,4]. Chromatography on thin layer proved the presence of rutine (by using reference substance and based on fluorescence at 254 nm and 365 nm). By using Borntrager's colored reaction, the presence of anthraquinone was determined. These researches significantly confirm ethical – botanical experiences with buckwheat at these areas and indicate beyond doubt huge possibilities of buckwheat in modern nutrition and phyto-therapy of wide specter of metabolic diseases, cardio-vascular diseases, insomnia and urological disorders. **References:** [1] Redzic, S.J. (2006) Ecol. Food Nutr. 45:189 – 232. [2] Redzic, S.S. (2007) Collegium Antropol. 31:869 – 90. [3] Redzic, S. et al. (2005) Bosn. J. Basic Med. Sci. 5:53 – 8. [4] Redzic, S. et al. (2006) Bosn. J. Basic Med. Sci. 6:25 – 31.

PG61 Comparative analysis of anti-microbe activity of species of genus *Potentilla* L. (*P. anserina* L., *P. reptans* L., *P. palustris* L. and *P. tommassiniana* F.W. Schultez) from Bosnia and Herzegovina (W. Balkan)

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Most species of order *Potentilla* contain tannin [1] as active principle. Due to extinguished medical function many of them are used in traditional medicine for treatment of inner and outer fevers, against diarrhea, hart diseases or for heeling of ponderous wounds [2]. High level of chlorophyll and carotenoides, in addition to dominating secondary metabolite, influence distinguished anti-oxidant, anti-inflammatory and anti-microbial activities [3,4]. In these researches, tested was anti-microbial activity of rhizome with small roots of widely spread species *P. reptans*, edible plant *P. anserina*, very rare species *P. palustris* and endemic Balkan plant *P. tommassiniana*. Plant material was gathered in September 2006 and it was dried at room temperature. Species *P. tommasiniana* was used to obtain dried extract with Soxhlet apparatus. Other species were used in obtaining ethanol mazzerato, obtained through double mazzeration. Also used were concentrate and attenuated extracts of mazzerato (%: 50, 0, 5 and 0, 1), while dried extract was used in attenuate (%): 0, 1, 0, 01 and 0, 05. Used were test micro-organisms: *Staphylococcus aureus* (ATCC 6538P), *Escherichia coli* (ATCC 8739) and *Candida albicans* (ATCC 10231) for dry and mazzerato extracts and *Pseudomonas aeruginosa*, *Proteus mirabilis* for dry extracts of species *P. tommassiniana*. Diffuse method was used for developing an anti-biogram. Best effects were obtained in inhibition of growth of *Staphylococcus aureus*. Treatment with mazzerato extracts provided zones of inhibition in range 4 – 11 mm, and treatment with attenuated dried extracts provided extremely high zones of inhibition in range 11 – 13 mm. Same extracts provide high zones of inhibitions with *Proteus mirabilis* (8 – 13 mm) and species *Pseudomonas aeruginosa* (10 – 13 mm). With *Escherichia coli* best effects are given by mazzerato extract of species *Potentilla reptans* (10 – 15 mm). *Candida albicans* is resistant to all treated extracts.
Reference: [1] Tomczyk, M. and Latté, K.L. (2009) J. Ethnopharmacol. 122:184 – 204. 2. Redzic, S.S. (2007) Collegium Antropol. 31:869 – 90. 3. Redzic, S. et al. (2007) Planta Med. 73:887. 4. Pilipovic, S. et al. (2008) Planta Med. 74:989.

PG62 Fingerprinting of formulation of Indian system of medicine: Triphala Churna

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Herbal formulations of Indian system of medicines, especially Ayurveda are well known for their therapeutic efficacy. These formulations lack quality assurance specifications to maintain batch to batch uniformity. The authenticity, safety and efficacy of any formulation are ensured by testing tools in the global market. The present research work has been attempted to provide the tool in the form of fingerprints of one of the most common and popular Ayurvedic formulation – Triphala Churna. The Triphala churna is an Ayurvedic medicine official in Ayurvedic formulary of India (2003) and enjoys great reputation in Ayurvedic text as tonic, blood cleanser and gentle laxative. The accepted botanical sources of herbal ingredients of Triphala are dry fruits of *Emblica officinalis, Terminalia chebula* and *Terminalia belerica.* The fingerprints were developed using physicochemical (ash value, acid value, saponification value etc), macroscopic (morphological parameters), microscopic analysis. The instrumental methods were developed with ultra violet spectroscopy (UV), High Performance liquid Chromatography (HPLC) & High Performance Thin Layer Chromatography (HPTLC) using accepted markers. UV Fingerprint of Triphala Churna was determined at λmax 276 nm (tannic acid marker). The HPLC on C_{18} (5 micron 25 cm x 4.6 mm) column from Phenomenex in binary gradient mode with mobile phase acetonotrile:-methanol:phosphate buffer (pH 3.0) (10:5:85) at flow rate 1.2 ml/min and effluent monitored at 264 nm using gallic acid and tannic acid markers. The HPTLC fingerprint was specified by Densitometric Methods using gallic acid as a marker. The fingerprinting methods were validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day assay precision, repeatability of measurement, and repeatability of sample application. The finger printing methods are simple, sensitive, precise and accurate and can be adopted for the routine quality control of Triphala Churna.

PG63 Comparative chemical and biological analyses of *Aloe schweinfurthii* and *Aloe vera* for laxative activity

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Aloe species have been widely used for folk remedies in many countries and considered useful for a variety of diseases for several thousand years [1]. The genus comprises approximately 400 species [2]. *Aloe vera* (Linn.) Burm. f. has a legendary medicinal reputation, appreciably recorded in literature owing to its multipurpose medicinal properties while *A. schweinfurthii* Baker is cultivated in Nigeria. Identification, differentiation, authentification and quality assurance of the two *Aloe* species growing in Nigeria have been studied by Odeleye [3]. *Aloe schweinfurthii* Baker, *Aloe schweinfurthii* Baker, indigenous to Nigeria, and the imported but official *Aloe vera* (Linn.) Burm. f., both of Liliaceae family, were assayed spectrophotometrically for combined anthraquinone contents and also pharmacologically for their laxative properties in male albino rats, using official senna leaf (*Senna acutifolia* Del.) as the reference standard. The leaf exudates of *A. schweinfurthii* and *A. vera* were found to possess significant laxative activities higher than that of the official senna leaf. Statistical comparison of *A. schweinfurthii*, *A. vera* and *S. acutifolia* (positive control), with water as the negative control, revealed that there were significant differences between their laxative activities. The contents of the total free anthraquinones and the total combined anthraquinones in *A. schweinfurthii* exudates were remarkably higher than those of *A. vera*. Consequently, the use of laxative index is proposed for comparative study of *Aloe* (or related) species, and as a possible quality control tool.
References: 1. Suga, T. and Hirata T. (1983) Cosmet.-Toiletries 98:105 – 108. Kimberley, M.J. (1991) An Update of the T.B.C Harding 1979 Checklist, Index and Code of Aloes of the World. Excelsa No 15. Odeleye, O.M. (2004) M. Sc. Thesis, Obafemi Awolowo University. Nigeria.

Topic H: Prevention of metabolic diseases by medicinal plants and nutraceuticals

PH1 *In vivo* assessment of hypoglycaemic and antioxydant activities of aqueous extract of *Terminalia superba* in alloxan-diabetic rats

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Terminalia superba, used in traditional medicine in Cameroon for the treatment of diabetes [1,2], is widely accepted as one of the medicinal herb with the highest antioxidant activity. Accordingly, the present study was designed to investigate the possible actions of aqueous extract of the roots of *Terminalia superba* on glucose homeostasis and on MDA, SOD and catalase homolysat of diabetes rats. In the first set of experiments, hypoglycaemic effect of oral administration of various doses (75, 150 and 300 mg/kg) of the extract were examined in normoglycaemic and diabetes rats. Male Wistar rats were injected with 120 mg/kg alloxan monohydrate. Tolbutamide was used as a reference drug at a dose of 80 mg/kg. Optimal effect was observed in both of the animal groups with a dose of 300 mg/kg of the extract. In another part of experiments, oral administration of this extract was given to diabetic rats at the dose of 300 mg/kg for 14 days. Blood glucose level was measured on day 0, 7 and 14. At the end of experiment, the rats were anaesthetized with ether and blood sample was collected and homolysat obtained for the study of the effect on MDA, catalase and SOD. The experimental data indicated that this extract demonstrates significant hypoglycaemic effect thus reduces the antioxidant parameters above in alloxan-induced diabetes rats which in confirmation with the folkloric utilization. This plant has been revealed to be rich in flavonoids, saponnins [3] which may be responsible for the hypoglycemic and antioxidant

activity. **References:** [1] Dimo, T. et al. (2006) *Pharmazie*, 61:470 – 473. [2] Dongmo, A.B. et al. (2006) *Pharmacologyonline*, 2:171 – 177. [3] Momo, N.E.C. et al. (2008) International Conference on Natural products-For Quality and Health. 12 – 14 November 2008 Yaounde, Cameroon.

PH2 Effect of crocin on the progression and treatment of diabetic neuropathy in mice

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Oxidative stress is a mechanism which has been implicated to play a major role in pathogenesis of diabetic neuropathy. Antioxidant agents have an important place in prevention and treatment of diabetic neuropathy. In previous studies, the antioxidant effects of saffron and two active constituents (crocin and safranal) have been reported [1]. Thus, in this study, the effects of crocin on diabetic hyperalgesia are investigated in mice. Diabetes was induced by single injection of streptozocin (200 mg/kg).Three doses of crocin (50, 100 and 200 mg/kg/day) were administered subcutaneously in three groups of normal and diabetic animal for one or four weeks or in single administration. Hyperalgesia was evaluated using tail flick test. Four weeks of treatment with doses of 100 and 200 mg/kg/day of crocin prevented the induction of hyperalgesia (from 44 to 52%) in diabetic mice ($P < 0.05$ and $P < 0.01$, respectively). Also after induction of hyperalgesia, the treatment of diabetic mice by administration of crocin (50, 100 and 200 mg/kg/day) for one week ($P < 0.05$) or single dose ($P < 0.001$) was effective to prevent the induction of hyperalgesia (up to 50%). It can be concluded that the crocin is effective in prevention and treatment of hyperalgesia in diabetic mice. **Reference:** [1] Assimopoulou, A.N. et al. (2005) Phytother. Res. 19:997 – 1000.

PH3 Stimulation of AMPK and enhancement of glucose uptake in muscle cells by quercetin and quercetin glycosides, the actives principles of *Vaccinium vitis-idaea*

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As a part of an ongoing project aiming to develop culturally-adapted diabetes treatment for Canadian native populations, our team has identified several medicinal plants that stimulate glucose uptake in C2C12 muscle cells from among species used by the Cree of Eeyou Istchee (James Bay area of northern Quebec) to treat symptoms related to diabetes. The goal of this study was to elucidate the mechanism of action of one of these plants, the berries of *Vaccinium vitis idaea*, as well as to isolate and identify its active constituents. Western immunoblot analysis in C2C12 cells revealed that the ethanol extract of the berries did not stimulate insulin signaling pathways, but instead activated the AMP-activated protein kinase (AMPK) pathway. The extract was observed to mildly inhibit ADP-stimulated oxygen consumption in isolated mitochondria, an effect likely responsible for metabolic stress and ensuing activation of AMPK. Fractionation guided by glucose uptake activity resulted in the isolation of 9 compounds. The 3 most active, quercetin, quercetin-3-O-galactoside and quercetin-3-O-glucoside, enhanced glucose uptake by 37 ± 9%, 38 ± 3% and 59 ± 2%, respectively, after an 18 hour treatment. These flavonoid glycosides, as well as the aglycone, were observed to stimulate the AMPK pathway. Quercetin was a powerful inhibitor of ATP synthase in isolated mitochondria. These finding indicate that quercetin and its glycosides are likely responsible for the antidiabetic activity of *V.vitis* crude berry extract mediated by AMPK. Quercetin and quercetin-3-glycosides thus potentially have applications for the prevention and treatment of metabolic diseases.

PH4 Effect of rice-bran water extract on the amelioration of pre-diabetic state in high-fat feeding rats

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The water extract of rice bran (RBE) was shown to reduce fasting blood sugar and glycosylated hemoglobin in diabetes [1]. Being a staple food among Thai, the effect of RBE on pre-diabetic state was interested. The study was done in high-fat (65% of total calories) feeding Sprague-Dawley rats. Four groups of 8 rats each were separately either co-treated daily with three doses (22.05, 220.5, 2205 mg/kg) of RBE or 9.55 mg/kg metformin, twice daily. After four weeks of treatments, RBE at the highest dose was able to significantly ($p < 0.05$) reduce the weight gain (125.98 ± 7.32 gm *vs* 160.72 ± 10.03 gm), visceral fat (8.99 ± 0.72 gm *vs* 13.95 ± 0.44 gm) and area under the glucose-clearance curve (1248.83 ± 189.62 *vs* 2787.75 ± 472.54). The percentage of homeostasis model assessment of B-cell function (HOMA-B) was reduced when rats were fed with high-fat diet but the value was increased when those rats were also received RBE, though there was no statistic significance. The value of the homeostasis model assessment of insulin resistance (HOMA-IR) was not affected by the treatment. *Acknowledgements: Research Unit, Faculty of Medicine, Thammasat University, the National Research Council of Thailand.* **References:** [1] Qureshi, A.A. et al. (2002) J. Nutr. Biochem. 13:175 – 187. [2] Matthews, D.R. et al. (1985) Diabetologia 28:412 – 419.

PH5 Effect of traditional ayurvedic arjuna formulations on cardiometabolic disorders

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Terminalia arjuna (Family-Combretaceae) herbal medicine is known as a remedy for cardiovascular disorders in traditional Indian System of Medicine. Arjaunaaristha and Arjunakwath are traditional ayurvedic formulations containing *Terminalia arjuna* [1]. The effects of these formulations are not scientifically documented on cardiometabolic disorders like hypertension and hyperlipidemia. In the present study, antihyperlipidemic and antihypertensive activity screening had been done for Arjunaaristha and Arjunakwath on Wistar rats. The hyperlipidemia was induced by Triton X-100 (100 mg/kg i.p.). The formulations and lovastatin as standard were administered by oral intubations simultaneously with Triton injection. Biochemical estimation was studied by monitoring the serum lipid profile before and after treatment using enzymatic assay kit. Hypertension was induced in rats by cadmium chloride (1 mg/kg, i.p.) and fructose for two weeks [2]. The rats were treated with formulations along with cadmium chloride and fructose for four weeks. Amlodipine was used as a standard. The data obtained was analysed statistically by ANOVA followed by Dunnett's post hoc test. Treatment with Arjunakwatha and Arjuanaristha reduces cholesterol, triglycerides, low density lipoprotein in Triton induce group. Lipid profile demonstrated by the standard lovastatin was found to be analogous to that of traditional arjuna formulations. Simultaneously there was significant decrease in elevated blood pressure in cadmium chloride as well as fructose induce hypertensive animals when treated with Arjunaaristha and Arjunakwatha. The present study shows the efficacy of *Terminalia arjuna* formulations as a hypolipidemic agent and antihypertensive agent and in over all management of cardiometabolic disorders. **References:** [1] Gauthaman, K. et. al. (2001) J. Ethnopharmacol. 75:287 – 289. [2] Shaila, H.P. et. al. (1998) Int. J. Pharmacol. 35:1 – 4.

PH6 Trapping of reactive dicarbonyl compounds by different *Opuntia ficus-indica* (L.) Mill. preparations *in vitro*

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The formation of advanced glycation end products (AGEs) is discussed to be a major pathogenic link between diabetes mellitus and its subsequent complications. Reactive dicarbonyl compounds such as methylglyoxal (MGO) and glyoxyal (GO) are precursors of AGEs and exert direct toxicity to cells and tissues [1]. The objective of this study was to test different preparations from the traditional antidiabetic plant prickly pear cactus (*Opuntia ficus-indica* (L.) Mill.) for their ability to trap reactive dicarbonyl compounds *in vitro*. Briefly, aqueous extracts from *Opuntia* cladodes and *Opuntia* fruit skin as well as the proprietary product OpunDia™ containing an extract of *Opuntia* cladodes and fruits were incubated with MGO or GO under physiological conditions (pH 7.4, 37 °C). Samples were collected after 1, 2, 4, 8 and 24 hours of incubation, and the remaining MGO and GO activity was determined by HPLC. The activity of the *Opuntia* preparations was compared to an extract from *Rosmarinus officinalis* L. enriched in 45% carnosic acid. All tested *Opuntia* preparations were able to effectively trap MGO after 24 hours of incubation, whereas the trapping of GO was less pronounced. *Opuntia* fruit skin extract and OpunDia™ were the most active compounds (46% and 44% MGO trapping activity after 24 hours), followed by *Opuntia* cladode extract (32%). In conclusion, the observed trapping activity of the studied prickly pear cactus preparations on the reactive dicarbonyl compound MGO in vitro might be one explanation for the mode of action of the antidiabetic plant *Opuntia ficus-indica* (L.) Mill. **Reference:** [1] Singh, R. et al. (2001) Diabetologia 44:129 – 146.

PH7 The flavonoid rich fraction of *Coreopsis tinctoria* promotes glucose tolerance regain in streptozotocin-induced glucose-intolerant rats

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Infusions of *Coreopsis tinctoria* Nutt. flowering tops have been used traditionally in Portugal to control hyperglycaemia [1]. A previous study revealed marein as the main chalcone and that daily administration of the infusion during a 3 week period (500 mg containing 20 mg of marein/Kg/day) promoted the recovery of glucose tolerance in a streptozotocin-induced glucose intolerant rat model [2]. In order to identify the active principles we studied the AcOEt fraction of the infusion and its chemical characterisation was achieved by HPLC-DAD-ESI-MS/MS and HPLC-electrochemical detector, allowing the identification of chalcones and flavanones as main constituents and potential strong radical scavengers, their structure being confirmed by NMR studies. For the pharmacological evaluation, glucose intolerance induction was achieved using streptozotocin (40 mg/Kg) and blood glucose levels were monitored by weekly OGTT as described previously [3]. This fraction was administered to male Wistar rats (n = 8) at the concentration of 125 mg/Kg daily for 3 weeks. Normal (n = 8) and glucose-intolerant (n = 11) rats were used as controls. The results show that after 2 weeks oral administration of *C. tinctoria* AcOEt fraction (125 mg containing 20 mg of marein/Kg/day) the animals were no longer glucose-intolerant ($p < 0,01$), an effect maintained over the remaining experimental period. The oral treatment caused no hepatotoxicity, as determined by blood ALT and AST. **Conclusions:** AcOEt fraction, containing the same amount of marein as the infusion, promoted glucose tolerance regain in the rats more quickly, which means that the bioactivity is probably due to more of the several flavonoids present in infusion and AcOEt fraction and not to marein alone. The possibility of a pancreatic citoprotective antioxidant action is now being considered as the probable mechanism responsible for reverting this glucose-intolerant state. The work is still ongoing and additional results will complete the study. *Acknowledgments: Science and Technology Foundation, for the PhD grant* **References:** [1] D'Oliveira Feijão, R. (1973) Medicina pelas Plantas. Progresso Ed. Lisboa. [2] Dias, T. et al. (2008) Planta Med. 74:PA61.

PH8 Inhibition of human LDL lipid peroxidation by black pepper extracts and piperine

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Atherosclerosis, a chronic inflammatory and progressive disease, is a major cause of coronary heart disease resulted from the accumulation of plague inside the arteries [1]. The oxidized low-density lipoprotein is strongly implicated to play an important role in the generation and progression of the atherosclerotic plaque. Black pepper (*Piper nigrum*) has been reported as the anti-inflammatory and antioxidant agent. This study aimed at investigating the black pepper extracts and its major alkaloid, piperine, as antioxidants in preventing LDL oxidation. Black pepper extracts were prepared by maceration technique with water, methanol, dichloromethane, hexane and diethyl ether. LDL was isolated by ultracentrifugation and dialysed before subjected to copper-induced oxidation. Using two different approaches, conjugated diene formation during lipid peroxidation was monitored continually by UV-spectrophotometer, and changes in the relative electrophoretic mobility (REM) of lipoprotein were monitored by agarose gel electrophoresis. Suppression of copper-induced LDL oxidation was measured by change in 234 nm absorbance in the presence and absence of either black pepper extracts or piperine. Using each compound at 10 μg/ml, diethyl ether, dichloromethane extracts and piperine significantly inhibited *in vitro* oxidation, and extracts from methanol and hexane had stronger inhibitory activity against LDL oxidation than water. Cotreatment of LDL with copper and one of these extracts -methanol (100 μg/ml), dichloromethane and diethyl ether (25 – 100 μg/ml)- significantly decreased REM, indicating the inhibition of LDL oxidation. In conclusion, this study shows that particular black pepper extracts and piperine could reduce *in vitro* LDL oxidation, suggesting their role in lowering the atherosclerosis risk. **Reference:** [1] Ross, R. (1999) N. Engl. J. Med. 340:115 – 126.

PH9 Antioxidant and antimicrobial properties of Malaysian *Uncaria longiflora* var. *pteropoda*

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Uncaria is a genus of woody climbers from the Rubiaceae family which can be found in many parts of the world. Many *Uncaria* species are used as traditional medicine for the treatment of cancer and other diseases. The most popular and extensively studied species is the Peruvian medicinal plant *Uncaria tomentosa* known as Cat's Claw which has been marketed in the form of various medicinal products [1]. In Malaysia, the plants of the genus are generally known as "gambir" or "kekait" and are used to treat various diseases including diarrhea, dysentery and rheumatism [2]. However, not much has been reported on their biological activities. In this study, the methanolic extracts of a local *Uncaria* species, *U. longiflora* var. *pteropoda (Miq.) Ridsdale* was evaluated for its antioxidant, radical-scavenging and antimicrobial potential. The leaves and stems extracts exhibited stronger antioxidant potential than vitamin E and quercetin (> 90% inhibition) and showed strong radical-scavenging properties with IC_{50} values of 8 – 10 μg/ml, comparable to vitamin C. In the antimicrobial assay, both leaves and stems extracts displayed good inhibition against Gram-positive bacteria and were found to inhibit *Candida albicans* with MIC values of < 1.0 μg/ml, comparable to cyclohexamide. Phytochemical screening of the extract and TLC screening of fractions revealed the presence of flavonoids, alkaloids and tannins. This study provides evidence for the antioxidant and

antimicrobial properties of *U. longiflora.* However, further studies are required to identify compounds responsible for these activities. **References:** [1] Heitzman, M.E. et al. (2005) Phytochemistry 66:5 – 29. [2] Kam, T.S. et al. (1992) Phytochemistry 31:2031 – 2034.

PH10 *Malva sylvestris* water extract: A potential anti-Inflammatory and anti-ulcerogenic remedy

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Malva sylvestris, family Malvaceae, has been grown as a medicinal plant and pot herb since Roman times. It is found in subtropical and temperate latitude of both hemispheres. The present study investigates the role of the aqueous extract of *its* aerial part upon lipemia, glycemia, inflammation and gastric ulcer using rats as a model. After one month of extract intake via drinking water (100, 400 and 800 mg/kg body weight) the 400 and 800 mg/kg body weight doses resulted in significant increase in serum triglyceride, while other lipid and glycemic parameters and liver enzyme activities (AST, ALT, LDH, ALP) were unaffected. About 10% increase in stool water content was observed at highest dose used. Doses of 50, 100, 250 and 500 mg/kg body weight were used in acute and chronic inflammation models induced by carrageenan and formalin respectively [1]. Significant anti-inflammatory activity was observed at most doses used with an optimum inhibition at 100 mg/kg body weight (60% inhibition) in both models. Protection against ethanol-induced gastric ulcer was investigated [2]. Results showed maximum protection (37%) at 500 mg/kg body weight, a value higher than that observed with cimetidine (30%), a reference drug. The assessment of antibacterial activity against 11 bacterial hospital isolates and the antifungal effect against *Candida albicans* using disk diffusion technique showed no potentials in this respect. In conclusion, *Malva sylvestris* water extract showed no liver toxicity, and exhibited a positive effect on ulcer and inflammation with relatively a neutral effect on lipemia and glycemia. *Acknowledgments: Mr. Jean Karam.* **References:** [1] Jose, N. et al. (2004) Phytother. Res. 18:43 – 46. [2] Alkofahi, A. and Atta, A.H. (1999) J. Ethnopharmacol. 67:341 – 345.

PH11 Effect of plant derived-phenolic extracts on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats

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Gastric and duodenal ulcers are illnesses that affect a considerable number of people in the world and they are induced by several factors, for example, stress, smoking, nutritional deficiencies and ingestion of non-steroidal-antiinflammatory drugs. Plant extracts are some of the most attractive sources for new drugs and have been shown to produce promising results in the treatment of gastric ulcer [1]. The object of the study deals with the antiulcerogenic effect of an hdroacoholic extracts obtained from of *Acacia nilotica* leaves and *Retama raetam* seeds was investigated using indomethacin-induced ulcer model in rats. Antiulcerogenic activities of hydroacoholic extract of *A. nilotica* (30 mg/kg) and hydroalcoholic extract of *R. raetam* (25 mg/kg) were determined by comparing the negative treated only with (indomethacin) and positive (ranitidine) control group. Although the dose of the hydroacoholic extract of *A. nilotica* and *R. raetam* showed significant antiulcerogenic activity as compared to negative control, the highest activity was observed with mixed (30 mg/kg) of *A. nilotica* and (25 mg/kg) of *R. raetam* (81%). The activities of enzymes superoxide dismutase (SOD), catalase (CAT) and gluthione-S-transferase (GST) were determined in the stomach tissues of rats and compared with those of negative and positive control groups. The enzymes activities in indomethacin-administrated were reduced significantly SOD (38.50 ± 4.16), CAT (43.20 ± 3.09), GST (22.80 ± 2.25) and increased by the plants extracts SOD (48.90 ± 4.07), CAT (37.50 ± 3.26), GST (32.00 ± 2.06) and ranitidine SOD (52.30 ± 3.51), CAT (41.90 ± 2.67), GST (23.70 ± 2.2.14), ($p < 0.05$). Phytochemical investigation of *Acacia nilotica* leaves and *Retama raetam* seeds afforded of catechin gallate esters and isoflavonoids C-glycosides derivatives. The presence of these phenolic compounds may probably explain the antiulcerogenic and antioxidant effect of the plant extracts. **Reference:** [1]Toma, W. et al. (2002) Biol. Pharm. Bull. 25:1151 – 1155.

PH12 Antihyperglycemic activity of aqueous stem bark extract of *Eugenia jambolana* in alloxanized rats

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The antidiabetic potential of the aqueous stem bark extract of *Eugenia jambolana* (EJ) Linn. (Myrtaceae) was evaluated in the Alloxan induced diabetic model [1]. *Eugenia jambolana* Linn. a medicinal plant widely used in the traditional Ayurveda and Siddha systems of medicine for the treatment of diabetes mellitus and several other diseases [2]. Graded doses of the aqueous stem bark extract of EJ were administered to normal and experimental diabetic rats for 28 days. Blood glucose levels were evaluated on the 7th, 14th, 21st and 28th days. Doses of 500 and 1000 mg/kg body weight of both extracts produced significant ($p < 0.05$) hypoglycemic activity in alloxanized rats when compared with diabetic control. The biochemical parameters studied were serum triglycerides, cholesterol, high density lipoproteins (HDL), and low density lipoproteins (LDL), and glycosylated hemoglobin level. In addition, changes in body weight was assessed in the EJ extract treated diabetic rats were compared with diabetic control and normal animals. Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the indigenous system of medicine. **References:** [1] Madhavan, V. et al. (2007) Pharm. Biol. 45:18 – 21. [2] Nadkarni, A.K. and Nadkarni, K.M. (2007) Indian Materia Medica, Bombay Popular Prakashan, India.

PH13 *In-vivo* antidiabetic effect of *Cistus laurifolius* L. leaves

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The genus *Cistus* (Cistaceae) is one of the characteristic genera of the Mediterranean region, colonizing degraded areas [1]. In Turkish folk medicine, various parts of *Cistus laurifolius* L. are used to treat peptic ulcer and various types of pains, i.e., rheumatic, back pain, etc. It has been used for the treatment of stomach ache and gastric ulcer in the folk medicine since the time of Dioscorides, i.e., for at least 2000 years in Anatolia. The tea prepared from the leaves is used to decrease symptoms of diabetes hypoglycemic [2,3]. In the present study, the hypoglycemic and antihyperglycemic effects of water and ethanol extracts of *C. laurifolius* were evaluated by using *in vivo* methods in normal, glucose loaded hyperglycemic and streptozocin-induced diabetic rats. Diabetes was induced by intraperitoneal injection of streptozocin at the dose of 55 mg/kg. Blood glucose levels of animals were measured by the glucose oxidase method. Tolbutamide (100 mg/kg) was used as reference drug. All data were compared to control group. Results indicated that blood glucose levels of the streptozocin induced diabetic rats were decreased by loaded ethanol extract at the doses of 250 and 500 mg/kg as compared to control group (16 – 34%). On the other hand all extracts tested in the experiments were found inactive totally on blood glucose level of normal rats. Water and ethanol extracts have shown a weak hypoglycemic effect in glucose loaded animals (11 – 20%). The experimental data obtained from water and ethanol extracts of leaves confirmed the folkloric utilization. *Acknowledgements: This study was financially supported by the Research Fund of Gazi University (Project no:EF-02/2003 – 02)* **References:** [1] Attaguile, G. et al. (2000) Cell. Biol. Toxicol. 16:83 – 90. [2] Sezik, E. et al. (1991) J. Ethnopharmacol. 35:191 – 196. [3] Yesilada, E. et al. (1995) J. Ethnopharmacol. 46:133 – 152.

PH14 Antidiabetic effect and antioxidant potential of *Rosa canina* fruits

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Rosa canina L. fruits (Rosaceae) are used to treat diabetes in Anatolia traditionally [1,2,3]. In this study, the ethanol extract of *R. canina* fruits and its fractions were screened for their antioxidant, hypoglycaemic and antidiabetic activities. Two doses of ethanol extract (250 and 500 mg/kg) was administered to streptozotocin (STZ) induced diabetic rats for 7 days. The extract possessed a remarkable hypoglycemic effect at 250 mg/kg dose. Then it was fractionated through successive solvent extractions to yield chloroform fraction ($CHCl_3$ Fr.), ethyl acetate fraction (EtOAc Fr.), *n*-butyl alcohol fraction (*n*-BuOH Fr.) and remaining water

fraction (R-H_2O Fr.) respectively. These fractions were administered to normal plus glucose hyperglycaemic rats. Additionally the subacute antidiabetic activities of the fractions were studied in diabetic rats for 7 days (10, 40, 150 and 300 mg/kg dose respectively). The experimental data indicated that R-H_2O Fr. possessed significant antidiabetic activity (50 – 62%) in diabetic rats. Also, a minor hypoglycemic effect was observed in normoglycemic plus glucose-hyperglycemic animals treated with R-H_2O Fr. (15%). *In vitro* antioxidant experiments revealed that EtOAc Fr. showed the highest radical scavenging activity on DPPH (79.5 ± 0.4%), whereas $CHCl_3$ Fr. exhibited the maximum reducing power. Total phenol contents of the fractions were determined as gallic acid equivalent (GAE) using Folin-Ciocalteau's reagent. The highest total phenolic content was observed in $CHCl_3$ Fr. (18.5 ± 0.6% gallic acid equivalent g/g fraction) but no correlation was observed between the antidiabetic activity of fractions and their phenolic contents. Our findings support the traditional usage of *R. canina* fruits as a folk remedy in the treatment of diabetes in Turkey. **References:** [1] Yesilada, E. et al. (1999) J. Ethnopharmacol. 64:195 – 210. [2] Sezik, E. et al. (2001) J. Ethnopharmacol. 75:95 – 115.

PH15 Restoration of altered homeostasis in type 2 diabetic animals by an Ayurvedic formulation

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Diabetes mellitus is of world wide significance and increasing prevalence. Plant remedies have played an important role in traditional treatment of diabetes. In the present study effect of *Limit,* an Ayurvedic formulation of nine plant extracts with antidiabetic and antioxidant principles (viz., *Gymnema sylvestre, Momordica charantia, Cassia auriculata, Syzium cumini, Phyllanthus emblica, Melia azadiracta, Trigonella foenum graceum, Coccinia indica, Tinospora cordifolia*) were studied on type 2 diabetic rats. Diabetes was induced in Albino rats (streptozotocin, 65 mg/kg and nicotinamide 110 mg/kg, i.p). *Limit* (150 and 300 mg/kg), administered orally daily for 40 days to diabetic animals. Plasma glucose and body weight, urine volume, urine analysis and other biochemical parameters were monitored on every 10th day over a 40-day period of the experiment. Insulin and glycosylated haemoglobin levels were monitored on 0 day and 40th day. On 40th day, glucose uptake was measured using isolated hemidiaphragms. Glibenclamide (4 mg/kg, p.o) is used as a reference standard. Treatment with *Limit* (150 and 300 mg/kg) significantly reduced plasma glucose levels (24.4% & 52.8% respectively) on 40th day of experiment. Reduction in glycosylated haemoglobin (19.1% & 30.5%), triglyceride (2.0&3.6%) and cholesterol (2.9&12.6%) levels were also observed ($p < 0.05$). Significant improvement in insulin (11.8% and 24.3%), body weight, urine volume and biochemical parameters were observed in treated groups compared to diabetic control. Hemidiaphragms treated with *Limit* showed significant enhancement of glucose uptake (34.6% and 61.4%, $p < 0.001$) compared to diabetic control. Thus *Limit* restored biochemical parameters & enhanced glucose uptake in diabetic rats. **References:** [1] Grover, J.K. et al. (2001) J. Ethnopharmacol. 76:233 – 238. [2] Ghosh, R. et al. (2004) Ind. J. Pharmacol. 38:222 – 225.

PH16 The effect of a mixture of 6 plant extracts on blood insulin levels in diabetic rats

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Diabetes mellitus is one of the most common diseases associated with carbohydrate metabolism. In the present study, the effect of six plant extracts and their mixtures was evaluated on diabetic rats. Plants used in this study included: *Rosmarinus officinalis*, *Arctium lappa*, *Vaccinium myrtillus*, *Urtica dioica*, *Rosa canina*, and *Citrullus colocynthis*. Diabetes was induced by intraperitoneal injection of streptozotocin (STZ, 80 mg/kg in citrate buffer, 0.1 M, at pH of 4.5). After 24 hours of food deprivation, blood samples were collected from the orbital sinus before oral administration of the extracts, immediately, and after 1, 2, 3, and 48 hours. Blood glucose level was determined by glucose oxidase, and blood insulin level was determined by standard radioimmunoassay method under light ether anesthesia. A rat was declared diabetic if it was found to have glucose levels greater than 12.0 mmol/l. In diabetic rats, *R. canina*, and *R. officinalis* had no effect. *C. colocynthis*, *A. lappa*, and *U. dioica* increased blood insulin levels ($p < 0.05$). Oral administration of a mixture of the six extracts induced increased blood insulin levels throughout the 3 hour sampling period ($p < 0.01$), and this effect continued for 48 hours thereafter ($p < 0.05$).

PH17 Licorice extract ameliorates diabetic nephropathy in rats

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The objective of this study was to investigate the protective action of licorice in diabetic nephropathy in male rats. Diabetes was induced in male Wistar rats using streptozotocin (60 mg/kg body weight). Daily oral ingestion (1 g/kg body weight) of licorice extract for 60 days after the onset of diabetes reversed the adverse effect of diabetes on rats. Licorice extract alleviated blood glucose levels, restored renal function, and attenuated body weight loss. In addition, licorice extract modulated the adverse effect of diabetes on renal malondialdehyde, glutathione, superoxide dismutase and catalase activity. Furthermore, licorice extract restored the total antioxidant capacity of diabetic rat kidneys. The biochemical analyses were reinforced by the histological investigations where focal segmental glomerulosclerosis (FSG), tubular damage and hyperemic kidney were the histological changes seen in diabetic rats but not in treated rats. In conclusion, the biochemical analysis and light histological investigations of diabetic rat kidneys treated with licorice extract revealed that licorice may have a potential therapeutic effect for diabetes due to its antioxidant and anti-hyperglycemic properties. *Acknowledgements: United Arab Emirates University, Hazem Kataya* **References:** [1] Evans, J.L. et al. (2002) Endocr. Rev. 23:599 – 622 [2] Yorek, M.A. (2003) Free Radical Res. 37:471 – 480. [3] Johansen, J.S. et al. (2005) Cardiovasc. Diabetol. 4:5.

PH18 Chemical and immunopharmacological studies of two crude macromolecular fractions isolated from *Telekia speciosa* Baumg.

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Telekia speciosa Baumg. (Asteraceae family) is an indigenous plant, used in vernacular Romanian medicine for the treatment of some inflammatory diseases (rheumatism, liver and urinary disorders); the therapeutical remedies are administrated both by internal or external ways. Two crude macromolecular fractions have been isolated from the leaves and roots of *Telekia speciosa* (codified as PTfol and PTrx). After a partial purification, PTfol and PTrx have been submitted to a chemical analysis consisted of: anion-exchange column chromatography, high-pressure gel-permeation chromatography and TLC. The immunostimulatory activity of PTfol and PTrx was further investigated by a series of tests on rats (after 7 days p.o. treatment with 100.0 mg/kg): phagocytosis capacity of PMN cells by NBT test, serum complement activity and determination of the activity on spleenic T-lymphocytes [2]. In all experimental procedures Levamisole® 10.0 mg/kg p.o. was the reference compound; statistical significance was established by the Student's "t" test. The results showed that the PTfol has a good immunostimulatory activity, comparative to the Levamisole and the investigated fractions showed the big advantage of total lack of acute and subacute toxicity. **References:** [1] Milosavljevic, S. et al. (1999) J. Serb. Chem. Soc. 64:397 – 442. [2] Embuscado, M.E. et al. (1996) Carbohyd. Polym. 31:1 – 9.

PH19 Anti-inflammatory effect of *Mitragyna speciosa* crude methanol extract on the guinea pig ileum

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In a recent study, we have shown that the crude methanol extract of *Mitragyna speciosa* (of the family Rubiaceae) exerted significant *in vivo* anti-inflammatory activities in rodents as evident in the carageenan-induced paw edema and cotton pellet-induced granuloma tests [1], but the underlying mechanism is poorly understood. Our present study aims to explore the anti-inflammatory activities of this plant *in vitro* in order to rationalize the traditional use of this plant in the treatment of some stomach ailments [2]. The pharmacologic actions of *M. speciosa* were assessed by measuring the mechanical activity of isolated guinea pig ileum strips in an organ bath. The resultant methanol extract (0.01 – 0.05 mg/ml) caused a stimulatory effect followed by a relaxation of ileal activities at a higher dose (0.3 – 5 mg/ml) ($p < 0.05$). These results indicate that *M. speciosa* exert spasmogenic effects at a lower dose and a spasmolytic effect at a higher dose thus corroborating the use of plant in the treatment of diarrhoea and constipation. Moreover, results indicated that pretreatment with *M. speciosa* (0.3 – 5 mg/ml) which were tested positive for flavonoids, alkaloids, saponins, sterols and tannins produced significant concentration-dependant inhibition of spasmogenic activities when exposed to single submaximal contraction induced by histamine (H) and bradykinin (B). These results suggest that the anti-inflammatory activity of *M. speciosa* is mediated possibly through the H and B receptor antagonism, thus providing a scientific basis for the folkloric use of this plant in stomach disorders. *Acknowledgements: Faculty of Medicine and Health Sciences, UPM Fundamental Research Grant Scheme (FRGS/FASA1 – 2006/(Sains Perubatan)/UPM/179) from the Ministry of Higher Education Malaysia.* **References:** [1] Shaik Mossadeq, W.M. et al. (2009) J. Med. Princ. Prac. (Accepted for publication-in print). [2] Chittrakarn, S. et al. (2008) J. Ethnopharmacol. 116:173 – 178.

PH20 In vitro hypoglycaemic activity of Senecio nemorensis subsp. stabianus Lacaita (Asteraceae)

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Senecio species are used in folk medicine in the treatment of various diseases [1 – 3]. Interesting, in Mongolia *S. nemorensis* subsp. *fuchsii* was used as herbal tea for the treatment of diabetes [4]. Some *Senecio* species, like *S. inaequidens* and *S. vulgaris*, recently, were found to be active as antidiabetic [5]. In our continuing search of hypoglycaemic compounds from *Senecio* species in the present investigation we have tested *S. nemorensis* subsp. *stabianus* for inhibition of α-amylase and α-glucosidase. The digestive enzymes inhibitory activity was analyzed in order to evaluate the potential use of this plant in the control of the high postprandial blood glucose peaks in diabetics. Dried and powdered aerial parts of *S. nemorensis* subsp. *stabianus* were extracted as previously described [6]. The pyrrolizidine alkaloids, the most characteristic secondary metabolites of the *Senecio* genus, suitable in literature as hepatotoxic, are easily removed by extraction with chloroform [7]. *n*-Hexane extract exhibited the highest activity with the IC_{50} of 291 μg/ml. This extract submitted to column chromatography separation led to isolation of seven fractions that were tested for digestive enzymes inhibitory activity. A selective α-amylase inhibition was observed (IC_{50} values ranging from 287 to 744 μg/ml). Chemical composition of these fractions was analysed by GC-MS. The most active fraction was characterised by an high content of mono- and sesquiterpenes. **References:** [1] Christov, V. et al. (2002) Z. Naturforsch. C 57:780 – 784. [2] Bautista Peres, J. et al. (1991) Guia de las Plantas Medicinales de la Communidad Valenciana. Las Provincial, Valencia . [3] Torres, P. et al. (1988) Planta Med. 54:257 – 258. [4] Wiedenfeld, H. et al. (2000) Sci. Pharm. 68:207 – 211. [5] Conforti, F. et al. (2006) Int. J. Food Sci. Nutr. 57:1 – 8. [6] Tundis, R. et al. (2007) Nat. Prod. Res. (2007) 21:396 – 400. [7] De Vivar, A.R. et al. (1996) Biochem. Syst. Ecol. 24:175 – 176.

PH21 Chemical composition and antimicrobial properties of *Piper ovatum* Vahl.

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Piper ovatum Vahl (Piperaceae), an herbaceous plant occurring throughout Brazil, is popularly known as "joão burandi" or "anesthetic." It is used in traditional medicine for the treatment of inflammations [1] and as an analgesic [2]. The chemical composition of essential oil obtained from the leaves of *Piper ovatum* by hydrodistillation was analyzed by GC-MS. The main constituents were δ-Amorphene (16.5%), *cis*-Muurola-4(14),5-diene (14.29%) and γ- Muurolene (13.26%). The hydroalcoholic extract of *Piper ovatum* leaves and isolated compounds piperovatine, piperlonguminine and essential oil were screened for their antimicrobial activity by microdilution MIC and disc diffusion method respectively. The amides were made determination adherence inhibition assay and cytotoxicity assay. Hydroalcoholic extracts of different parts of *Piper ovatum* Vahl, essential oil, and amides isolated from leaves were tested against Gram-positive and Gram-negative bacteria and *Candida* species. All extracts and amides were active against *Bacillus subtilis* and *Candida tropicalis*, including clinical strains. Essential oil was active against *C. tropicalis*. These amides showed an inhibitory effect on the adherence of *C. tropicalis* ATCC 28707 on cover glasses at 10 μg/ml, but did not show morphological alterations at the tested concentrations. Amides were identified as piperovatine and piperlonguminine, and showed MIC values of 15.6 and 31.2 μg/ml to *B. subtilis* and 3.9 μg/ml to *C. tropicalis*, and low toxic effects to confluent Vero cells monolayers and adherent J774G8 macrophages cells. *Acknowledgements: The authors are grateful to CNPq for providing a research grant and fellowships* **References:** [1] Rodrigues-Silva, D. et al. (2008) J. Ethnopharmacol. 116:569 – 573. [2] Correa, M.P. (1984) Dicionario das Plantas Uteis do Brasil e das Exoticas Cultivadas, vol. 1. Instituto. Brasileiro de Desenvolvimento Florestal, Rio de Janeiro.

PH22 The effect of total extract of *Securigera securidaca* L. seeds on serum lipid profiles and vascular function in hypercholesterolemic rats

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Seeds of *Securigera securidaca* (Fabaceae), also called goat pea, *are* used for the treatment of disorders such as hyperlipidemia, diabetes, and epilepsy in folk medicine [1]. In this study the effect of total extract of *S. securidaca* seeds on serum lipid profile and on function of isolated thoracic aorta in high-fat fed rats was investigated. High-fat fed wistar rats received 50 – 200 mg/kg/day of the extract orally for 20 days. At the end of the experiment vena cava vein blood was collected for lipids measurement and then thoracic aorta was excised. The extract in doses of 100 and 200 mg/kg/day reduced the level of LDL significantly ($p < 0.05$) from 158 ± 17 mg/dl in hypercholesterolemic rats to 107 ± 14 and 99 ± 11 mg/dl in treated groups, respectively. These declines were accompanied by a significant reduction of serum triglyceride ($p < 0.05$; max: 40%) and liver deposition of lipids in all treated groups. The extract also produced a marked ($p < 0.001$) antioxidant activity by suppressing the hypercholesterolemia induced elevation of malondialdehyde levels both in serum (max 73%) and liver (max 80%). In hypercholestrolemic group carbachol-induced endothelium-dependant vasodilatation was decreased significantly from 95 ± 12% in control to 34 ± 7% ($p < 0.001$).

The extract improved significantly ($P < 0.01$) the endotheliuml-dependant relaxation in hyperlipidemic animals. So that, the maximum relaxation in thoracic aorta isolated from the rats treated by 100 mg/kg of the extract was 82 ± 6.5%. The results of this study indicated that the total extract of *S. securidac*a seeds in addition to having a considerable antioxidant and anti-hyperlipidemic effects, is able to improve vascular endothelium dependent relaxation in hypercholesterolemia. **Reference:** [1] Hosseinzadeh, H. et al. (2002) Phythother. Res. 16:745 – 747.

PH23 Effect of caffeine on the neuromuscular system in rats: an immunohistochemical study

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Caffeine (Caf) is a central nervous system stimulant, it is used to reduce physical fatigue and restore mental alertness. Caffeine (1,3,7-trimethylxantine) is one of the most consumed drugs in the world. It is a natural alkaloid present in coffee, tea and mate plants. There is a strong belief that Caf is an ergogenic aid to sports performance [1,2,]. Although much evidence suggests that Caf may improve endurance exercise performance, questions still remain with regard to its effects on neuromuscular function. At the cellular level, it stimulates the central nervous system (CNS), enhances neuromuscular transmission and improves skeletal muscle contractility [3]. Caf enters the bloodstream through the stomach and small intestine and can have its effects as soon as 15 minutes after it is consumed. Once in the body, it takes about 6 hours before to be eliminated. Since in scientific literature there are only few morphological studies about neuromuscular system, we performed an immunohistochemical study on neuropeptides expression in rat skeletal muscle after Caf oral administration: thirty male rats were divided into 3 groups, the first one with 16 mg/kg Caf, the second one with 24 mg/kg Caf, the last one was control group. The immunohistochemical study was performed with antibodies against Protein Gene Product 9.5 (PGP 9.5), Serotonin (5-HT), Vasointestinal Peptide (VIP) and Substance P (SP), on rat skeletal muscle. We observed an accentuated immunoreactivity for peptides in samples with higher concentration administration (24 mg/kg Caf), compared with normo-fed rats (control group), in which was observed a small positivity for tested substances. Our results suggest that caffeine is able to increase muscular performance with a dose-dependent effect. **References:** [1] Tarnopolsky, M.A. (2008) Appl. Physiol. Nutr. Metab. 33:1284 – 1289. [2] Keisler, B.D. and Armsey, T.D. (2006). Curr. Sports Med. Rep. 5:215 – 219. [3] Paluska, S.A. (2003) Curr. Sports Med. Rep. 2:213 – 219.

PH24 Low density lipoproteins and cardiovascular disease – cause or consequence?

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Saturated fatty acids can be found in certain vegetable or animal-derived foods (cocoa butter, coconut oil, dairy fat). Saturated fats have been shown to exert positive effects e.g. being antibacterial and antifungal. However some long chain saturated fats have been shown to increase low density lipoprotein (LDL) serum levels, which is considered to be a determinant of the metabolic syndrome. Concerning the aetiology of atherosclerosis, the general consensus is that "high circulating levels of LDL damage the arterial wall", "at the site of injury, LDL can enter the arterial wall more easily" and "in the arterial wall, LDL becomes oxidized and this leads to even more cholesterol depositing and thus atherosclerosis". Decreasing the level of LDL (via diet or medicine) is generally seen as beneficial for decreasing cardiovascular disease (CVD) development, though this view is challenged by several groups. We therefore hypothesize that the increase of LDL levels is rather a concomitant event in the development of CVD than a causal factor. Therefore, it was tested whether high concentrations of LDL negatively affect endothelial cells *in vitro*. Endothelial function was assessed by NO-release, intracellular cholesterol content and intracellular adhesion molecule (ICAM) expression in primary human aortic endothelial cells (HAEC). The results demonstrate that uptake of LDL by HAEC was increased with increasing exogenous LDL-concentrations; however, this did not induce endothelial dysfunction as measured by the mentioned parameters. Based on this, it can be concluded that saturated fats might lead to increased LDL levels, but this does not seem to be detrimental to endothelial cell function. Our data support reports stating beneficial effects or lack of detrimental effects of any type of dairy (skimmed or full fat) on cardiovascular health.

PH25 Effect of *Oenothera paradoxa* defatted seed extracts and penta-*O*-galloyl-β-D-glucose on human polymorphonuclear leukocyte function

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Polymorphonuclear leukocytes (PMN) are suggested to be implicated in vascular and heart diseases [1]. Particularly, activated PMN produce and release reactive oxygen species (ROS), proteolytic enzymes and neutral endopeptidase (NEP). NEP degrades the atrial natriuretic peptides, which are protective factors in circulation and heart. Previously, we have demonstrated that *Oenothera paradoxa* defatted seed extracts inhibited the NEP activity on isolated enzyme [2]. In this study, we investigated the effect of those extracts on human PMN function such as: NEP activity inhibition, ROS production and elastase release. The aqueous and 60%ethanolic extracts at concentration of 5 – 50 μg/ml inhibited in dose dependent manner the NEP activity, ROS production was inhibited at concentration of 0.2 – 20 μg/ml and the elastase release was slightly reduced. The HPLC-DAD analysis showed that the dominating compounds in both extracts are: gallic acid (3.7 ± 0.1 and 2.2 ± 0.1 mg/g), (+)-catechin (23.4 ± 0.8 and 30.4 ± 01.5 mg/g) and penta-*O*-galloyl-β-D-glucose PGG (12.1 ± 0.5 and 16.8 ± 0.6 mg/g). PGG appeared to be partly responsible for observed effects: IC_{50}= 7 μM for NEP activity inhibition, IC_{50}> 0.2 μM for ROS production and IC_{50}= 15 μM for elastase release. These results indicate that *Oenothera paradoxa* defatted seed extracts down-regulated the PMN function and may provide a protective effect against vascular and heart diseases. **References:** [1] Ernst, E. et al. (1987) JAMA 257:2318 – 2324. [2]Kiss, A.K. et al. (2008)J. Agric. Food. Chem. 56:7845 – 7852.

PH26 Phytochemical and antidiabetic investigations of *Otostegia persica* from Iran

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Otostegia persica (Burm.) Boiss. is an endemic plant of Iran and Pakistan [1]. The people in the south of Iran used the flowering aerial parts of *O. persica* for antidiabetic and anti-inflammatory properties. *Otostegia persica* was collected at flowering stage in May 2005, around of the Taftan mountain of Sistan & Baluchestan Provinces, Iran. The dry-powdered aerial parts of plant were extracted with petroleum ether, chloroform, ethyl acetate (EA), butanol and methanol (ME); the fractions concentrated in vacuum. Antioxidant activity of all fractions was measured with DPPH method [2]. ME and EA fractions were showed potent and moderate activities (91.53% and 82%) on inhibition of free radicals, respectively. ME fraction was showed equal activity with vitamin E (94.69%) and BHT (90.6%) with $P > 0.05$. Other fractions did not have potent antioxidant activity. Therefore ME and EA fractions subjected to antidiabetic investigation. Male NMRI mice, after 12 hours fasting, received a single dose of STZ (200 mg/kg) with i.p. injection [3]. Three days later fast blood sugar reached more than 250 mg/dl and stabilization of blood glucose level occurred, then fresh dilution of ME and EA fractions in normal saline were prepared and administered by i.p. injection at different doses in a fixed volume of 0.5 ml. Results showed that response of ME fraction at dose of 400 mg/kg had equal effect with glibenclamide (P= 0.45) and NPH Insulin (P= 0.06). EA fraction did not show any reduction on blood sugar of mice. Four compounds were isolated and purified by paper and Sephadex LH20 column chromatography. 6-methyl apigenin, apigenin-7-o-glycoside, echinaticin from ME

fraction and chrysoeriol from EA fraction were isolated and identified with spectroscopic methods. **References:** [1] Recshinger, K.H. (1982). In Flora Iranica: *Otostegia persica* (Labiatae), Rechinger KH, ed. Akademische Druck-u. Verlagsanstalt, Graz- Austria. [2] Sánchez-Moreno, C. et al. (1999) Food Res. Int. 32:407 – 412. [3] Hayashi, K. et al. (2006) Biol. Pharm. Bull. 26:1110 – 1119.

PH27 Computational evaluation of Isoorientin (*C*-glycosyl flavone) on PPAR-gamma receptors and HMG- CoA reductase using MOE 2008.10

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Peroxisome proliferators-activated receptor-gamma (PPAR-gamma) plays an essential role in lipid and glucose homeostasis. Numerous studies and comprehensive reviews have documented various naturally derived ligands as PPAR-γ a potential source of novel anti-diabetic compounds from plants and herbs [1]. Isoorientin, a C-glycosyl flavone, has been isolated as a antidiabetic and antihyperlipidemic agent from aerial parts of *Gentiana ollivieri* Griseb. [2]. The objective of this study is to find out, the relation between these receptors and ligand. We used docking property and site finder and electrostatic map tools of molecular operating environment (MOE) 2008.10 computer programme from Chemical computing group. Protein structures were taken from Protein Data Bank PDB and operated with Protonate 3D and minimized. Ligands were designed by LigX. Results shown that, E score1: -16.0501 and E refine: -32.3072 for Isoorientin-PPAR gamma docking study, E score1: -9.8957 E refine: -17.2581 for Isoorientin-HMG-CoA docking study. Data obtained from experiments demonstrated that isoorientin can be candidate as a good multi-target drug template. *Acknowledgements: Authors to thank Ms. Patricia Middleton from Chemical Computing Group INC. for supply MOE 2008.10 programme* **References:** [1] Salam, N.K. et al. (2008) Chem. Biol. Drug Des. 71:57 – 70. [2] Sezik, E. et al. (2005) Life Sci. 76:1223 – 1238. [3] Labute, P. et al. (2008) Electrostatic Maps, Chemical Computing Group, Montreal, Canada.

PH28 *Brassica oleracea* L.var. *italica*: A nutritional supplement for weight loss

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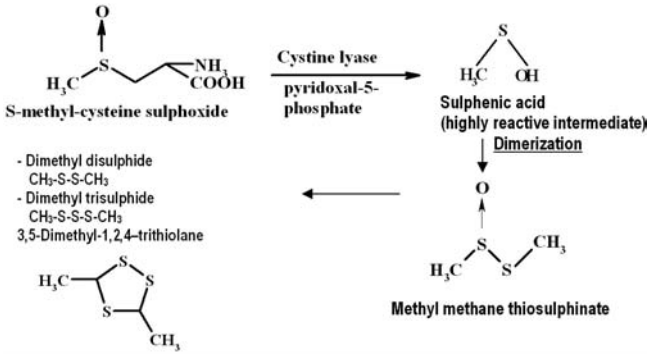
The florets of *Brassica oleracea* L. var. *italica* (Brassicaceae) are an important nutritional supplement containing a high percentage of sulphur compounds. GC/MS analysis of the volatiles produced by the action of endogenous cystine lyase on S-methyl-cysteine sulphoxide present in Broccoli florets showed three sulphur components: dimethyl disulphide, dimethyl trisulphide and 3,5-dimethyl-1,2,4-trithiolane. Four isothiocyanate compounds were also present. Cystine lyase enzymatic fission of endogenous myrosinase gave the unsaturated glucosinolates ethenyl isothiocyanate and allylisothiocyanate, together with the saturated 4-methylthiobutyl isothiocyanate (Erucin) and the aromatic 2-phenylethyl isothiocyanate. When an LD_{50} test was performed on the crude extract of broccoli, no toxicity was observed up to 10 g/kg body weight in rats. The chloroform extract, the combined ethyl acetate and ethanol extracts and the crude extract of broccoli florets showed significant loss in body weight of female rats at 5% (LSD 24.9) and 1% (LSD 33.7) of diet, according to statistical tests (1) (180, 85, and 75 g total loss in body weight, respectively). When these results were compared with the water extract of green tea (117 g loss), the chloroform extract was more active.



References: [1] Snedecor, W.G., Cochran, G.W. (1982) Statistical Methods, 10th ed., Iowa State University Press, USA.

PH29 Evaluation of hepatoprotective activity of the *Retama raetam* seeds on carbon tetrachloride-induced liver damage in rats

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In this study, the hepatoprotective effect of the aqueous methanolic extract of *Retama raetam* seeds was investigated against CCl_4-induced liver damage in rats. The extract was tested in two different treatments (20 and 40 mg/kg b.wt.) and three different durations (2, 3 and 4 weeks). Serum samples were taken to determine the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The histopathological and histochemical effects on the liver tissue were also investigated to support the above parameters. The results of the present study indicated that the levels of serum AST, ALT and ALP were significantly ($P < 0.05$) elevated by CCl_4 administration as compared with the control group and significantly reduced at $P< 0.05$ by the treatment with the plant extract (20 and 40 mg/kg/b.w for 2, 3, or 4 weeks) in the CCl_4-intoxicated rats. Microscopic examination of liver of CCl_4 treated animals revealed focal necrosis and lymphocytic infiltration in the periportal areas with massive fatty infiltration. The histopathological examination also showed clearly that the extract of *Rateam ratem* seeds reduced the alterations that induced in liver by CCl_4. The maximum protection against CCl_4-induced hepatic aberrations was achieved with the optimum dose (40 mg/kg b. wt.) of the extract and the effect of *Retama raetam* seems dose- and time-dependant. Flavonoid derivatives are commonly found in plants and have been shown to display remarkably of biological activities, such as hepatoprotective, anti-inflammatory and antiviral activities [1]. Therefore, our findings suggest that the flavonoid derivatives are the major active compounds responsible for the hepatoprotective activity of *Retama raetam*. **References:** [1] Middleton, E. et al. (2000) Pharmacol. Rev. 52:673 – 751.

PH30 Hypoglycemic effect of methanol and chloroform extract of *Cuscuta reflexa* Roxb. in mice

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Cuscuta reflexa is a parasitic vine prevalent throughout Bangladesh. The plant is used in folk medicine as remedy for prostate cancer, impotency, scabies, fevers, diarrhea, and throat pains. In this study, we examined the hypoglycemic activity of methanol and chloroform extracts of the whole vine in glucose-loaded mice (oral glucose tolerance test). Control mice received distilled water, while experimental mice received methanol or chloroform extract at oral doses of 50, 100 and 200 mg/kg body weight. A further group was orally administered glibenclamide (10 mg/kg body weight). All mice received oral glucose at 2 g/kg body weight, 60 min after extract or glibenclamide administration. Blood samples were collected at 120 min following glucose loading and serum levels of glucose determined. The results are expressed as mean ± SEM. The significance of the results was calculated using Student's t-test and were considered statistically significant when $P < 0.05$. Both methanol and

chloroform extract demonstrated significant hypoglycemic activity; however, the effects were lower than that obtained with glibenclamide. Chloroform extract demonstrated higher hypoglycemic activity than methanol extract. Serum glucose concentrations in control, glibenclamide-administered, and 50 mg/kg body weight methanol extract- and chloroform extract-administered mice were respectively, 87.0 ± 1.7, 37.8 ± 1.8, 74.6 ± 1.5, and 54.1 ± 1.3 mg/dL. Overall, the results demonstrate significant hypoglycemic activity, particularly in the chloroform extract of *Cuscuta reflexa*.

PH31 Anti-inflammatory effect of *Curcuma longa* (turmeric) rhizome when administered topically in gel form

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Curcuma longa L. (Zingiberaceae) rhizome (turmeric) is widely used in folk medicine of the Indian subcontinent for inflamed joints. The objective of the present study was to investigate the anti-inflammatory effects of turmeric when administered topically in gel form to carrageenan-induced paw edema in rats. Gels contained polyethylene glycol-6000, sodium carboxymethyl cellulose and isopropyl alcohol without and with turmeric powder or diclofenac. The right hind paw of all rats were pre-treated twice daily for 2 days with gel without (Group 1) or containing turmeric powder at 3.33, 10 and 33.3% w/v (Groups 2, 3 and 4, respectively). Group 5 rats were pre-treated with gel containing the standard anti-inflammatory drug diclofenac (1% w/v). Edema was induced on Day 3 by injecting 0.1 ml of 1% carageenan solution (in normal saline) into the plantar surface of the right hind paw of each rat. Increase in paw volume was monitored up to 5 hours after injection. Significance levels of the results were calculated using Student's t-test and date were considered statistically significant when $P < 0.05$. Pre-treatment with turmeric-containing gel produced a significant and dose-dependent inhibition of rat paw edema. Compared to controls, at the highest dose (33.3% turmeric), edema was inhibited by 53.4 ± 2.6, 40.0 ± 3.3, 36.3 ± 2.8, 33.5 ± 3.7 and 31.4 ± 3.4%, respectively, at the first, second, third, fourth and fifth hour following carrageenan injection. These results compare favorably with diclofenac, where the respective inhibitions were 35.5 ± 3.4, 36.5 ± 3.3, 33.5 ± 3.7, 29.5 ± 3.0 and 24.5 ± 4.0%. Even at the lowest dose (3.33%), turmeric inhibited paw edema by 27.4% at the first hour following carrageenan injection. The results validate the folk medicinal use of turmeric as an anti-inflammatory agent.

PH32 An ethnomedicinal survey of several regions of Pabna district, Bangladesh

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The predominantly rural population of Bangladesh relies on traditional medicinal practitioners (Kavirajes) for primary treatment of their various ailments. The medicinal plants used by the Kavirajes for treatment can vary considerably from region to region. We accordingly conducted an ethnomedicinal survey of several areas within Pabna district, Bangladesh to learn more about medicinal plants used by the Kavirajes. Interviews were conducted with the help of a semi-structured questionnaire and plant specimens as pointed by the Kavirajes were collected and identified at the Bangladesh National Herbarium. Some of these plant species (with ailments treated given in parentheses) included *Tecoma stans* (pain, piles), *Asteracantha longifolia* (insomnia, kidney stones), *Holarrhena antidysenterica* (rheumatism), *Trigonella foenum-graecum* (to ease delivery pain, chicken pox), *Lectuca sativa* (rheumatism, sprain, asthma), *Butea monosperma* (to increase skin brightness), *Bixa orellana* (to stop menstruation), *Brassica orellana* (filariasis), *Nigella sativa* (helminthiasis), *Uraria picta* (coughs, pain), *Mimosa pudica* (waist pain), *Fumaria indica* (irregular menstruation), *Cynodon dactylon* (sex stimulant), *Musa sapientum* (irregular menstruation), *Abrus precatorius* (hair loss), *Guizotia abyssinica* (hair loss, skin infections), *Thespesia populnea* (skin infections, scabies, eczema), *Piper cubeba* (sex stimulant, asthma), *Arachis hypogaea* (diabetes, weakness), *Pterocarpus santalinus* (fever, blood in urine), *Luffa cylindrica* (headache, sinusitis), *Rauwolfia serpentina* (hypertension), *Mucuna pruriens* (erectile dysfunction, rheumatic pain), *Acacia nilotica* (to increase semen count, breast infections), *Zingiber officinale* (to increase memory, headache), *Morinda citrifolia* (snake bite), *Azadirachta indica* (skin diseases, chicken pox, strengthen gums and teeth), *Artocarpus heterophyllus* (to increase fetal safety), and *Plumbago rosea* (to increase memory).

PH33 Effects of STW 5 and its single extracts on ileal oxygen radical production induced by histamine

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STW 5 (Iberogast®) is a well established mixture prepared from nine plant extracts (angelika root, bitter candy tuft, camomile flowers, caraway fruits, greater celandine herb, liquorice root, milk thistle fruits, lemon balm leafs, peppermint leafs) with clinically proven efficacy in several digestive diseases, especially in therapy of Irritable Bowel Syndrome (IBS). Recent findings favour that inflammation seems to play an important role in IBS, suggesting that oxidative stress could be involved in the pathogenesis. Here we could show by luminol-enhanced chemiluminescence that histamine was able to increase oxygen free radical production of incubated samples of mouse intestine. In addition, the antioxidative effects of STW 5 and its constituent extracts were studied. The evaluated data show that STW 5 as well as the constituent single extracts could reduce the induced radical production dose-dependently. Peppermint and camomile showed highest quenching effects, whereas the lowest effects were observed with greater celandine herb and bitter candy tuft, these results were similar to the in-vitro-data [1]. Further experiments should show whether the effects of the extracts can be explained either by direct anti-oxidant properties or by inhibition of the enzymes/enzyme systems producing free radicals. **References:** [1] Germann, I. et al (2006) Phytomedicine 13(Suppl.V):45 – 50.

PH34 Synergistic anti-inflammatory activity of papaya and kale in colitis induced by trinitrobenzenesulfonic acid in the rat

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Papaya and kale are usual vegetables in the Brazilian diet that have antioxidant activity. This study proposed to evaluate if the concurrent administration of both dried vegetables results in a synergism in their intestinal anti-inflammatory activity in the TNBS model of rat colitis. Five groups of rats were used (n = 7); non-colitic (NC) and control-group (C) did not receive treatment; one group received orally 130 mg/rat/day of papaya (P), other group received the same dose of kale (K) and the last one, 78 mg K plus 52 mg P (M). The dose 130 mg/rat/day of vegetables has the highest butyrate production, as shown by an *in vitro* study [1]. After two weeks, colitis was induced by intracolonic administration of TNBS (10 mg), and, one week after, damage score (method described by Bell) [2] and biochemical parameters were evaluated. The administration of the mixture showed intestinal anti-inflammatory effect, but this effect was not observed with each vegetable. This was evidenced by a reduction in damage score (6.4 ± 0.9 vs. 7.5 ± 0.4; $p < 0.05$). The colonic iNOS expression was downregulated by M administration. In addition, M and K treatments significantly inhibited the increased production of the TNFα (796 ± 160 and 706 ± 122, M and K, respectively vs. 1657 ± 180 pg/g tissue; $p < 0.01$); only M administration significantly inhibited the increased production of the IL-1β (12482 ± 2100 vs. 17744 ± 1118 pg/g tissue; $p < 0.01$). The combination of dried papaya and dried kale results in an increased anti-inflammatory effect in the TNBS model of rat colitis, when compared with each single vegetable. *Acknowledgements: Fapesp and Capes* **References:** [1] Dias, J.C. et al. (2008) J. Pharmaceut. Biomed. 49:1128 – 1132. [2] Bell, C.J. et al. (1995) Am. J. Physiol.- Gastr. L. 278: H377-H383.

PH35 Evaluation of the synergism of papaya and kale in their prebiotic effect in rats

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Papaya fruits contain insoluble diet fibre and are used in popular medicine as a laxative, whereas kale leaves contain mainly soluble diet fibre that has beneficial effects on intestinal flora. The aim of this study is to evaluate if the concurrent administration of both vegetables to rats results in a synergism in their prebiotic effects. Four groups of rats were used: control group without treatment, and three treated groups, which received orally papaya (P), kale (K) or a mixture of both (M) (60% K and 40% P) at 130 mg/rat/day. Lactobacilli and bifidobacteria (beneficial bacteria) as well as aerobic and enterobacteria (potential pathogens) counts were determined in the colonic and ceacum contents. The percentage of water was evaluated both in faeces and in intestinal contents. The administration of M significantly increased the ratio of the beneficial bacteria to potential pathogens in both intestinal segments analysed, colon (1.43 ± 0.2 vs. 1.32 ± 0.01; $p < 0.01$) and ceacum (1.4 ± 0.2 vs. 1.3 ± 0.01; $p < 0.01$); however this effect was not observed with each vegetable. In addition, all treatments significantly increased the percentage of water in the faeces ($52.3 \pm 7.3\%$, $51.9 \pm 8.4\%$ and $51.6 \pm 8.9\%$, K, M and K, respectively, vs. $48.5 \pm 0.8\%$ in controls; $p < 0.01$); whereas only the mixture significantly increased this in ceacum contents ($79 \pm 12\%$ vs. $75 \pm 0.5\%$; $p < 0.05$) and colonic contents ($78 \pm 12\%$ vs. $70 \pm 2.6\%$; $p < 0.05$). The combination of both vegetables facilitates the prebiotic effects showed by each one when administered separately. *Acknowledgements: Fapesp and Capes*

PH36 Effect of grape seeds on the IL-10 and IL-12 in the trinitrobenzenesulfonic acid model of rat colitis

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The pathogenesis of inflammatory bowel disease (IBD) is not completely understood, a loss of immune tolerance toward the enteric flora it is mediated by different molecules. Among this molecules are included the cytokines that are key signals in the intestinal immune system and are important in the pathogenesis of IBD [1]. Grape seeds (GS) have been reported to possess a broad spectrum of pharmacological and therapeutic effects including antiinflammatory activity [2]. In this context, the aim of this study is to evaluate the efficacy of GS administration on reduction of proinflammatory cytokine interleukin-12 (IL-12) and anti-inflammatory interleukin-10 (IL-10) production in TNBS model of rat colitis. Three groups of rats were used ($n = 8$); non-colitic (NC) and control groups (C) did not receive treatment, and the treated groups were given orally GS at 5 g/rat/day. After two weeks, colitis was induced by intracolonic administration of TNBS (10 mg), and, one week after, biochemical parameters (IL-10 and IL-12) were evaluated. The administration of the GS reduced the IL-12 expression when compared to TNBS group (182 ± 10 vs. 115 ± 11 pg/p tissue; $p < 0.001$). In addition, GS treatment significantly increased production of the IL-10 (111 ± 16 vs. 164 ± 9 pg/g tissue; $p < 0.01$). The efficacy of GS treatment for the reduction of intestinal inflammation in rats is a result of both anti-inflammatory and immunosuppressive activity. *Acknowledgements: Fapesp* **References:** [1] Andoh, A. et al. (2008) World J. Gastroenterol. 14:5851 – 5856. [2] de la Lastral, C.A. and Villegas, I. (2007) Biochem. Soc. T. 35:1156 – 1160.

PH37 Antiestrogenic properties of Griffonianone C in U2OS human osteosarcoma cells

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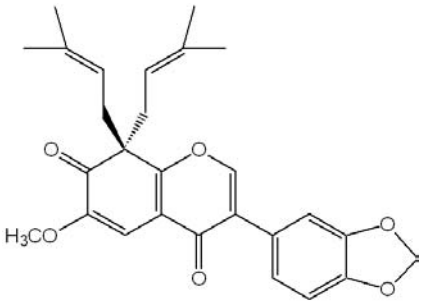
Griffonianone C (Griff C) [1], the most potent isoflavone derived from *Millettia griffoniana* Baill. (Fabaceae), has been shown to be a weak activator of estrogen receptor-α (ERα) [2] and results in vivo suggested a possible interaction with estrogen receptor-β (ERβ) [3]. The aim of this study was to investigate the interaction of Griff C with both estrogen receptors in more details.



Griffonianone C

For this purpose the human osteosarcoma U2OS cells either stably transfected with ERα or transiently with ERβ were used in a luciferase reporter gene assay. Cells were treated with different concentrations (10^{-8} M, 10^{-7} M and 10^{-6} M) of Griff C for 24 hours and the relative luminescence units were determined. The results showed an inactivation of residual ERα and β in the presence of Griff C in a dose dependent manner in this particular experimental setup. In addition, competition experiments showed an antagonisation of estradiol-induced activation by Griff C. *In vivo* experiments in our lab are ongoing to investigate a potential tissue selective effect of Griff C. *Acknowledgements: Dr C.B. Magne Ndé was a research fellow supported by the German Academic Exchange Service (DAAD), This work is further supported by the DFG/BMZ grant NoVO 410/11 – 1 to Prof Dr G. Vollmer and Prof.Dr. D. Njamen.* **References:** [1] Yankep, E. et al. (2001) Phytochemistry 56:363 – 368. [2] Ketcha Wanda, G.J.M. et al. (2006) Phytomedicine 13:139 – 145. [3] Ketcha Wanda, G.J.M. et al. (2007) Planta Med. 73:512 – 518.

PH38 Antihyperglycaemic activity of *Hunteria umbellata* (K. Schum) seed extract in experimental diabetes

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The present study investigates the antihyperglycaemic activity of the aqueous seed extract of *Hunteria umbellata* K. Schum (Apocynaceae) (*HU*) in alloxan-induced, high fructose- and dexamethasone-induced hyperglycaemic rats. Single, daily oral administration of 1 mg/kg of glibenclamide, 50 mg/kg, 100 mg/kg and 200 mg/kg of *HU* to alloxan-induced hyperglycaemic rats in groups III, IV, V and VI, respectively, for 14 days [1] caused significant dose related ($p < 0.05$, $p < 0.01$ and $p < 0.001$) reductions in the fasting blood glucose when compared to the values obtained for model control (Group II) rats. In the high fructoseinduced hyperglycaemic model, daily oral administration of 66.7 g/kg fructose [2] to rats for 8 weeks was associated with significant ($p < 0.001$) hyperglycaemia, elevations in plasma glycosylated haemoglobin (HbA1c), free insulin, fasting insulin resistance indices, serum triglyceride, and cholesterol. However, concomitant oral treatments with 1 mg/kg of glibenclamide, 50 mg/kg, 100 mg/kg, and 200 mg/kg of *HU* extract significantly and dose dependently ($p < 0.05$, $p < 0.01$, and $p < 0.001$) attenuated development of hyperglycaemia, and decreased

levels of plasma HbA1c, free insulin, serum triglyceride and cholesterol, in the groups III, IV, V and VI rats, respectively, when compared to model control (Group II) rats. Similar effect was also recorded in the dexamethasone-induced hyperglycaemic rats. The results showed that the antihyperglycaemic and antihyperlipidaemic effects of *HU* are mediated via enhanced peripheral glucose uptake and improvements in hyperinsulinaemia. **References:** [1] Adeneye, A.A. et al. (2007) Fitoterapia 78:502 – 505. [2] Fasanmade, A.A. and Alabi, O.T. (2008) African Journal of Biomedical Research 11:191 – 196.

PH39 Ameliorative effect of *Rhodotorula glutinis* and its two mutants on histopathological and biochemical changes induced by Ochratoxin A in rat kidney

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Rhodotorula glutinis (*R. glutinis)*, red soil yeast have proved safe and non-toxic in experimental animals. Two mutant strains (Col-1R1 and Col-1R3) were obtained from (Plant Pathology department, NRC, Cairo, Egypt), to improve cell contents (carotenoids, β-1, 3 glucane,) and to be used as safe biocontrol in harvesting crops. This study was designed to evaluate the possible curative effect of the wild strain and its two mutants against renal toxicity induced by ochratoxin (OTA) in rat. *Rhodotorula glutinis* containing higher amount of carotenoids [1]. Eight groups were used as follows: group 1 (the control group) was the vehicle (10 ml/Kg); group 2 treat with OTA (1 mg/Kg); groups 3, 4 and 5 treatment with yeast and its two mutants at dose (10^5 CFU/ml liquid media); groups 6, 7 and 8 orally treat with yeast and its two mutants then OTA administration to animal after 1 h of treatment with yeast and its two mutants. The experimental period for this study was 15 successive days. The blood samples were collected for assign serum biochemical parameters (creatinine and uric acid). Biochemical results revealed that OTA significantly elevated kidney function (creatinine and uric acid) than normal control group. The two mutants and the wild strains of *R. glutinis* significantly decreased these increased values toward the normal level. The changes in the kidney tissues of control, OTA and OTA+ *R. glutinis* and two mutants-treated rats was evaluated by histopathology, histochemistry and DNA ploidy measurement using image analysis. There were no changes in the kidney tissues of the control rats. Histopathological examination of kidney of rats treated with OTA showed tubular epithelial cells degeneration, necrosis, proliferation and karyomegaly in the epithelial cells nuclei. Peritubular and periglomerular lymphocyte infiltration, fibrous tissue proliferation and hypercellularity of glomeruli were also observed in OTA group. The cytometric results revealed that the rats treated with OTA induced an increase in the aneuploidy cells and decrease in diploid cells. These findings were ameliorated by *R. glutinis* and two mutants when compared to the OTA-treated group. The resultant effect indicated that the two mutant strains had powerful effect more than the parent *R. glutinis* to ameliorate renal dysfunction in ochratoxicosis-rat; specially Col-1R3 more effective than Col-1R1 due to its higher contents of carotenoids, glucane and chitine, which act as antioxidants. **Reference:** [1] Bhosale, P. et al. (2002) Curr. Sci. India 83:303 – 308.

PH40 Medicinal plants used against syphilis and gonorrhea by traditional medicinal practitioners of Bangladesh

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Sexually transmitted diseases like syphilis and gonorrhea are prevalent worldwide and are also present in both rural and urban areas of Bangladesh. Most people suffering from these diseases, particularly the rural population seek remedy from traditional medicinal practitioners (Kavirajes) rather than visiting modern doctors either because of lack of access or because of hesitancy in telling about these diseases to an unknown doctor. The remedies offered by the Kavirajes, although based primarily on Ayurvedic medicine, relies more on their knowledge of medicinal plants and their healing properties. We conducted an ethnomedicinal survey amongst the Kavirajes of Bangladesh to gather information on medicinal plants used by the Kavirajes to treat syphilis and gonorrhea. Plants were collected from the Kavirajes and herbarium specimens were deposited and identified at the Bangladesh National Herbarium. A total of 21 plants were identified as to their being used to treat syphilis or gonorrhea. The plants used to treat gonorrhea (with family name in parenthesis) include *Amaranthus spinosus (*Amaranthaceae), *Piper betle (*Piperaceae), *Pongamia pinnata (*Leguminaceae), *Sida cordifolia (*Malvaceae), *Ocimum tenuiflorum (*Labiateae), *Curcuma longa (*Zingiberaceae), *Swertia chirata (*Gentianaceae), *Phyllanthus niruri (*Euphorbiaceae), *Abrus precatorius (*Leguminaceae), *Aloe vera* (Asphodelaceae), *Senna alata (*Leguminaceae), and *Pistia stratiotes (*Araceae). Plants used to treat syphilis include (with family name in parenthesis) include *Cassia fistula (*Leguminaceae), *Mucuna pruriens (*Leguminaceae), *Solanum surattense* (Solanaceae), *Azadirachta indica (*Meliaceae), *Terminalia chebula (*Combretaceae), *Phyllanthus niruri (*Euphorbiaceae), *Gloriosa superba* (Colchicaceae), *Areca catechu (*Arecaceae), and *Gmelina arborea (*Labiateae). The plant *Phyllanthus niruri (*Euphorbiaceae) was used as remedy for both syphilis and gonorrhea.

PH41 Mechanisms underlying the vasorelaxant effect induced by *Anacardium occidentale* L. leaf fraction in rat small resistance mesenteric arteries

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Anacardium occidentale L. (*A. occidentale*), belonging to Anacardiaceae family, has been documented as traditional plant for the treatment of diabetes and hypertension [1]. Four extracts of *A. occidentale* were used for this study, namely AOL1 (from cyclohexane), AOL2 (from CH_2Cl_2), AOL3 (from EtOAc) and AOL4 (from MeOH). The most potent antiradical reactivity was observed in AOL4 with 2023 TE/g of extract compared to chlorogenic acid (2976 TE/g of compound) as positive standard. In the other hand, our data showed a significant inhibitory effect of EtOAc and MeOH extracts on BSA glycoxidation measured in terms of advanced glycation end products (AGEs). In isolated mesenteric artery rings precontracted with phenylephrine [2], AOL1, AOL2, AOL3, and AOL4 induced a concentration-dependant relaxation respectively with IC50 values of 120 μg/ml, 100 μg/ml, 70 μg/ml and 70 μg/ml. Exposure of Eahy cells to high glucose for 7 days significantly ($p < 0.05$) decreased, cell viability (22%), the level of antioxidant gluthatione and expression of protein kinase C. Incubation with low concentrations (7 μg/ml, 12.5 μg/ml) of AOL3 and AOL4 for 7 days significantly ($p < 0.05$) attenuated high glucose-induced dysfunction of EAhy cells. Preliminary phytochemical investigations of AOL3 and AOL4 by HPLC-DAD analyses suggested the presence of flavonoids and biflavonoids as major compounds in both extracts. *Anacardium occidentale* leaf extract induces a vasodilatation in mesenteric arteries precontracted with phenylephrine. Ethylacetate and methanol extracts improved high glucose-mediated endothelial dysfunction and thus may be potential new therapeutic agents for diabetic cardiovascular complications. **References:** [1] Runnie, I. et al. (2004)J. Ethnopharmacol. 92:311 – 316. [2] Henrion, D. et al. (2008) Cardiovasc. Res. 77:600 – 608.

PH42 Hypotensor effects of consumption of garlic "*Allium sativum*"

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Traditional medicine used among the methods of treatment the virtues of the plants to fight against the diseases [1]. In our country, arterial hypertension became extensive, this frequent disease settle without precursory sign [2], and the best means of countering this type of disease it is early tracking and the prevention. Accordingly the use of a

therapy at base of a mode is much indicated. Our work is a study of the effectiveness of a garlic mode "A*llium sativum*" introduced into the diet in addition to the treatment containing drugs with a troop of 30 voluntary people in a medical centre (50% of each sex) old between 50 and 75 years and presenting an essential HTA. The results obtained after 3 months were conclusive, insofar as the diet recommended at the subjects reaches of an essential hypertension stabilized their blood pressures towards the 8th week which spent 17, 5 at 14, 5 for the systolic pressure (PS) and from 10 to 7, 8 for the diastolic pressure (PD) with the difference in the reference group receiving only the drugs whose tension was variable and unstable. **References:** [1] Valn, J. (1983) Aromathérapie, traitement des maladies par les essences des plantes, Edition Maloine. [2] Kemali, Z. (1999) L'hypertension artérielle au Maghreb, Edition Dahleb, Algérie.

PH43 *In-vitro* anticandidal activity of endemic *Salvia potentillifolia* Boiss & Heldr. Ex Bentham and *Origanum hypericifolium* O. Schwartz & P.H. Davis in Turkey

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The present study established baseline data on anticandidal lytic activities of endemic species *Origanum hypericifolium* and *Salvia potentillifolia* naturally distributed Denizli and its environment. Steam distillation was used to isolate the unfatty polar part and clinical isolated *Candida* spp. strains were subcultured to sabouraud dextrose agar. Lytic anticandidal activities of unfatty polar parts were evaluated by enzym linked calorimetric method [1] against 93 clinical isolates belong to *Candida albicans, C. tropicalis, C. glabrata, C. krusei, C. kefyr* and *C. parapsilosis*. As a result, two (2.15%) strains of *Candida glabrata* amongst tested patogenic 93 clinical isolates of *Candida* strains were found to be sensitive to *S. potentillifoli*. However, each strain of *Candida albicans* and *Candida tropicalis* was found to be sensitive to *Origanum hypericifolium*. Results indicated that *O. hypericifolium* and *S. potentillifolia* had a potential of being used in food and medicine because of its anticandial activity. **References:** [1]Sally, N. et al. (2002) J. Micro. Methods 49:1 – 9.

PH44 *Plantago holosteum* Scop. as a potential natural antioxidant and antiinflammatory agent

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The genus *Plantago* L. (Plantaginaceae) comprises about 275 species found all over the world. Ancient use of plantains as herbal remedies is a consequence of their astringent, anti-toxic, antimicrobial, expectorant and diuretic properties. In order to valorize medicinal use of *Plantago holosteum* Scop., some tests on antioxidative and antiinflammatory activities of methanolic extract of *P. holosteum*, collected from mountain Kopaonik (Serbia) have been undertaken. The extract has been characterized regarding composition by LC-MS/MS and by different colorimetric techniques [1,2]. Flavonoids luteolin, luteolin-7-*O*-glc, apigenin and apigenin-7-*O*-glc, also iridoid aucubin were identified and quantified. The content of total phenolic compounds expressed as mg gallic acid equivalents/g of dry extract was 68.2 ± 3 and flavonoids was 13.1 ± 0.7 mg quercetine equiv/g of d.e. The radical scavenger capacity (RSC) was evaluated towards several radicals using spectrophotometry [3] and following IC_{50} were found: diphenylpicrylhydrazyl (6.1 ± 0.6 μg/ml), hydroxyl (127.2 ± 6.8 μg/ml), superoxide anion (73.3 ± 2.5 μg/ml) and nitric oxide radical (0.67 ± 0.06 mg/ml), inhibition of lipid peroxidation (11.5 ± 1.8 μg/ml). These results indicate comparable or higher extract activity than activity of synthetic antioxidants as BHT or BHA (butylated hydroxytoluene/hydroxyanisol). Antiinflammatory activity was examined by means of 12-lipooxygenase (12-LO) and cyclooxygenase-1 (COX-1) inhibition, quantifying the 12-LO product 12-HETE (12-hydroxy-5,8,10,14-eicosatetraenoate) and COX-1 product 12-HHT (12-hydroxy-5,8,10-heptadecatrienoic acid) by RP-HPLC-MS/MS. At concentration of 4 mg/ml extract showed 12-LO and COX-1 inhibitory activity of 60 and 42%. In this study, we report for the first time about antioxidant and antiinflammatory activity of *P. holosteum*, and accordingly assesses this species as a promising source of natural antioxidant and antiinflammatory agents. 1. Singleton, V.L. et al. (1999) Methods in enzymology, Acad. Press, New York. 2. Chang, C.C. et al. (2002) J. Food Drug Anal. 10:178 – 182. 3. Sanchez-Moreno, C. (2002) Food Sci. Tech. Int. 8:121 – 137.

PH45 Antiulcer activity of the ethyl acetate fraction of *Anchomanes difformis*

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The tuber of *Anchomanes difformis* is used by local herbalists in Nigeria especially in the western part of the country for the treatment of peptic ulcer disease. This study was carried out to evaluate the possible gastroprotective properties of the ethylacetate fraction of *A. difformis* on lesions induced by indomethacin, ethanol and pylorus ligation in rats. Oral administration of the extract (200 mg/kg and 500 mg/kg) caused a dose-dependent and significant ($p < 0.001$) reduction in total acid output and severity of ulceration in the pylorus ligation model. These same doses of the extract also produced dose-dependent and significant ($p < 0.001$) protection against ethanol-induced and indomethacin-induced ulcerations. The protection conferred by the extract was comparable to the effect of the standard ulcer drug – ranitidine – on these same models. Addition of the extract to 0.1 N HCl caused very little variation in pH suggesting a lack of buffering ability. Results obtained suggest that the ethylacetate fraction of *Anchomanes difformis* possesses clear gastroprotective activity. This activity may not be due to neutralization of gastric acid but may result from its ability to reduce total acid output, or via the production of prostaglandins and free radical scavengers which protect the gastric mucosa. Drugs with multiple mechanisms of protective action, including antioxidant properties, may be a way forward in minimizing injury in human disease [1]. The effectiveness of the extract in the three ulcer models studied points to its usefulness in the management of ulcer and this may provide the basis for its local use in this ailment **Reference:** [1] Barry, H. (1991) Drugs 42:569.

PH46 Inhibition of morphine dependence by *Withania coagulans* in mice

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It is clear that repeated use of opioid drugs cause physical dependence and tolerance. Dependence can be measured by evocation of abstinence signed by abrupt drug withdrawal or administration of narcotic antagonist or both. Jumping is most suitable sign for measuring abstinence quantitatively as jumps are easily counted and jumping rate increases when dependence increases or dose of antagonist increased. Investigation of root extract of *Withania coagulans* revealed its beneficial effects to decrease dependence sign produced by morphine in mice. After induction of dependence by morphine, mice were divided in to 7 groups. Then, distilled water was injected to control group and specific concentrations of the extract were administered to the other groups. To assess morphine withdrawal, mice were given naloxone (5 mg/kg) on the day 4th after three consecutive days of morphine injection, intraperitoneally. Withdrawal syndrome was assessed by placing each mouse in a 30 cm high glass box and recording the incidence of escape jumps for 60 minutes. Data were analyzed by one-way ANOVA followed by Student-Newman-Keuls'test ($p < 0.05$). The results showed that animals received acute treatment with morphine displayed dependence. The animals treated with different concentrations of root extract of *Withania coagulans* could decrease or increase incidence of escape jumps in number following naloxone administration. The study showed that *Withania coagulans* can decrease development of morphine dependence.

Although, mechanism of action of this plant for inhibition or decrease of abstinence syndrome in dependent mice is unknown.

PH47 The protective role of *Sphenocentrum jollyanum* (Pierre) root ethanol extract on alloxan -induced diabetic rabbits

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The protective role of *Sphenocentrum jollyanum* (SJ) (Pierre) root used locally for diabetic treatment was assessed against alloxan diabetic rabbits. Fifteen rabbits randomly divided into three groups (5 in each), received oral treatment as follows: group I- root extract (100 mg/kg); group II- glibenclamide (10 mg/kg); group III- diabetic control. A week later (day 0), basal glycaemia was determined followed by alloxan challenge (170 mg/kg). Blood was collected at days 0, 3, 5, 7, 9, 11, 13, 15 and 17 and analyzed by glucose oxidase method. Oxidative activity was evaluated by C – reactive protein (CRP) analysis [1]; Superoxide dismutase (SOD), catalase and lipid peroxidation assays, as described by Rukkumani *et al*. [2]. Results showed that alloxan influenced slight glycemic increase in extract treated that peaked at day 3 (165.0 ± 6.7) of treatment followed by rapid decline to basal glycaemia (94.0 ± 1.5). The difference in value between the treated and the untreated was marked ($P < 0.01$). This implied that the diabetogenic activity of alloxan was effectively checked by the plant extract. The extract group showed slight increase in CRP concentration at day 3 (10.34 ± 1.0 mg/dl) but decreased appreciably at day 17 (7.6 ± 0.4 mg/dl). The activities of SOD and catalase were considerably higher in the extract treated compared to the diabetic group. However, decrease in lipid peroxidation occurred indicating that the plant inhibited oxidative damage. The photomicrograph of extract treatment showed no lesion while mild cellular injury occurred in glibenclamide specimen. Diabetic control showed shrunken mass of amorphous eosinophilia. The extract provided effective protection against alloxan diabetogenic activity with comparably higher protection than the control drug. *Acknowledgement: Omolere Aye Medical Herbalist Society, Oru, Ijebu, Ogun State. Nigeria. Chief (Dr.) Adeyemi Adebambo* **References:** 1. Eda S. et al. (1998) J. Clin. Lab. Anal. 12:137 – 144. 2. Rukkumani R. et al. (2004) J. Pharm. Sci. 7:274 – 283.

Topic I: Cosmetics, flavours and aromas

PI1 Chemical constituents and antibacterial activity against bacteria causing foot odor of three extraction methods from the pummelo peel oil

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The flavedo from fresh peels of Pummelo (*Citrus maxima* (Burm) Merr.) [1] were extracted by three different extraction methods; hydrodistillation [2], steam distillation and hexane extraction. The total yields from hydrodistillation, steam distillation and solvent extraction were 2.25, 1.83 and 1.47% w/w, respectively. Each oil samples were analyzed for chemical components by gas chromatograph and mass spectrometer (GC-MS). The highest content of monoterpene hydrocarbons i.e., limonene, phellandrene, α-pinene, were found in the extracts obtained from hydrodistillation (95.12%, 0.65%, and 0.61%, respectively). They were also investigated for their antimicrobial activities against bacterial causing foot odor, including *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), and *Staphylococcus epidermidis* (ATCC 12228) [3], using broth microdilution method [4]. The minimum inhibitory concentration (MIC) of the oil obtained from hydrodistillation with the most potency is 0.125, 0.125 and 0.03125% v/v for *Bacillus subtilis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, respectively. **References:** [1] Smitinand, T. (2001) Thai plant names. The forest research office. Bangkok. [2] Atti-Santos, A.C. et al. (2005) J. Braz. Arch. Biol. Techn. 48:155 – 160. [3] Katsutoshi, A. et al. (2006) Can. J. Microbiol. 52:357 – 364. [4] Ferraro, M.J. et al. (2000) Methods of dilution antimicrobial susceptibility test for bacteria that grow aerobically; approved standard-fifth. NCCLS. USA.

PI2 *In vitro* anti-inflammatory and antioxidant activities of stilbenoids of *Vanda coerulea* (Orchidaceae)

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In the framework of our investigations towards the isolation of biologically active constituents from Orchidaceae, we carried out biological screening of various Orchids. Among the metabolites isolated from *Vanda coerulea*, three stilbenoids corresponding to imbricatin, methoxycoelonin and gigantol, display protective antioxidant and anti-inflammatory properties via complementary mechanisms: free radical scavenging activity and inhibition of PGE-2 liberation on HaCAT irradiated cells with UV_B (60mJ/cm^2). Imbricatin, the major stilbenoid in *V. coerulea* stem extract, displays an excellent hydroxyl radical scavenging activity (IC_{50} 8.8 μM) on HaCAT cells model in a concentration-dependant manner evaluated by dichlorodihydrofluoresceine (DCFH) assay [1]. It also inhibits significantly PGE-2 liberation, implied in skin inflammation, induction of various matrix metalloproteinases or inhibition of collagen synthesis in fibroblasts [2]. Besides, this stilbenoid shows the best scavenging activities on 2,2-diphenyl-1-picrylhydrazyl, DPPH (IC_{50} 11.0 μM) and hydroxyl radicals *in tubo* with an IC_{50} of 0.11 μM, twice as lower as reference molecule quercetol.

	IC_{50} (μM)				
	In tubo			*In vitro* (HaCaT)	
compounds	**DPPH**	**$O_2^{\cdot-}$**	**$HO^{\cdot}$**	**$HO^{\cdot}$**	**PGE-2**
Imbricatin	11.0 ± 0.7	98.3 ± 16.8	0.11 ± 0.04	8.8 ± 1.7	12.2 ± 2.9
Methoxycoelonin	15.9 ± 1.9	722.7 ± 111.4	0.82 ± 0.17	10.2 ± 2.6	19.3 ± 3.8
Gigantol	Nd	Nd	1.7 ± 0.04	20.6 ± 0.5	Nd
Standard Quercetol	2.9 ± 0.4	6.2 ± 2.3	0.29 ± 0.01	13.8 ± 2.2	Nd
Standard Resveratrol	Nd	Nd	Nd	Nd*	6.6 ± 0.8

IC_{50}: half maximal Inhibitory Concentration Nd: not determined Nd*: resveratrol shows pro-oxidant properties in DCFH assay

References: [1] LeBel, C.P. et al. (1992) Chem. Res. Toxicol. 5:227 – 231. [2] Seo, J.Y. et al. (2003) Mech. Ageing Dev. 124:903 – 910.

PI3 Analysis of *Rhaponticum carthamoides* (Willd.) Iljin crude extracts composition and ability to simulate cell proliferation

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The presented study concerns the activity of leaves crude extracts, isolated from ecdysteroid-rich plant *Rhaponticum carthamoides* (Willd.) Iljin. Plant material, collected in Koriażma (Archangielsk district), was obtained through the courtesy of FITOSTAR™ company. Composition analysis of chloroform, methanol and water extracts was performed using GC-MS. The presence of plant sterols: campesterol, β-sitosterol and stigmasterol were previously observed in extract of *R. carthamoides* seeds [1], whereas α- and β-amyrin were detected in this plant for the first time. The ability of *R. carthamoides* crude extracts, α-amyrin, β-sitosterol and stigmasterol to stimulate the proliferation of human keratinocytes (HaCaT cell line) was analyzed. Keratinocytes were grown in the presence of analytes (10 – 40 μg/ml of pure compounds or 10 – 80 μg/ml of crude extracts). The ability to influence cell growth and prolifera-

tion was determined using methylene blue assay (after 24 and 48 h of incubation) and BrdU incorporation assay (after 24 h of incubation). Both sterols and α-amyrin enhanced the proliferation of HaCaT cells up to 30% after 48 hours of incubation (methylene blue assay) and up to 60% after 24 h incubation (BrdU incorporation assay). Cholesterol (10 – 40 μg/ml), serving as a control, stimulated cell proliferation in a dose response manner (up to 300% after 24 h; BrdU assay). Chloroform crude leaves extract inhibited cell growth and turned to be highly cytotoxic even in the lowest concentrations applied (10 μg/ml). Methanol extract exhibited mild cytotoxicity at 80 μg/ml, whereas water extract did not influence cell growth. According to our knowledge the ability of α-amyrin, β-sitosterol and stigmasterol to enhance human cells proliferation was shown for the first time. Obtained data indicate that the other component(s) of *R. carthamoides* can in certain conditions mask the biological activity of the sterols present in their leaves tissue. *Acknowledgements: this research was supported by the European Union within the European Social Fund in the framework of the project "InnoDoktorant – Scholarships for PhD students, I edition" and by the Polish Ministry of Research and Higher Education. Projects numbers: DS 8200 – 4-0085 – 9 and N302 3566 33.* **Reference:** [1] Stránsky, K. et al. (1998) Russ. J. Plant Physiol. 45:333 – 338.

PI4 Absolute configuration of α- and β-pinene in essential oils of two *Nigella* species

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Plants of the genus *Nigella*, belonging to the family Ranunculaceae, play an important role in folk medicine in India and Arabian countries [1]. This family is generally considered as a taxon not containing essential oils [2]. However, during the last years several papers were published on essential oils in seeds of four species of the genus *Nigella* [3 – 6]. Therefore, we have carried out analysis of *Nigella nigellastrum* seed essential oil by GC and GC-MS and compared the composition with a related species, i.e. *Nigella arvensis*. As the biological activity often depends on the absolute configuration, we have paid attention to this aspect, too. The *N. nigellastrum* oil consisted mainly of monoterpene hydrocarbons, namely α-pinene (43%) and β-pinene (46%). Enantioselective GC separation on a permethylated β-cyclodextrine column showed that both pinenes were present in a high enantiopurity: (-)-α-pinene, 90% e.e. and (-)-β-pinene, 96% e.e. Sesqui- and diterpene hydrocarbons formed about 3% of the total oil. The *N. arvensis* essential oil contains a substantial amount of the same monoterpenes (α-pinene, 6%, and β-pinene, 21% [6]). In this species, (-)-enantiomers of both pinenes also predominated, however, their enantiopurities were lower: (-)-α-pinene, 72% e.e. and (-)-β-pinene, 80% e.e. *Financial support by the Czech Science Foundation (#525/08/1179) is gratefully acknowledged.* **References:** [1] Liu, Y.M. et al. (2004) Chem. Pharm. Bull. 52:454 – 455. [2] Watson, L. et al. (1992) The families of flowering plants: descriptions, illustrations, identification, and information retrieval (available from: http://delta-intkey.com). [3] Moretti, A. et al. (2004) J. Essent. Oil. Res. 16:182 – 183. [4] Fico, G. et al. (2003) J. Essent. Oil. Res. 15:57 – 58. [5] Kokoska, L. et al. (2005) Flavour Fragr. J. 20:419 – 420. [6] Havlik, J. et al. (2006) Flavour Fragr. J. 21:713 – 717.

PI5 Composition of the essential oil from aerial parts of *Lepechinia meyenii*

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Lepechinia meyenii (Walp.) Epling (Lamiaceae), locally known as "pacha salvia" or "puna salvia", is a stoloniferous perennial herb which grows in high altitudes in Peru, Bolivia and Argentina. In the communities from the high Andes of Peru (Ayacucho), it is used to treat respiratory ailments, cough and bacterial infections. It is also used to flavour fresh pasteurized milk together with roasted sweet corn and as a source of natural dyes used to colour vicuña fiber and other fibers [1,2]. Ethanolic extracts from aerial parts of *L. meyenii* have shown antimicrobial and antioxidant activities [3,4]. Although several caffeic acid derivatives, carnosol, ursolic acid and diosmetin have been isolated and identified [5], the composition of its essential oil has not been previously investigated. In the present work, the essential oil from fresh leaves and flowering young stems of *L. meyenii* was obtained by hydrodistillation and subsequently analysed by GC-FID, GC-MS and ^{13}C NMR. The identification of the constituents was achieved from their GC retention indices (both, relative to alkanes and to fatty acid methyl esters) in two columns of different stationary phases (SPB-1 and Supelcowax® 10) and by comparison of their MS fragmentation patterns with those stored in our own database and with literature data. Major compounds were also identified by NMR. Sixty-three constituents, representing 99% of the total oil, were identified. It is characterised by a high content of monoterpenes (83.3%), 53.8% being hydrocarbons and 29.5% oxygenated. The major ones were found to be limonene (23.0%), trans-pinocarvyl acetate (12.7%) and β-pinene (12.6%). Among sesquiterpenes (14.7%), guaiol (7.4%) and bulnesol (2.1%) are the main components. **References:** [1] Carhuapoma, Y.M. (2002) Taxonomía de las plantas medicinales aromáticas nativas de la provincia de Huamanga y sus perspectivas económicas, UNSCH, Ayacucho. [2] Sotta, N. (2000) Plantas aromáticas y medicinales de la región de Arequipa, El Taller, Arequipa. [3] Rojas, R. et al. (2003) J. Ethnopharmacol. 88:199 – 204. [4] Lock, O. et al. (2005) Acta Horticulturae 675:103 – 106. [5] Castillo, R. et al. (2005) Rev. Soc. Quím. Perú 71:227 – 236.

PI6 Isolation and structural identification of stilbenoids from *Vanda coerulea* (Orchidaceae)

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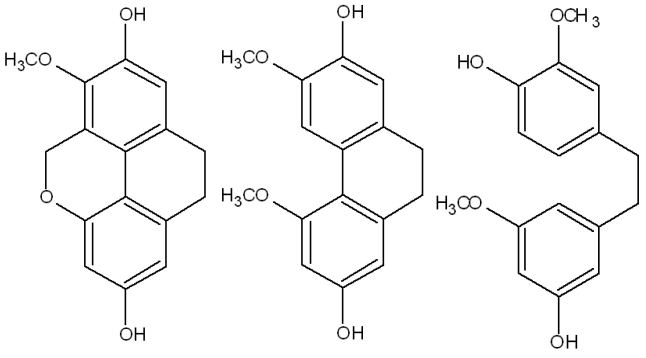
Vanda coerulea is an epiphytic orchid found in tropical areas such as Thailand. In the course of phytochemical studies on Orchidacae, we have identified phytosterols, terpenoids, carbohydrates and stilbenoids from *V. coerulea* stem extract. Among those stilbenoids, two dihydro-phenanthrens corresponding to imbricatin (**1**) and methoxycoelonin (**2**) and one dibenzyl compound corresponding to gigantol (**3**), were purified. A new efficient and more sensitive on line LC-SPE-NMR/MS coupling was used for their separation and characterization. The identification of these key orchid biomarkers was so established by means of spectral data analysis including1D and 2D NMR, MS, and UV. [1 – 3]. These metabolites were already described in some Orchidaceae such as *Agrostophyllum* and *Coelogyne* for **1-2**, considered as phytoalexins [4]; *Dendrobium* and *Cymbidium* for **3**. However this is the first time that they are described together in the genus *Vanda*.



References: [1] Majumder, P.L. et al. (1982) Ind. J. Chem. 21B:829 – 831. [2] Majumder, P.L. et al. (1995) Phytochemistry 39:649 – 653. [3] Juneja, R.K. et al. (1987) Phytochemistry 26:1123 – 1125. [4] Kovács, A. et al. (2008) Phytochemistry 69:1084 – 1110.

PI7 Inhibitory activity of nine essential oils on nitric oxide production by human leukocytes

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Nitric oxide (NO) plays a key role in the production of reactive nitrogen species (RNS), which have cytotoxic properties against pathogenic microbes and, at the same time, can damage host tissues [1]. Some essential oils have antioxidant properties and their consumption can influence immune cell functions [2,3]. In order to widen our knowledge of the antioxidant properties of essential oils we studied their effect on NO production induced by LPS in human blood leukocytes. NO was determined in 96-well microtiter plates by the Griess reaction [4]. N^G-methyl-L-arginine acetate (L-NMMA, IC_{50}= 38.2 ± 1.4 µg/ml) was used as positive control. The essential oils investigated were obtained from commercial sources: nutmeg (NM) (*Myristica fragans* Houtt), clove leaves (CL) (*Syzygium aromaticum* (L) Merr. et L.M. Perry), tarragon (TR) (*Artemisia dracunculus* L.), juniper berries (JB) (*Juniperus communis* L.), rosemary (RO) (*Rosmarinus officinalis* L.), lemon grass (LG) (*Cymbopogon martinii* Roxb. Wats.), lemon (LE) (*Citrus limon* (L.) Burman fil.), thyme (TH) (*Thymus zygis* (Loefl) L.) and Spanish oregano (SO) (*Thymbra capitata* Griseb.). In addition, nutmeg terpenes (NT, a fraction of nutmeg oil) and the pure compounds eugenol (EU), thymol (TM) and carvacrol (CR) were also tested. All the essential oils were chemically characterised by GC-FID and GC-MS. Results showed that CL (IC_{50}= 39.8 ± 6.3 µg/ml) and its major constituent, EU (IC_{50}= 19.0 ± 1.8 µg/ml), were the most active. JB, NM, NT, TR and SO had an IC_{50}> 50 µg/ml, and CR, the main component of SO had an IC_{50}= 39.3 ± 6.8 µg/ml. LE, LG, RO, TH and TM showed no measurable activity. The results confirmed the good antioxidant profile of CL [3].
Acknowledgements: Thanks are due to Lidervet S.L. (Tarragona, Spain) for the financial support. The work of R. Perez-Roses was supported by the Department of Education and Universities of the Generalitat de Catalunya and the European Social Fund.
References: [1] Martínez, M.C. et al. (2009) Antioxid. Redox Sign. 11:669 – 702. [2] Pérez-Roses, R. et al. (2007) Planta Med. 73:976. [3] Pérez-Roses, R. et al. (2008) 39th ISEO, Quedlingburg (Germany). [4] Green, L.C. et al. (1982) Anal. Biochem. 124:131 – 138.

PI8 Topical application of solubilized *Reseda luteola* extract inhibits ultraviolet B-induced inflammation in human volunteers *in vivo*

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The flavone luteolin displays numerous anti-inflammatory effects at micromolar concentrations which cannot be completely explained by its anti-oxidant capacities. In the present work we investigated a dry extract from *Reseda luteola* rich in flavones (40% w/w), especially luteolin, some of its glucosides, methylethers, and apigenin, obtained by a multistep extraction process using water and ethanol as solvents (drug to extract ratio 26 – 28:1). We investigated the skin tolerance and anti-inflammatory potential of a nanoparticular solubilisate of the *Reseda* extract (s-RE) in two independent studies *in vivo*. *Reseda luteola* extract was solubilized with polysorbate, resulting in product micelles with a diameter of 10 (± 1.5) nm. Standardized inflammation was induced by irradiating test areas on the back of healthy volunteers with defined doses of ultraviolet B (UVB). In the first study different concentrations of s-RE were tested in 10 volunteers to evaluate dose-dependency of anti-inflammatory effects of s-RE. In the second randomized, double-blind, placebo-controlled study a defined concentration of s-RE (2.5% w/w) was tested in 40 volunteers in comparison to the vehicle (glycerol) and hydrocortisone (1% w/w). s-RE dose-dependently inhibited UVB-induced erythema when applied 30 minutes before irradiation. Topical application of s-RE after irradiation also prevented UVB-induced erythema. S-RE was as effective as hydrocortisone, whereas the vehicle had no effect. Occlusive application of s-RE on non irradiated test sites did not cause any skin irritation. Due to excellent skin tolerance combined with potent anti-inflammatory properties s-RE bears potential especially for the prevention but also for the treatment of inflammatory skin conditions such as UV-induced erythema.

PI9 Essential oil composition of *Perilla* L. cultivated in Lithuania

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Perilla L. is a genus of annual herbaceous plants of Lamiaceae family, originated from Eastern Asia [1]. *Perilla* L. has been cultivated in the collection of medicinal plants at Kaunas Botanical Garden of Vytautas Magnus University in Lithuania since 1990. The investigations of plant growth, vegetation rhythmic and its dependence upon ecological factors, has been carried out since 1998 [2,3]. As biogenesis and composition of essential oil depend on geographical location, environmental factors, plants with same chemotypes but growing in different conditions have different composition of major components. The plant material (leaves) was collected from collection and trial area of Kaunas Botanical Garden of Vytautas Magnus University in August 2007. Essential oil of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, *Perilla ocymoides* L. var. *bicolorlaciniata* was obtained by hydrodistillation. Analysis was performed using GC-FID and GC-MS. Compounds were identified by comparing retention indices and mass spectra. Thirteen constituents of essential oil, representing 96.42% of identified compounds were identified in the essential oil of *Perilla frutescens* (L.) Britton. Principal compounds were perillaketone (55.60%) and egomaketone (28.12%). In *Perilla ocymoides* L. var. *bicolorlaciniata*, 97.70% of essential oil components were identified with abundant amounts of perillaldehyde (72.07%) and limonene (13.15%). In the essential oil of *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, 83.18% of compounds were identified. Principal compounds were perillaldehyde (49.47%), limonene (11.76%). Considering a broad diversity of *Perilla* L. species and chemotypes, it is important to discriminate cultivated *Perilla* L. species with identified chemotypes.
References: [1] Nitta, M. et al. (2003) Econ. Bot. 57:245 – 253. [2] Ragažinskienė, O. et al. (2006) Medicina (Kaunas) 42:667 – 672. [3] Ragažinskienė, O. et al. (2007) Ekologija. 53:45 – 50.

PI10 Essential oil components of turmeric leaves and rhizome cultivated in Iran

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Curcuma longa L. (Turmeric) belongs to Zingiber family. Turmeric is an important herb in all over the world as medicines, condiment, dye and cosmetic. The essential oil composition is known to be dependent on the plant part it is obtained from. The aim of this work was to compare the essential oils composition of leaves and rhizome of *Curcuma longa* cultivated in Isfahan, Iran. The essential oils from leaves and rhizome of *Curcuma longa* were obtained by steam distillation and analyzed by GC and GC-MS. The main components of the essential oil from the leaves were determined as α-phellandrene, carene, eucalyptol and the main components of rhizome essential oils were determined as α-tumerone, zingiberene, carene, and phellandrene, respectively. Remarkable differences between the two plant parts were detected in the mono and sesquiterpenoids pattern of essential oils compositions and the corresponding content. The rhizome showed a higher accumulation of sesquiterpenes, whereas the monoterpene contents were rather similar. The result indicates that the leaves of the *Curcuma longa* may present the flavour of turmeric rhizome. It seems that green turmeric leaves can be cured and dried for condiment used.

PI11 The antioxidant and free radical scavenging activities of peacock petal extracts

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The main objective of this study was to evaluate the anti-oxidant properties of the ethanolic extract of the petals of *Caesalpinia pulcherrima* (L.) Sw. (Caesalpiniaceae). The DPPH radical scavenging and ABTS cation radical scavenging assays were used to evaluate the anti-oxidant properties compared to the standards gallic acid and rutin. The results of the DPPH radical scavenging assay demonstrated the strongest activity of the crude extract of the red petals (IC_{50}= 34.74 μg/ml) followed by the extract of the orange petals (IC_{50}= 35.63 μg/ml) and the yellow petals (IC_{50}= 102.27 μg/ml), respectively compared with gallic acid and rutin (IC_{50}= 5.21 and 23.11 μg/ml). The ABTS cation radical scavenging assay demonstrated the strongest activity (IC_{50}= 227.66 μg/ml) for the orange petals followed by the red petals (IC_{50}= 243.01 μg/ml) and the yellow petals (IC_{50}= 338.72 μg/ml) compared with gallic acid and rutin (IC_{50}= 6.73 and 257.82 μg/ml). The phenolic compounds consisting of tannins and flavonoids in the red and orange petals of *C. pulcherrima* may be a good source of natural antioxidants which could be incorporated into a range of cosmetics and health products [1,2]. *Acknowledgements: Thailand Research Fund (TRF) through Industrial and Research Projects for Undergraduate Studies (IRPUS) 2550, University Mobility Asia Pacific (UMAP)* **References:** [1] Samee, W. et al. (2007) Thai Pharm. Health Sci. J. 2:131 – 137. [2] Srinivas, K.V.N.S. et al. (2003) Phytochemistry 63:789 – 793.

PI12 Identification of volatile constituents of beer using resin adsorption and chiral GCMS. Correlation with malt type and brewing method

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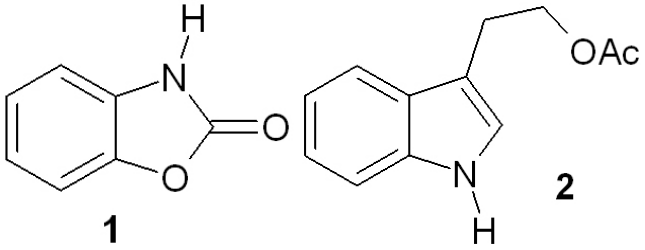
Beer is a widely consumed and widely studied alcoholic beverage. The beer aroma is influenced by several factors like the plant origin of the used malt (barley, wheat), the use of additional cereals (corn, wheat, barley, rice), the brewing methodology, the hop variety etc. A new methodology for the study of the volatile constituents of beer using adsorption resin was carried out for the first time. The same methodology had been successfully applied in grape distillates [1]. The isolation of volatile components was based on their adsorption on XAD4 resin. The beer sample without any treatment was passed through a column containing XAD4 resin and the adsorbed volatile components were desorbed using diethylether/pentane (1:1) which was then carefully evaporated. The residue was analyzed by GC-MS on a chiral β-Dex sm column. Twenty commercial samples belonging to the three major beer classes (lager, ale, lambic) were studied. In all cases phenylethanol (25 – 45%) and 3-methylbutanol (20 – 40%) were identified as the major constituents. Several differences were observed among the > 70 identified constituents related mainly with the malt type and the brewing method. Interestingly, 2(3 H)-benzoxazolone (**1**), a known phytoalexin of wheat, was found only in beers prepared using wheat malt. Additionally, tryptophol acetate (**2**) was for the first time identified as a beer constituent and it was found to be restricted to ale beers.



Reference: [1] Lelis, K. et al. (2008) Planta Med. 74:1186.

PI13 *In vitro* anti-acne inducing bacteria activity of mangosteen fruit rind extract gel

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The extract of fruit rind of mangosteen (*Garcinia mangostana* Linn.) was reported to possess a strong inhibitory effect against *Propionibacterium acnes* which is a major bacteria involves in acne development [1]. Therefore, it is interesting for developing anti-acne gel preparation from this plant. The ethanolic extract of mangosteen fruit rind was prepared using Soxhlet extraction. Hydro-alcohol gel preparation containing 1% w/w of the extract was formulated. The antibacterial activity of the extract and its preparation against *P. acnes* was evaluated using broth microdilution method [2]. Based on MIC and MBC values, the extract promoted a good inhibitory effect against *P. acnes* (MIC = 7.81 μg/ml, MBC = 15.63 μg/ml), while the 1%w/w mangosteen fruit rind gel showed similar anti-acne activity with standard 2.5% benzoyl peroxide anti-acne gel (Pan-Oxyl 2.5%gel) at MIC and MBC 1.56 mg/ml. The results showed that anti-acne gel with mangosteen fruit rind extract promoted good effect against acne inducing bacteria. The stability of this preparation is being investigated. *Acknowledgements: This study is a part of Ph.D. thesis of Mahidol University and was granted by the University Research Fund.* **References:** [1] Chomnawang, M.T. et al. (2005) J. Ethnopharmacol. 101:330 – 333. [2] NCCLS (2008) Performance Standards for Antimicrobial Susceptibility Testing. Wayne. PA.

PI14 Turmeric cream: Irritation test on human skin

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Turmeric (*Curcuma longa* Linn.) is a medicinal plant in Zingiberaceae family. The rhizome of this plant has been used in traditional medicines for treatment of carminatives, gastric ulcer, inflammation, and fungal and bacterial skin diseases. The main components promoting anti-inflammatory and antimicrobial activities are curcuminoids and essential oil, respectively. In this study, turmeric oil and crude curcuminoids were extracted from dried rhizomes of *C. longa* and incorporated in a cream base. Five formulations consisting of cream base, 0.026% crude curcuminoid cream (CC), 6% turmeric creams with and without curcuminoids (6TC+CC, 6TC) and 20% turmeric cream with curcuminoids (20TC+CC), were tested for human skin irritation. A 21-day cumulative irritation method [1] was used for irritation test in 22 volunteers (10 women aged 21 – 35 and 12 men aged 21 – 32). The irritation reaction of 20TC+CC, 6TC+CC and 6TC could be visually observed from day 7, 14 and 10 on, respectively while their IT50 [2] was found to be 14.5, 17.2 and 15.9 days, respectively. It was found that 6TC+CC and 6TC caused irritancy potential significantly ($p < 0.05$) lower than 20TC+CC while the cream base and CC showed very low irritation score (< 0.5). This study indicates 6% turmeric cream produced only mild irritation. Thus, turmeric cream should be of great benefit for dermatophytosis treatment. *Acknowledgements: This study was granted by Thai Traditional Medicine Development Foundation, Thailand.* **References:** [1] Dreher, F. et al. (1996) Skin Phar-

macol. 9:124 – 129. [2] Kligman, A.M., Wooding, W.M. (1967) J. Invest. Dermatol. 49:78 – 94.

PI15 Clinical evaluation of fitness cream "HOOTAN"

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Obesity is the major problem in developed and developing countries. There are a lot of ways to reduce fats in the body particularly in accumulated area which induce serious side effects. The aim of this study is to evaluate the effect of special formulated topical 2% cream based on glycyrrhetinic acid, the active principle of licorice roots extract, in reducing the fats of the skin. The extract and cream were standardized using HPLC method. The project was first approved by the university ethic board and carried out on double blind level .Twenty healthy volunteers girls aged 20 – 30 years with almost same level of life style were chosen, checked for blood pressure, triglycerides and cortisol levels before and after the study [1]. They were adviced to keep their overall food regime during the study and use 4 grams of the cream and the placebo twice daily which remains at least for half an hour, and for one month. Their right or left thighs were selected randomly for the cream and the placebo, respectively. The thighs circumference and the skin thickness were measured by one of the research team members who did not know the kind of the sample. The measurements were carried out on three points of the thighs before and after each week of the application using a strip ruler and special clipper with 0.1 cm sensitivity and averaged. Statistical t- student and ANOVA tests were applied to the results for conclusion. The results revealed that 18 of the volunteers (90%) showed a significant decreasing in their thigh circumferences compared to the placebo while the thickness of the skin showed higher significance differences for all volunteers. For most volunteers this reduction has no longer happened in the first two weeks. In addition the level of the blood triglycerides and cortisol did not raise compared to the beginning. Unless other parameters like feeding that interfere the results, it would be concluded that the cream could reduce the skin fat of the thigh and potentially to do so for other areas particularly for the thicker parts like belly. **Reference:** [1] Armini, D. et al. (2005) Steroids 70:538 – 542.

PI16 Topical therapy with the betulin based triterpene extract (TE) in patients with chronic pruritus

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The betulin (BE) based triterpene extract (TE) from birch cork contains 81% BE, betulinic acid (4%), lupeol (3%), erythrodiol (1%) and oleanolic acid (1%). The TE is able to stabilize a W/O emulsion (BE emulsion) of water and jojoba oil without any other additives [1]. Experimental studies suggest that the TE components induce anti-inflammatory [2] and wound healing effects [3] in the skin, but no antipruritic activity is published, yet. An open-labelled trial aimed to investigate the antipruritic effects of the BE emulsion. 23 patients with chronic pruritus on unchanged skin as well as 20 patients with pruritus and chronic scratch lesions received the BE emulsion. It was applied for a period of two weeks twice daily on the affected areas followed by 2 weeks without cream and a follow-up visit. Before and after therapy, patients received a detailed clinical investigation with documentation of present scratch lesions assessed by the prurigo-score. For daily documentation of pruritus intensity patients used the visual analogue scale (VAS) from 0 to 10. Statistical analysis was done by intention-to-treat analysis. A significant antipruritic effect was documented in 56.2% of patients of group 1 and 70.0% of patients of group 2. The dynamic score (reduction of pruritus intensity in percent) in responsive patients was 66.8% in group 1 and 82.7% in group 2. The analysis of the VAS data before and after therapy showed a 2.6-fold better response of group 2. Patients of group 2 showed a slight regression of scratch lesions within two weeks of cream application. Nearly all patients (95.3%) tolerated the therapy well. The present results suggest that the topical use of TE within a BE emulsion is an effective, adjuvant antipruritic treatment option with good compatibility in patients with chronic pruritus, especially in patients with chronic scratch lesions. **References:** [1] Daniels, R. (2008) Pharm. Ztg. 11:34 – 35. [2] Alakurtti, S. (2006) Eur. J. Pharm. Sci. 29:1 – 13. [3] Harish, B.G. (2008) Phytomedicine 15:763 – 767.

PI17 A new emulsifier-free w/o system based on a triterpene extract from the outer bark of birch

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Surfactants are critically seen due to their impairment of skin barrier function. Therefore surfactant-free emulsions become more and more important. Here we present a new w/o emulsion system based on a triterpene dry extract from the outer bark of birch (TE) that is supposed to be emulsifier-free. Beside the galenic properties the TE display various pharmacological activities also important for dermatology [1]. 80% of TE is betulin, a pentacyclic triterpene with a polar group (alcohol) on each side of the molecule, respectively [1]. Investigations on the surface tension were done using axisymmetric drop shape analysis (ADSA) method. To further clarify the stabilizing action of TE on emulsions, Raman microscopy was carried out and the solubility of the TE was determined in the Jojoba oil by gas-chromatography [1]. In comparison to classical emulsifiers, the TE reduces the interfacial tension between oil and water by only 5 mN/m. 2 – 6% (w/w) of TE are necessary to prepare a stable emulsion. Only 0.28% (w/w) of TE are soluble in Jojoba oil and less than < 0.0001% (w/w) are soluble in water [3]. Thus TE-particles are present in the lipid phase forming a Pickering emulsion [2]. Accordingly, Raman microscopy shows that the TE particles surround the water droplets and they additionally form a network like structure in the lipid phase. The surface of the water droplets is not completely covered in contrast to a classic Pickering emulsion showing long term stability. Presumably, the stability of the w/o emulsions is enhanced by the lipophilic gel phase which is formed by the TE. In conclusion the TE allows to formulate a plant based and long-term stable emulsifier-free w/o system without any further ingredients. **References:** [1] Laszczyk, M.N. et al. (2006) Planta Med. 72:1389 – 1395. [2] Stiller, S. et al. (2004) Colloid Surface A 232:261 – 267. [3] Jäger, S. et al. (2008) Molecules 13:3224 – 3235.

PI18 Bornyl acetate conversion enhancement by pervaporation in ionic liquid

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The case study selected in this work involves the esterification of (-)-borneol with acetic acid in order to produce (-)-bornyl acetate. This compound is a valuable chemical for industries fresheners; (-)-bornyl acetate is above all used as an intermediate for the production of camphor. A promising way to improve conversion consists in coupling the esterification reaction with a pervaporation process, able to selectively recover the reaction products in-situ [1]. This work is focused on the study of a catalyzed esterification reaction, taking place in the ionic liquid ([bmim] [BF_4]), while one of the reaction products (water) is removed by pervaporation. In-situ extraction of water from the reaction medium allowed shifting the reaction towards formation of the desired product. Conversion of the reactants during esterification was followed with and without integration of the pervaporation process, under exactly the same conditions (flow regime, volume of ionic liquid, concentration of reactants and catalyst, temperature), in order to determine the exact impact of pervaporation on the overall process performance. Due to the selective removal of water from the reaction medium by using an integrated reaction-pervaporation system it was possible to increase the

reaction conversion from 22% to 44%. *Acknowledgements: The financial support of The Czech Science Foundation for grant No. 104/08/0600 is gratefully acknowledged.* **Reference:** [1] Gubicza, L. et al. (2003) Green Chem. 5:236 – 238.

PI19 The whitening effect of Acai berry extract on the B16F10 melanoma cells

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The Acai berry (*Euterpe oleracea*) is a tropical fruit from the Amazon region [1]. It has long been consumed as part of the traditional Brazilian diet. The main component of Acai berry is anthocyanin, which has been reported to have antioxidant effects [2]. In this study, we investigated the whitening effects of the Acai berry on the B16F10 melanoma cell. The powder of freeze-dried Acai fruit was extracted by water at 60°C temperature. We examined melanin contents (IC_{50}= 99.96 μg/Ml) dose-dependently, and investigated as to whether ERK activation by water extract is related to MITF and tyrosinase down-regulation. The ERK pathway is involved in the melanogenic signalling cascade [3], and that ERK activation by water extract reduced melanin synthesis via MITF downer-regulation, tyrosinase levels also decreased. In conclusion, the Acai berry indicated that it could decrease the melanin content. Therefore, we expect the whitening effects of the Acai berry may be a possible candidate for further functional foods or cosmetic research. **References:** [1] Lichtenthaler, R. et al. (2005) Int. J. Food Sci. Nutr. 56:53 – 64. [2] Schauss, A.G. et al. (2006) J. Agric. Food Chem. 54:8604 – 8610. [3] Englaro, W. et al. (1998) J. Biol. Chem. 273:9966 – 9970.

PI20 Antimelanogenesis effect of *Vaccinium uliginosum* L. extract on B16F10 melanoma cell and UVB-irradiated C57BL/6 mice

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Melanogenesis is a physiologic process resulting in the synthesis of melanin pigments. Although melanin plays an important role in protection against UV [1], the overproduction of melanin can cause a large number of skin diseases, including hyperpigmentaion such as melasma, freckles, solar lentigo, etc. [2] *Vaccinium uliginosum* L. is one of the berries of *Vaccinium* genus in Ericaceae family. Its habitat includes Backdoo Mountain North Korea, Europe, and North America. Organic acids, vitamins, glycosides and anthocyanins are known as main components of this plant. However, few biological studies have been reported about it. To investigate the physiological new function of *Vaccinium uliginosum* L, the effects on melanogenesis were studied. Treatment with *Vaccinium uliginosum* L. extract for 72 h inhibited melanogenesis (IC 50:500 μg/ml) and tyrosinase activity (64% in highest dose, % of CTL) in B16F10 melanoma cells. However, *Vaccinium uliginosum* L. extract showed hardly inhibitory effect on mushroom tyrosinase. (93.5% in highest dose, % of CTL) Also, the present study was conducted to investigate antimelanogenesis effect of *Vaccinium uliginosum* L. extract on ultraviolet radiation B (UVB)-irradiated C57BL/6 mice. Histological examination revealed that the number of 3,4-dihydroxyphenylalanin (DOPA)-positive melanocytes increased by ultraviolet radiation B (UVB) irradiation was decreased by oral administration of *Vaccinium uliginosum* L. extract. These results suggest that *Vaccinium uliginosum* L. has an inhibitory effect on melanogenesis and its inhibitory effect was associated with indirect inhibitory effect of tyrosinase **References:** [1] Oetting, W.S. (2002) Pigm. Cell Res. 13:320 – 325. [2] Sugumaran, M. (2002) Pigm. Cell Res. 15:2 – 9.

PI21 Effect of essential oil from *Citrus aurantium* and its main compound limonene on quantity of PGE2 and mucus production in gastric mucosa

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The previous finding of an antiulcerogenic effect from essential oil of *Citrus aurantium* L. (Rutaceae) (OEC) and its main compound limonene (LIM) has provided continuity for research seeking to clarify their antiulcerogenic action mechanisms. Models for gastric ulcer induction by non-steroidal antiinflammatory drugs (DAINE) [1], for the quantification of gastric mucus [2] and the quantification of prostaglandin PGE2 in the gastric mucosa have been established [3]. The dose of LIM (245 mg/kg) used in the experiments was calculated based on both the amount of the compound in the OEC (97%) and on determination of the most effective OEC dose (250 mg/kg). In models of gastric ulcers induced by DAINE, the OEC and LIM were effective in gastric protection, both showing 99% protection ($p < 0.05$) compared to control animals. In the PGE2 quantification model, even with the joint administration of DAINE (Indomethacin 30 mg/kg sc), a PGE2 inhibitor, the OEC and LIM were able to maintain high PGE2 levels similar to control groups, without changing basal PGE2 levels ($p > 0.05$). The same did not occur in groups that were treated with vehicle and DAINE, since DAINE provoked a 60.2% drop in PGE2 levels in the gastric mucosa of these animals ($p < 0.05$) in relation to control group. Groups treated with OEC and LIM presented significantly augmented gastric mucus (mg/g tissue) secreted in the stomach: vehicle 1.8 ± 0.17, OEC 3.0 ± 0.26 ** and LIM 2.7 ± 0.23 * ($p < 0.05$). The data show that OEC and LIM modulated the amount of PGE2 in the gastric mucosa without inducing indomethacin-level reductions, which justifies the antiulcerogenic results and observed rise in gastric mucus production. **References:** [1] Puscas, I.A. (1997) Arznei-Forschung. 47: 568 – 572. [2] Rafatullah, S. (1990) J. Ethnopharmacol. 29:25 – 34. [3] Curtis, G.H. (1995) Can. J. Physiol. Pharmacol. 73:130 – 134.

PI22 Researches regarding in vivo skin imagistic dermatologic evaluation of evening primrose oil (*Oenothera biennis* L.)

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Evening primrose (*Oenothera biennis* L.) oil is known as a cosmetic ingredient, having moisturizing and nutritional properties in skin, because of its content in essentials fatty acids, which involve skin ceramide synthesis. At present, EPO is one of the most important sources of γ-linolenic acid (GLA; 18:3ω6), which is in growing demand for its clinical and pharmaceutical applications [3]. In this paper we present a study on the cosmetic proprieties of the evening primrose oil (*Oenothera biennis* L.) using an imagistic evaluation with Proderm Analyser on human volunteers. The evening primrose oil (*Oenothera biennis* L.) was extracted from seeds using the Soxhlet method and hexane as solvent. It was established that the oil content was between 20 and 25% and the distribution of fatty acids using HPLC method [1 – 2]. Evening Primrose oil was applied to dry skin and the imagistic skin evaluation was pointed to the following parameters: skin texture and wrinkles involution, both after 28 days of daily treatment. The clinical study was realised on 24 volunteers, ages between 33 – 59 years, by applied the Evening Primrose Oil one time/day, for each volunteer, on a photoaged skin area. The imagistic evaluation performed with Proderm Analyser was performed after 14 days, 21 days, and respectively 28 days. An improvement of skin texture consisted in 20% photoaged wrinkles involution was registered after 28 days of daily treatment. **References:** [1] Czauderna, M., Kowalczyk, J. (2002) Chem Anal-Warsaw 47:867 – 882. [2] Czauderna, M., Kowalczyk, J. (2001) J. Chromatogr. B 760:165 – 178. [3] Senanayake, S.P.J.N. and Shahidi, F. (2004) Food Chem. 85:489 – 496.

PI23 Leaves extract of a Lamiaceae genus *Lepechinia*: screening issue and development of a new active cosmetic ingredient

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Apoptosis is a physiological process leading to a programmed cell death [1]. The survivin protein, an inhibitor of apoptosis (IAP), is not expressed in differentiated tissues except the skin, especially by keratinocytes of the basal layer [2], so survivin is involved in cutaneous aging by its implication in cellular mecanism (apoptosis of keratinocytes). Screening of hundred plant extracts resulted in the selection of leaves extract of a *Lamiaceae* as the best activator of survivin (ELISA measurement in ker-

atinocytes lysates after 16 h of treatment with extract at 3.125 µg/mL). The impact of the extraction process was studied in a second time (biological activity and organoleptical caracteristics): discoloration, type of solvent, temperature, and fractionation study. Even if active carbon did not affect the biological activity, we chose to test different extraction solvents on the plant material, to reduce the cost and number of steps. We have demonstrated that the active compounds are extracted by the alcoholic or hydroalcoholic solvents with intermediate polarity (examples: 37% overexpression of survivin rate with ethanol/water (50/50 v/v) extract, or +15% with butanol extract). This study allowed us to make the choice of the best solvent of extraction according to criteria of technical and economic practicability. More, in a worry of security and respect for environment, we turned to green techniques. **References:** [1] Bowen, A.R et al. (2003) J. Investig. Dermatol. 120:48 – 55. [2] Deveraux, Q.L. et al. (1999) Genes Dev. 13:239 – 252.

PI24 Free radical scavenging and antityrosinase activities of guava leaf extract

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The aim of this study was to evaluate free radical scavenging and antityrosinase activities of the ethanolic extract of guava leaf (*Psidium guajava* Linn.). Anti-free radical and antityrosinase activities were used as the outcome of the antioxidant and whitening properties. The percentage of free radical inhibition was evaluated by DPPH assay compared to the standard gallic acid. The percentage of tyrosinase inhibition was examined by Dopachrome method compared to the standard arbutin. The results of free radical scavenging activity of the guava leaf extract was found to be IC_{50}= 53.19 µg/ml compared with gallic acid (IC_{50}= 5.21 µg/ml). The antityrosinase activity was shown to be IC_{50}= 9.86 mg/ml compared with arbutin (IC_{50}= 7.3 mg/ml). Gallic acid, one of the phenolic compounds in the ethanolic extract of guava leaf, was measured by HPLC technique to be 3.07 µg/ml. The guava leaf may be a potential source of natural antioxidant and whitening agents which could be incorporated into a range of cosmetics and health products [1 – 3]. *Acknowledgements: Thailand Research Fund (TRF) through Industrial and Research Projects for Undergraduate Studies (IRPUS) 2550, Chophaya Abhaibhubejhr Hospital Foundation.* **References:** [1] Tachakittirungrod, S. et al. (2007) Food Chem. 103:381 – 388. [2] Lim, Y.Y. et al. (2007) Food Chem. 103:1003 – 1008. [3] Yan, L.Y. et al. (2006) Sunway Acad. J. 3:9 – 20.

PI25 Saffron (*Crocus sativus*) decoction induces intra abdominal fat deposition in pregnant mice

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Objectives: In this study the effects of non toxic [1] concentrations of saffron decoction on fat deposition in pregnant mice was assessed. **Methods:** 130 adult female mice were mated overnight and checked daily for vaginal plaque until yielding 65 pregnant mice. The vaginal plaque observation was considered as the1st day of pregnancy. The reminder mated females were considered as non pregnant. Each category of pregnant and non pregnant animals was randomly and equally divided into 13 groups. In each category animals in control group received tap water while the animals in test groups received different concentration of saffron decoction (0.2, 0.4 and 0.8%) in 4 different time course during 3 weeks of gestational period (1st, 2nd, 3 rd week and complete duration of pregnancy) *ad libitum*. 18 days after the onset of the experiments animals were sacrificed by deep anesthesia and striping fats deposited in the abdominal cavity from diaphragm to the genitalia were precisely removed and weighted. Result: there was no significant difference between the fat deposition in pregnant and non pregnant animals but the intra abdominal fat deposition in all test groups was significantly reduced in both pregnant and non pregnant animals as compared with their own control group ($p < 0.05$).The effect of saffron on intra abdominal fat deposition was dose and time dependant and maximum effect was seen in animals receiving 0.8% saffron decoction throughout the gestational period. Conclusion: according to our data, saffron is a potent attenuator of fat deposition probably due to its action on food intake and its antioxidative effect [1,2]. **References:** [1] Abdullaev, F.I., Espinosa-Aguirre, J.J. (2004) Cancer Detect. Prev. 28:426 – 432. [2] Abdullaev, F.I. (1993) Biofactors 4:83 – 86. [3] Rios, J.L. et al. (1996) Phytother. Res. 10:189 – 193.

PI26 Evaluating the effect of saffron (*Crocus sativus*) on prevention and treatment of Alzheimer's disease in mice by the "one way active avoidance learning and memory" tests

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This study was conducted to examine the effects of saffron hydro-alcoholic extract on learning and memory function in mice model of Alzheimer's disease (AD). In this study 20 ovariectomized mice were randomly and equally divided into 4 following groups: healthy control (Distilled water), AD control (D-galactose and NaNO2 solution [1]), AD prevention (saffron extract with D-galactose and $NaNO_2$ solution), and AD treatment (saffron extract 15 consecutive days following AD induction). All injections were administered intraperitoneally (IP) for 60 consecutive days. The cognitive functions were examined using " one way active avoidance learning and memory" test in a shuttle box apparatus [2] and the mean number of shock free trials (M.Sh.F.T.) was considered as an index for learning (1 day after the treatments), short term memory (one week after training) and long term memory (one month after training). M.Sh.F.T. for healthy control, AD control, AD prevention and AD treatment groups was 13.93 ± 0.8667, 1.933 ± 1.235, 9.000 ± 2.200, 17.47 ± 3.459, respectively in learning, 18.47 ± 1.733, 1.933 ± 0.5207, 11.93 ± 1.733, 20.00 ± 0.5292, respectively in short term memory and 19.07 ± 2.210, 3.667 ± 0.9684, 13.53 ± 0.5207, 27.80 ± 0.1155, respectively in long term memory. In all 3 sets of experiments (learning, short and long term memory), M.Sh.F.T. in AD control group was significantly less than all other groups ($p < 0.05$), but there was no significant difference between AD treatment and healthy control groups ($p > 0.05$). Our findings indicate that saffron hydro-alcoholic extract prevents the induction of AD and improves the learning ability and memory recall in AD mice model. **References:** [1] Hua, X. et al. (2007) Life Sci. 80:1897 – 1905. [2] Das, A. et al. (2003) Indian J. Pharmacol. 35:47 – 50.

PI27 Antioxidant and whitening effects of *Lindera obtusiloba* BL. 70% EtOH extract

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Lindera obtusiloba BL. is known to contain various bioactive constituents such as geranyl acetate, L-phellandrene, linderic acid and tsudzuic acid [1], it have antioxidative activities and liver protection. This study investigated the effects of reactive oxygen species scavenging activities and melanogenesis inhibition of leaf and branch extracts from *Lindera obtusiloba* BL. It had scavenging activities on DPPH radical, superoxide anion radicals, and hydroxyl radical dose-dependently. Also, leaf extract inhibited tyrosinase activity and melanogenesis in B16 melanoma F10 cells. ERK pathway activation is related to MITF and tyrosinase down-regulation The ERK pathway is involved in the melanogenic signaling cascade [2], and that ERK activation by leaf extract reduced melanin synthesis via MITF down-regulation, also tyrosinase level decreased. From the above results, it is possible that *Lindera obtusiloba* BL. may be developed to be the health functional food and functional cosmetics that have anti-melanogenesis. **References:** [1] Elwood, J. M., Jopson, J. (1997) Int. J. Cancer. 73:198 – 203. [2] Englaro, W. et al. (1998) J. Biol. Chem. 273:9966 – 9970.

PI28 The abortificant effects of different doses of saffron (*Crocus sativus*) decoction in mice

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The aim of this study was to assess the evidences for the effects of saffron consumption on abortion and congenital disorders [1 – 3]. 65 female BALB/c mice, weighting 25 – 30 grams, were breed in animal house of medical college. The first day of pregnancy was the day on which the vaginal plaque was observed. The pregnant mice were divided into13 subgroups. Each pregnant animal was placed in a separate cages throughout the gestational period and were fed in the same conditions. Animals in control group received tape water but the test groups received different concentration (0.8, 0.4&0.2%) of aqueous saffron decoction in whole or only in1st, 2nd or 3 rd trimesters of gestational period. In 18th day of pregnancy, animals were anesthetized and their fetuses were extracted through a cesarean section. The placenta was excised, weighed, and the number and placement of implantation sites, Live, dead and resorbed fetuses were recorded. All fetuses were stereo microscopically examined for any morphological abnormalities. According to our findings the mean number of live fetuses in animals receiving 0.8% saffron solution and mostly those who were received the decoction on 2nd trimester or whole gestational period were significantly less than control group while the mean numbers of resorbed and dead fetuses in test groups were dose dependently greater than control group ($p < 0.05$). Maximum number of dead fetuses was for animals receiving saffron solution on 2nd trimester. Some developmental abnormalities were observed only in animals using solutions in whole gestational period. Saffron's components especially in high doses and in 2nd gestational trimester affect embryonic implantation, organogenesis and may lead to abortion. **References:** [1] Abdullaev, F.I., Espinosa-Aguirre, J.J. (2004). Cancer Detection and Prevention 28:426 – 432 [2] Abdullaev, F.I. (1993) Biofactors 4:83 – 86. [3] Rios, J.L. et al. (1996) Phytother. Res 10:189 – 193.

PI29 The effects of different concentrations of saffron (*Crocus sativus*) decoction on preterm delivery in mice

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It has been suggested that saffron increases the myometrial contractions and may lead to the abortion1. The present study was conducted to assess the effects of non toxic concentrations of aqueous saffron decoction on preterm delivery in mice. In this study 65 female BALB/c mice, weighting 25 – 30 grams, were breed in the animal house of medical college by keeping the ratio of male: females as 1:3, in each plastic cage. The females were checked daily for vaginal plaque and the day on which the vaginal plaque was observed, was considered as the first day of pregnancy. The pregnant rats were divided into 7 subgroups and placed in separate cages throughout the gestational period and were fed in the same conditions. Animals in control group received tape water but the test groups received different concentrations (0.8%, 0.4% & 0.2%) of aqueous saffron decoction in whole gestational period or only in 1st, 2nd or 3 rd trimesters of gestational period. All animals had natural childbirth. Parturitions in 19th, 20th and 21th days of pregnancy was considered as normal delivery but deliveries before 19th day, were considered as preterm delivery. According to our findings the mean number of preterm delivery in animals receiving saffron decoction was dose dependently more than control group. Maximum preterm deliveries were observed in animals receiving different concentrations of aqueous saffron decoction on 3 rd trimester or whole gestational period especially for 0.4% decoction. Data collections in this study indicate that saffron consumption especially in last trimester of gestation can lead to preterm delivery. Preterm delivery may be due to increase in uterine contractions. References: 1. Sadraei, H. et al., Int. J. Aromatherapy 13:121 – 127.

PI30 Chemical composition and antimicrobial activity of essential oils from *Tanacetum parthenium* and *T. polycephalum* from Iran

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The Asteraceae is the largest plant family [1]. The genus *Tanacetum*, which is an important part of the Asteraceae family, consists of about 150 – 200 species [2]. These species have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically-active compounds [3, 4]. The essential oils of air-dried *Tanacetum parthenium* and *T. polycephalum* obtained by hydrodistillation were analyzed by gas chromatography-mass spectrometry (GC-MS). Seventy and twenty five components, respectively from the two plants, were identified in the essential oils. The main components of these species were isobornyl acetate and piperitone in the ratios of 15.3% and 20% from *T. parthenium* and *T. polycephalum*, respectively. The other major components of *T. parthenium* oil were camphene (11.12%), camphor (16.75%), bornyl isovalerate (5.98%), 10-epi-γ-eudesmol (2.45%), borneol (11.84%), isobornyl acetate (1.96%), and juniper camphor (5.71%). Camphor (35.11%), 1,8-cineol (9.4%) and borneol (15.4%) were found to be predominant constituents in the oil of *T. polycephalum*. Our data are different from previously reported ones and the studied specimens should be considered as chemotypes. The antimicrobial activities of the isolated essential oils of the plants was also investigated against four Gram-positive and four Gram-negative bacteria. They showed moderate to strong antibacterial activity against studied bacteria. The highest zones of inhibition were exhibited by the oil of *T. polycephalum*, ranging from 15 mm against *Citrobacter amalonaficus* to 26 mm for *Bacillus cereus*. Inhibition zones of *T. parthenium* varied from 9 mm against *Staphylococus subtilis* to 22 mm against *Bacillus megaterium.* The minimum inhibitory concentration (MIC) of *T. polycephalum* essential oil was close to that of streptomycin, while *T. parthenium* essential oil was less active. **References:** [1] Bremer, K. (1993) Asteraceae. Cladistics and classification. Portland: Timber Press. [2] Changqing, W. et al. (2006) Food Chem. 96:220 – 227. [3] Shinde, P.D. et al. (2007)J. Chromatogr. A 1138:184 – 189. [4] Tabanca, N. et al. (2007)J. Pharmaceut. Biomed. 45:714 – 719.

PI31 Polyphenol content of aqueous preparations of three chemotypes of *Lippia alba* Mill N. E. Brown (Verbenaceae) by HPLC/DAD/ESI-MS

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A number of *Lippia* species (Verbenaceae) [1,2] are used for food preparations and largely employed in folk medicines. In continuing our studies on *Lippia* genus, we now report on the polyphenol content of three different chemotypes of *Lippia alba* Mill N. E. Brown. *Lippia* species are characterised by the presence of phenylpropanoids, namely verbascoside and correlated molecules and flavonoids [3,4]. Recently, three chemotypes of *L. alba* have been classified according to the diffrent percentages of citral (chemotype I), carvone (chemotype II) and linalool (chemotype III) in the essential oil [5]. A rapid and efficient HPLC-DAD-MS assay was optimized and validated for the aqueous preparations of three chemotypes of *Lippia alba*. The analytical method attended a satisfatory accuracy, specificity and reproducibility. Furthermore, a good separation of the different classes of constituents, iridoids, flavonoids and phenylpropanoids was performed. The aqueos preparations were lyophilised and submitted to the HPLC analysis s such. All infusions had a lower content of polyphenols whencompared with the corresponding decoctions. The highest concentrations of total flavonoids were found in the decoctions of the leaves of chemotypes II (250 mg/g) and III (235 mg/g), while chemotype I showed a content of about 12 mg/g. Total phenylpropanoids of chemotypes I and III were similar (about 135 mg/g), while the content of phenylpropanoids in chemotype II was 180 mg/g. Chemotypes II and III of *Lippia alba* represent a good source of antioxidants, and decocting could be a simple and efficient extraction method of polyphenols. *Acknowledgements: This study was supported in*

part by the Programme Alβan, the European Union Programme of High Level Scholarships for Latin America, (scholarship n°:E06M104124BR to P.T.). The authors would like to thank Professor Lyderson F. Viccini, from Laboratory of Genetic, University of Juiz de Fora (MG, Brazil) for providing the plant material. **References:** [1] Bilia, A.R. et al. (2008) J. Pharm. Biom. Anal. 46:463 – 470. [2] Timóteo, P. et al. (2008) Nat. Prod. Commun. 3:2017 – 2020. [3] Pascual, M.E. et al. (2001)J. Ethnopharmacol. 76:201 – 214. [4] Valentão, P. et al. (2002) Biol. Pharm. Bull. 25:1324 – 1327. [5] Matos, F.J.A. et al. (1996) Brazilian J. Pharmacognosy 77:137 – 141.

PI32 Essential oils of *Dennettia tripetala* Bak. f. stem bark and leaf. Constituents and biological activities

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Dennettia tripetala Bak. f. (Annonaceae) is a rain forest tree esteemed for its fruits and young leaves which are chewed on account of their pungent spicy taste [1]. Previous workers have documented essential oil constituents of the fruits [2], while Adeoti et al. reported leaf oil constituents from Benin [3]. We therefore analysed constituents of the stem-bark and leaf oil of the Nigeria-grown plant by combined GC and GC-MS, and also evaluated them for antimicrobial and anti-trichomonal activities, and protective effect against UVC-induced peroxidation. Both oils showed distinct chemical composition, in that leaf oil comprised seven components while stem bark oil had thirty. In both cases, 2-phenyl nitroethane was the preponderant component (over 70%), in addition to linalool (17.8%). *Staphylococcus aureus* was the only susceptible microorganism to both oils, and stem bark oil showed better antimicrobial activity (MIC, 62.50 mg/ml). Both oils also showed moderate protective effect against UV radiation-induced damage in biomembranes, with the stem bark oil being more active. Anti-trichomonal activity of leaf oil was comparable to that of metronidazole. 1. Burkill, H.M. (1985) The Useful Plants of West Tropical Africa (Families A-D) Royal Botanic Gardens, London. 2. Osisiogu, I.U.W. et al. (1975) Planta Med. 27:287 – 289. 3. Adeoti, S.B. et al. (2000)J. Essent. Oil Res. 12:412 – 414.

PI33 Microwave-assisted hydrodistillation of essential oil from cherry laurel (*Prunus laurocerasus*) leaves

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Microwave-assisted hydrodistillation, a combination of microwave heating and hydrodistillation, was engaged to isolate essential oil from cherry laurel (*Prunus laurocerasus*) fresh leaves. Extraction experiments were carried out at atmospheric pressure in an electrically and mechanically modified microwave oven at different levels of power (150, 300, 450 W) and were continued until no more essential oil was obtained. The essential oil composition was determined by GC-MS. Each of the experiments were carried out in three replicate, and results were expressed as mean ± standard deviation. The optimum time was approximately 10 min for hydrodistillation under 450 W and 300 W, or 20 min under 150 W microwave power, ensuring nearly the maximum yield of oil, which was 0.38 ± 0.005 mL/100 g fresh leaves. When 450 W microwave power was used in microwave-assisted hydrodistillation, time was reduced twice or thrice compared with the processes under 300 W and 150 W, respectively. All the oils were rich in benzaldehyde (about 90%) a component of interest to the perfume and dyes industries [1 – 3] and also known as artificial almond oil [4] although 2-hexenal, benzoic acid and mandelonitrile were also present. Composition of the oils was similar to each other and depended on the microwave power input. The content of benzaldehyde was increased, while the content of benzoic acid and mandelonitrile were reduced by increasing the microwave power from 150 to 450 W. *Acknowledgements: Ministry of Science and Environmental Protection, Republic of Serbia project 142073b.* **References:** [1] MacEwen, E.G. (1986) Am. J. Vet. Res. 47:451 – 452. [2] Kochi, M. et al. (1980) Cancer Treat. Rep. 64:21 – 23. [3] Kochi, M. (1985) Cancer Treat. Rep. 69:533 – 537. [4] Burdock, G.A. (1996) Encyclopedia of Food and Color Additives. CRC Press. Boca Raton.

PI34 Hydrodistillation of essential oil from cherry laurel (*Prunus laurocerasus*) leaves: kinetics and chemical composition

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The essential oil was obtained from cherry laurel (*Prunus laurocerasus*) fresh leaves by hydrodistillation in a Clevenger-type apparatus. The resulting essential oil was dried over anhydrous sodium sulphate, filtered and stored at -4 °C in a well-filled, airtight container, protected from light, until GC-MS analysis. Waste liquid residue (hydrosol) was tested at total phenol and flavonoid content by Folin-Ciocalteu [1] and aluminium chloride colorimetric method [2], respectively. The optimum time for hydrodistillation process was approximately 40 min, ensuring nearly the maximum yield of oil, which was 0.39 ± 0.005 mL/100 g fresh leaves. To simulate the variation of the essential oil with time during the hydrodistillation process, the two-parameter kinetic model based on unsteady-state essential oil diffusion through plant material was used, where it was found that the increase in essential oil yield during hydrodistillation was in good agreement with a logaritmic kinetic equation. The essential oil analysis was performed by GC-MS and the main compound identified was benzylaldehyde (82.08%), which commercially is used in dyes, perfumes and flavourings and has also been tested for anticancer activity in humans and animals [3 – 5]. Minor constituents were 2-hexenal (0.63%), benzoic acid (1.12%) and mandelonitrile (15.40%). Total phenolics and flavonoids content were 4.9 ± 0.08 mg gallic acid equivalents (GAE)/ml hydrosol and 0.9 ± 0.01 mg rutin equivalents (RE)/ml hydrosol, respectively. *Acknowledgements: Ministry of Science and Environmental Protection, Republic of Serbia project 142073b.* **Reference:** [1] Singleton, V.L., Rossi, J.A. (1965) Am. J. Enol. Viticult. 16:144 – 158. 2. Chang, C. et al. (2002)J. Food Drug Anal. 10:178 – 182. 3. MacEwen, E.G. (1986) Am. J. Vet. Res. 47:451 – 452. 4. Kochi, M. et al. (1980) Cancer Treat. Rep. 64:21 – 23. 5. Kochi, M. (1985) Cancer Treat. Rep. 69:533 – 537.

PI35 The GC/MS characterization of the volatile oil from fresh leaves of *Alchornea cordifolia* leaves

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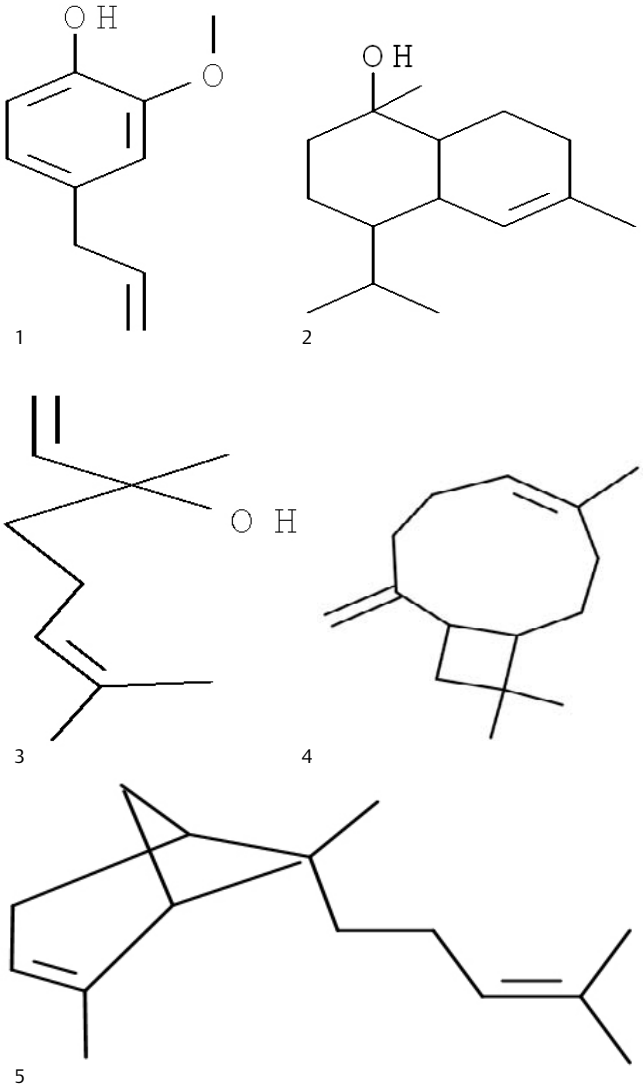
Alchornea cordifolia leaves have been the subject of many investigations [1,2]. This plant is used traditionally for the management of pain, arthritis and other inflammatory conditions. In this investigation, fresh leaves of *Alchornea cordifolia* were collected and the essential oil extracted by Clevenger apparatus. The chemical constituents of the extracted oil were analysed using GC/MS. Identification of the components of the oil were based on comparison of the retention times and computer matching of MS fragments with the NISTOL2. L library. The yield of the oil was 0.13% and 25 substances consisting 90.3% of the composition of the essential oil were identified. The major components were eugenol (**1**) (41.7%), cadinol (**2**) (2.46%), linalool (**3**) (30.6%), caryophyllene (**4**) (1.04%) and (E)-α-bergamotene (**5**) (4.54%). Some of these components have established anti-inflammatory activities and may contribute to the anti-inflammatory effect of *A. cordifolia* leaves previously reported [1,2].



Acknowledgements: Dr. Umaru FZ, Department of Pure and Applied Chemistry Usmanu Dan Fodiyo University Sokoto, Nigeria. **References:** [1] Osadebe, P.O. et al. (2008) Rec. Prog. Med. Plant Res. 22:571 – 577. [2] Marva-Manger, H. et al. (2008) J. Ethnopharmacol. 115:25 – 29.

PI36 *In vitro* evaluation of antioxidant, antibacterial, anti-tyrosinase activities of *Zanthoxylum planispinum*

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The methanolic extracts of *Zanthoxylum planispinum* leaves from south Korea were screened for their antioxidant properties, tyrosinase inhibition and antibacterial activities to apply as a functional ingredient for cosmetic products. The level of total phenolics and flavonoids of the leaves extracts were determined by UV/VIS spectroscopy. The extract of *Zanthoxylum planispinum* leaves had good phenolic (391 mg/g) and flavonoid (187 mg/g) contents and showed strongest activity of antioxidant-related enzymes including SOD, CAT, and APX. IC_{50} value of the extracts against mushroom tyrosinase was 5.3 μg/ml which is about 5 times more effective than arbutin and kojic acid. RS_{50} value of the DPPH free radical scavenging activity of the extracts was 2.7 μg/ml which is about 3 times more effect than BHT and α-tocoperol. The extracts also showed the best antimicrobial activity from gram-negative bacteria to gram-positive bacteria with MID value as low as 20 μg/disc. **References:** [1] Dewck, A.C. (2009) Clin. Dermatol. 27:148 – 158. [2] Kivrak, I. et. al. (2009) Food Chem. 116:470 – 479. [3] Larson, R.A. (1988) Phytochemistry 27:969 – 978.

PI37 Screening of the essential oil composition of Sicilian officinal plants

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The qualitative and quantitative composition of the essential oils obtained from wild Sicilian officinal plants has been investigated. The aim of this study is to promote the cultivation and the possible exploitation of the aromatic plants, which have for long time been considered a poor source of profit. Due to growing market demand and their low cost of cultivation, aromatic plants are being revaluated for their commercial exploitation [1]. The main goal of this study is thus to obtain extensive information on the wild species, in order to achieve the best methods for controlled cultivation and give a standardized product. This will avoid the indiscriminate harvesting of wild material which, among the other negative aspects, does not guarantee qualitative homogeneity in the long run. Oregano, rosemary, thyme, sage and fennel from the whole of Sicily were sampled. Altogether, 175 samples were collected during the years 2006 – 2007, hydrodistilled and analyzed by GC-MS in order to obtain aromatic profiles. More than 95% of the total composition for each sample was clarified and all data were statistically analyzed in order to determine the relationship between the different samples using the percentage composition of their essential oils. Euclidean distance was selected as a measure of similarity. A broad picture of Sicilian wild officinal plant composition emerges from this study. The characteristics and the phytochemical richness, reported here, represent a patrimony to be protected and, at the same time, exploited with the aims previously described. **Reference:** [1] Dordas, C. (2009) Ind. Crop. Prod. 29:599 – 608.

PI38 Chemical composition, antimicrobial and antioxidant activities of the essential oil of Guizotia scabra and Microglossa pyrifolia from Rwanda

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Guizotia scabra (Vis.) Chiov. and *Microglossa pyrifolia* (Lam.) Kuntze, Asteraceae, are collected and used for infection diseases in Rwanda [1]. Their essential oils obtained by hydrodistillation of leaves collected from Butare (Huye), Southern province of Rwanda, were simultaneously analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) [2,3,4,5]. As a result, a total of fifty components were characterized in *Microglossa pyrifolia*, representing 98% of the total oil with germacrene-D as the major component. 39 components were characterized in *Guizotia scabra*, representing 85.2% of the total oil with germacrene-D (26%) and limonene (10%) as the principal constituents. The essential oils were evaluated for their antimicrobial activity using a microdilution assay resulting in the inhibition of a number of common human pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli, Shigella flexneri*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The minimum inhibitory concentrations (MIC) of both *M. pyrifolia* and *G. scabra* varied between 0.25 and 0.5 mg/ml which is within a moderate antimicrobial activity range. Furthermore, the antioxidant capacity of the essential oils was examined using an *in vitro* radical scavenging activity test. The essential oils scavenged 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), resulting in IC_{50} > 30% Equivalent quercetine. **References:** [1] Rwangabo, P.C. (1993) La médicine traditionnelle au Rwanda. Paris, Karthala et ACCT. [2] Van Den Dool, H., Kratz, P.D. (1963) J. Chromatogr.11:463 – 471. [3] McLafferty, F.W., Stauffer, D.B. (1989) The Wiley NBS registry of Mass Spectral Data. 2nd Edition. J.Wiley & Son. New York. 4. Adams, R.P. (2001) Identification of Essential Oil Components by Gas chromatrography/Quadrupole Mass Spectroscopy. Allured Publishing

Corp., Carol stream, IL. [5] Joulain, D., König, W.A. (1998) The Atlas of Spectral Data of Sesquiterpene Hydrocarbons.E.B.Verlag, Hamburg.

PI39 Novel transferosomal cosmetic herbal formulation of curcumin for photo chemoprevention of skin

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Photo chemoprevention has become an important armamentarium in the fight against ultraviolet radiation induced damage to the skin. UV irradiation to skin results in erythematic, oedema, sunburn cells, hyperplasia, immunosuppressant, photo aging and photo carcinogenesis. Botanical agents play multiple roles in ameliorating the process of carcinogenesis. Curcumin, is an antioxidant, anti-inflammatory and photochemoprotective agent protecting skin against UV- A and UV- B radiations. The aqueous and alcoholic extract of curcumine was prepared as per the procedure of World Health Organization guidelines. The transferosomes (CT_1-CT_6, soya lecithin) of both aqueous and alcoholic curcumin extracts having various concentrations (i.e.0.5 – 2.0%w/w) were prepared. The transferosomes and conventional cosmetic formulations were prepared by incorporating transferosomes and curcumine equivalent to transferosomes concentration into the base cream. All formulations (NT_1-NT_6, CC_1-CC_6) were evaluated (long period study) for sun protective activity in terms of quantitative determination of erythematic (spring loaded pocket thickness gauge), epidermal cell count turnover (image analysis), oedema, sunburn cell count (sunburn cells per linear centimeter of skin), wrinkle volume determination (topometry), skin viscoelasticity (cutometer), *in vitro* sun protection factor (UV analysis), lipid concentration (sebumeter). All formulations showed photo protective, improved viscoelastic and antiwrinkle properties to the base line (study without formulation), but novel formulation showed 5 – 40 fold improved photo chemoprevention as compare to conventional formulations. Statistical analysis (SPSS Software) of all quantitative parameters were analyzed at 95% confidence level.

PI40 Essential oil constituents of rhizome oil of *Alpinia* species from South India

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Alpinia species (Family Zingiberaceae) are used as a food additive, spice and in indigenous system of medicine. In the present study, germplasm of three different species of *Alpinia* viz; *Alpinia galanga* (7 accessions), *Alpinia calcarata* (5 accessions), and *Alpinia officinarum* (1 accession), collected from different locations of South and Northeast regions of India were studied. The essential oils from rhizomes of these *Alpinia* species were isolated by hydrodistillation. The oil percentage was maximum in *A. calcarata* (0.73 – 1.26%), followed by *A. galanga* (0.27 – 0.56%) and *A. officinarum* (0.21%). These essential oils were analyzed by capillary GC and GC-MS. Around thirty compounds were identified in these species. The major component was 1,8-cineole, which was present in all three species, its percentage ranged from 40.92 – 72.49% in *A. galanga*, 32.5 – 46.77% in *A. calcarata* and 44.17% in *A. officinarum*. Our South Indian collections were found belonging to cineole rich chemotype, out of five chemotypes of the *A. galanga* reported in earlier studies [1 – 3]. These species differed as *A. calcarata* contained substantially high content of α-fenchyl acetate (13.74 – 27.39%) followed by *A. officinarum* (8.91%) whereas it was negligible in *A. galanga*. Other components found in appreciable amounts in *A. calcarata*[6] IC 210421 were: α-pinene (2.48%), camphene (6.02%), β-pinene (4.14%), camphor (5.27%), α-terpineol (6.44%) and methyl cinnamate (2.68%). Essential oil composition of *A. officinarum* showed α-pinene (1.99%), camphene (3.16%), β-pinene (5.68%), camphor (2.51%), α-terpineol (6.35%), α-fenchyl acetate (8.91%) and methyl cinnamate (1.88%). The drugs prepared from the *A. galanga* and *A. calcarata* are used in the treatment of rheumatism, bronchial catarrh, asthma and in reducing pain. 1,8-Cineole is an important aroma chemical reported to possess expectorant, antiseptic and anesthetic properties and is used widely in pharmaceutical preparations. Therefore, there is a promising possibility to utilize this plant species native to South India for industrial purpose. **References:** [1] Scheffer, C.J.J. et al. (1981) Sci. Pharm. 49:337 – 346. [2] Herman, L. et al. (1985) Phytochemistry 24: 93 – 96. [3] Charles, D.J. et al. (1992) J. Essent. Oil Res. 11:719 – 723.

PI41 Studing contraceptive effects of different doses of saffron (*Crocus sativus*) decoction in mice

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The aim of study was to assess the effects of non toxic doses [1] of aqueous saffron decoction (0.8% & 0.4%) on contraceptive attributes in mice. In this study 15 female BALB/c mice, weighting 25 – 30 grams, were randomly divided into 3 equal groups. 2 groups had fed by saffron solutions (0.4% & 0.8%) before the mating process.All groups were breed in the animal house of medical college. The day on which the vaginal plaque was observed, was considered as the first day of pregnancy. The pregnant mices were divided into 3 subgroups and placed in seperate cages throughout the gestational period and were acclimatized and fed in the same conditions. Animals in all group received tape water in whole gestational period. In 20th day of pregnancy, animals were anesthetized and their fetuses were extracted through a cesarean section. The placenta was excised, weighed, and the number and placement of implantation sites, live and dead fetuses and early and late resorptions were recorded. Mean number of live, dead or resorbed fetuses in animals receiving saffron extracts before the mating were dose dependently less than control group, maximum decrease were observed in animals receiving 0.8% saffron solution. Saffron and its components (2,3) may affect embryonic implantation and may result in contraceptive-like effects. Moreover, using saffron may cause long term effects because of its components and their metabolites like the effects on embryonic implantation if used just before breeding. **References:** 1. Rios, J.L. et al. (1996) Phytother. Res. 10:189 – 193. 2. Abdullaev, F.I. (1993) Biofactors 4:83 – 86. 3. Abdullaev, F.I., Espinosa-Aguirre, J.J. (2004) Cancer Detect Prev. 28:426 – 432.

Topic J: Free Topic

PJ1 Investigations on alkaloids and lipid constituents from *Mercurialis perennis*

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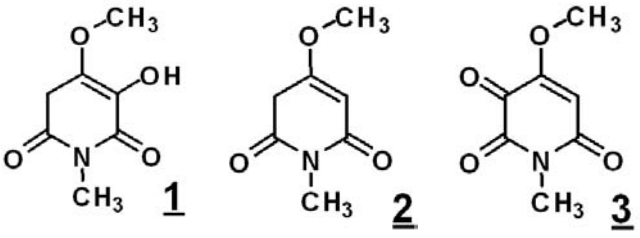
Dog's mercury (*Mercurialis perennis* L.) is a perennial herb, particular important in phytotherapy and anthroposophic medicine. Therefore, detailed studies on the phytochemical composition are of great interest. GC/MS investigations of chloroform extracts from the whole plant exhibited a broad spectrum of constituents, mainly alkaloids, sterols and simple phenolics. Due to the inherent instability of the piperidine alkaloid hermidin **1** towards oxygen, quantitative data were obtained using the more stable reference compound MMPD **2**. In addition, **2** was detected for the first time as a genuine compound in the plant. Hermidine quinone **3** and hermidin dimers originating from hermidin **1** via a free anionic radical reaction were also confirmed by GC/MS. Moreover, volatile compounds such as benzylalkohol, 2-phenylethanol, 4-methoxy- and 3,4-dimethoxyphenol, (-)-*cis*- and (+)-*trans*-myrtanol, (-)-*cis*-myrtanal as well as squalene were predominantly detected in *Mercurialis* roots. In contrast, aerial parts mainly contained phytol derivatives, sterols, and tocopherols. By changing solvent polarity, lipid and wax containing fractions were obtained. GC- and LC/MS-studies on hexane extracts showed the presence of several triglycerides of linolenic, linoleic, oleic, stearic and palmitic acids together with lutein, carotenes and pheophytins. The phytochemical data presented may complement our knowledge on the pharmacognosy of *M. perennis*.



PJ2 Effects of green tea, black tea and rooibos on angiotensin-converting enzyme activity in healthy volunteers

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Epidemiological studies show that tea drinkers exhibit lower risk for developing cardiovascular diseases. However, the pharmacological mechanism behind this effect is unknown. Previous studies *in vitro* have shown inhibition of angiotensin-converting enzyme (ACE) by green and black tea [1]. The aim of this project was to investigate the effect of *Camellia sinensis* L., green tea (Japanese Sencha), black tea (Indian Assam B.O.P.) and *Aspalathus linearis* Dahlg., Rooibos tea on ACE activity after oral intake. Seventeen healthy volunteers received a single oral dose of 400 ml green tea, black tea or Rooibos tea in a randomized three-phase cross over study. ACE activity was measured (at 0, 30, 60 and 180 minutes) in all three phases. ACE activity was analysed with a commercial radioenzymatic assay. In addition, ACE genotype was determined using a PCR method. After oral intake of a single dose of Rooibos tea a significant inhibition of ACE activity, $p < 0.01$ after 30 min and $p < 0.05$ after 60 min was seen. A significant inhibition of ACE activity was also seen with the green tea for the genotype II $p < 0.05$, 30 minutes after intake of the tea and for the genotype ID $p < 0.05$, 60 minutes after intake. A significant inhibition of ACE activity was also seen with the Rooibos tea for the genotype II $p < 0.05$, 60 minutes after intake. In conclusion, intake of green tea and Rooibos tea significantly inhibit ACE activity and may affect blood pressure regulation and thereby prevent cardiovascular diseases. **Reference:** [1] Persson, I.A-L. et al. (2006) J. Parm. Pharmacol. 58:1139 – 1144.

PJ3 Chemical composition and *in vitro* anticholinesterase inhibitory activity of *Citrus medica* L. cv. Diamante essential oil

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The interest in medicinal plant research has increased in recent years, especially for the treatment of pathologies related to aging of the population such as Alzheimer's disease (AD). Natural products such as rivastigmine and galantamine act as acetylcholinesterase inhibitors (AchE) and are actually the only effective treatment for AD. Plants of the genus *Citrus* are primarily valued for their edible fruit, but they also have traditional medicinal value. The peel of *Citrus* fruits has been used in traditional Asian medicine for centuries [1,2]. *Citrus medica* L. cv. Diamante (Diamante citron), known as Italian and Calabrese, is the cultivar more diffused in Italy and more sought by industry. Our previous study reported the chemical composition and the biological activity of Diamante citron peel *n*-hexane extract [3]. In this work the anticholinesterase activity by the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes of *Citrus medica* cv. Diamante peel essential oil assessed by the modified Ellman's method was investigated to explore the beneficial effects of this *Citrus* cultivar [4]. The chemical composition of the essential oil of *C. medica* L. cv. Diamante peel obtained by hydrodistillation was determined by GC/MS analysis. A total of forty-two components, representing 95.3% of the total oil, were identified. Limonene and γ-terpinene were the major components; the other most abundant were geranial, neral, α-pinene and β-pinene. The essential oil exerted an interesting inhibitory activity against BChE with an IC_{50} value of 154.6 μg/ml and AChE with an IC_{50} value of 171.3 μg/ml. **References:** [1] Blumenthal, M. et al. (1998) The Complete German Commission and Monographs: Therapeutic Guide to Herbal Medicines. American Botanical Council, Austin. [2] Wichtl, M. and Bisset, N.G. (1994) Herbal Drugs and Phytopharmaceuticals. Trans from 2nd German ed., Medpharm Scientific Publishers, Stuttgart. [3] Conforti, F. et al. (2007) Phytother. Res. 21:427 – 433. [4] Perry, N.S.L. et al. (1992) J. Pharm. Pharmacol. 52:895 – 902.

PJ4 Anti-*Candida* activity with alcoholic extracts of medical herbs

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The increasing resistance of *Candida* towards antifungal compounds and the reduced number of available drugs has resulted in a search for new therapeutic alternatives. The objective of this study was to examine *in vitro* antifungal activity and susceptibility of *Candida* species to *Solanum Lycopersicum* leafs and *Punica Granatum* in 60% ethyl alcohol extracts, and to present the results of studies concerning antifungal efficacy. *Candida* species were isolated from different hospital patient samples. The Mueller Hinton agar was inoculated with *Candida* species isolates and after inoculation 6 mm diameter wells were made in the agar. Leafs plant extract was added directly into each well. 60% ethyl alcohol without plant extract was added to one well as a control. The plates were incubated at 37 °C for 24/48 hour and the growth of *Candida* species was observed. The inhibition zone of antifungal susceptibility was measured in mm. The highest alcohol extract dilution added to the agar and showing no visible *Candida* species growth after incubation was regarded as the MIC. MFC is defined as the lowest concentration of alcohol extract which when added to an agar medium shows no *Candida* sp. growth after incubation. The 100% susceptibility results of *Candida* sp. are encouraging and indicate the potential use of *Solanum Lycopersicum* and *Punica Granatum* in the control of selected phytopathogenic fungi. The distinctive antifungal activities of commercial phytochemicals and their extracts provide some promising clues for anti-infective efficacy of the drug in clinical practice, which encourages us to elucidate the antifungal efficacy of phytochemical drugs. **References:** [1] Jorgensen, J.H. et al. (1999) Murray, P.R. (ed.) Manual of Clinical Microbiology. ASM Press. Washington, DC. [2] Chang, C. et al. (2002) J. Food Drug Anal. 10:178 – 182. [3] Tuner, R.A. and Hebborn, P. (1971) Screening Methods in Pharmacology, Academic Press New York, London, v:II. [4] Cota, B.B. et al. (2004) Biochem. Syst. Ecol. 32:391 – 397. [5] National Committee for Clinical Laboratory Standards: NCCLS document M 27-P (1992) 771 E. [6] Diaz, R. (2001) Fitoterapia 59:329 – 322.

PJ5 Ethnobotanical study of wound healing plants used in Lagos metropolis Nigeria

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Lagos is a huge metropolis which originated on islands separated by creeks. The city is the economic and financial capital of Nigeria. Intensive research in wound healing has not yielded, economic and efficacious pro-healing agents that could alleviate the long hospitalization of patients following surgery and wound infliction. An ethnobotanical study was carried out among women herb sellers who live in the central part of Lagos, Nigeria. Verbal information on the medicinal plants was obtained through unstructured questionnaire administered by interview in the local language spoken by the herb sellers in the study area. The local name, parts of plants used, mode of preparation and administration were recorded and literature searches carried out for the evaluation on the current status of investigations on these plants. Specimens were purchased in order to collaborate economically with their time and to gain their confidence. In this study, 22 species of plants, belonging to 18 plant families, which are commonly used for the treatment of wounds, are presented. The plants are used as first aids, in the washing of wounds, extraction of pus, as well as on infected wounds. Taxonomic distribution shows bark (36.7%), root (27.2%), leaves (9.1%), juice (22.5%) and rhizome (4.5%). Methods of preparation varies and they are species specific viz: plant parts applied as a paste, juice extracted from the fresh plant parts, powder made from fresh or dried plant parts, some fresh plant parts, and decoction. The most frequently used preparations are decoctions and powdered plant material.

PJ6 *In vitro* interaction of L-Dopa with bacterial adhesins of *Helicobacter pylori*: An explanation for clinicial differences in bioavailability?

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Recent investigations on the pharmacokinetics of levodopa (L-Dopa) indicated that the presence of *Helicobacter pylori* (*HP*) in Parkinson dis-

ease patients, orally treated with L-Dopa, influences the absorption of this compound, which consequently leads to decreased plasma levels [1,2]. Therefore this work aims to study a potential *in vitro* interaction of L-Dopa with *HP* and its surface adhesins. Free L-Dopa was quantified from the incubation supernatants with *HP* by HPLC. A flow cytometric assay with fluorescence labelled *HP* was used to investigate the influence of L-Dopa on the bacterial adhesion of *HP*. FITC-labelled bacteria were preincubated with L-Dopa, followed by incubation with gastric epithelial cells (AGS) and flow cytometric analysis. Quantitative evaluation of time and concentration dependent incubation experiments indicated a significant decrease of L-Dopa concentrations when getting in contact with *HP*. The reduction of L-Dopa concentrations was determined with 47 to 12% referred to the initial starting concentration, with time-dependency and dependency of the *HP* density. FITC-labelled *HP*, preincubated with differing L-Dopa concentrations, was shown to have a significant ($p < 0.05$) reduced bacterial adhesion to AGS cells with maximum reduction of 22 ± 9%. These results demonstrate a direct interaction of L-Dopa with outer membrane proteins of *HP*, responsible for the adhesion to gastric epithelial cells. By this interaction the unbound L-Dopa concentration in bacterial suspension was strongly reduced. This study suggests a potential *in vitro* interaction of L-Dopa with *HP* adhesins, confirming the clinical changes found in pharmacokinetics of L-Dopa therapy by *HP*-positive Parkinson patients. **References:** [1] Pierantozzi, M. et al. (2001) Ann. Neurol. 50:686 – 687. [2] Pierantozzi, M. et al. (2001) Neurol. Sci. 22:89 – 91.

PJ7 Dicaffeoylquinic acid ameliorates chronic dermatitis caused by trinitrochlorobenzene

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Although the animal models of skin inflammation designed to mimic atopic dermatitis (AD) do not completely reproduce the pathology, they are of importance as research tools to develop new approaches to therapy. Repeated application of 2,4,6-trinitrochlorobenzene (TNCB) at 2-days intervals for 3 – 4 weeks results in a site-restricted shift in the time course of antigen-specific hypersensitivity responses from a typical delayed-type to an immediate-type hypersensitivity followed by a late reaction, a finding often seen in skin lesions of AD patients [1]. This communication reports the effect of 2-isoprenylhydroquinone-1-glucoside (IHG) and 3,5-dicaffeoylquinic acid (DCA) isolated from *Phagnalon rupestre* (Asteraceae) [2], on a mouse model of dermatitis induced by repeated application of TNCB. BALB/c mice were sensitized with 0.3% TNCB applied to the both ears on day-7, followed by application to the same site three times a week from day 0 to 21 [3]. IHG and DCA (0.5 mg/ear) and the reference drug dexamethasone (0.025 mg/ear) were topically applied to both ears 30 min after challenge from day 0 to 21. DCA significantly reduced ear thickness at every time point and inhibited the edema over 40% from day 11 onward whereas the effect of IHG was lower (nearly 30% inhibition). DCA significantly inhibited myeloperoxidase (MPO) activity, a quantitative index of neutrophil infiltration into skin, by 44% on day 22. Dexamethasone almost abolished the edema and inhibited MPO activity by 78%. In our previous studies on murine hypersensitivity, DCA was more active than IHG, possibly due to the presence of an *ortho*-dihydroxycinnamic group which enhanced protection [4]. These results suggest that DCA seems to diminish the development of chronic dermatitis at least partially due to the reduction of ear swelling and cell infiltration at the site of inflammation. *Acknowledgements: This work was supported by the Generalitat Valenciana (GVPRE/2008/155) and by the Spanish Ministry of Science and Technology (SAF 2006 – 06726).* **References:** [1] Shiohara, T. et al. (2004) J. Dermatol. Sci. 36:1 – 9. [2] Góngora, L. et al. (2002) Planta Med. 68:561 – 564. [3] Harada, D. et al. (2006) Eur. J. Pharm. 532:128 – 137. [4] Olmos, A. et al. (2007) Br. J. Pharmacol. 152:366 – 373.

PJ8 Effect of alkylphenols from *Phagnalon rupestre* on trinitrochlorobenzene-induced hypersensitivity

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The three alkylphenols, 2-isoprenylhydroquinone-1-glucoside (IHG), 3,5-dicaffeoylquinic acid (DCA) and its methyl ester (DCE), isolated from *Phagnalon rupestre* (Asteraceae) [1], have previously been demonstrated to possess anti-inflammatory and anti-allergic properties [2]. The present communication reports their effects on the contact hypersensitivity (CHS) response to 2,4,6-trinitrochlorobenzene (TNCB) in mice assessed by ear swelling and cytokine production determinations. BALB/c mice were sensitized with 100 µl of 7% TNCB applied to the shaved abdomen on day-7, followed by epicutaneous application of 20 µl of 1% TNCB to the both ears on day 0 for elicitation [3]. IHG, DCA and DCE (0.5 mg/ear), and the reference drug dexamethasone (0.025 mg/ear) were topically applied to both ears 30 min after challenge. Ear thickness was measured immediately before sensitization and 24 h after elicitacion with TNCB. All three phenolics significantly reduced ear thickness at 24 h after challenge. IHG inhibited the edema by 50% whereas the effect of DCA and DCE was lower (38 and 39% inhibition, respectively). The levels of Th 1 cytokines of the homogenized ear tissues from the sacrificed mice were measured by ELISA. The three alkylphenols significantly inhibited IL-1β content by over 50%. DCA treatment also inhibited the TNF-α and IL-2 content by 53% and 39%, respectively, whereas DCE reduced these levels by 39 and 43%, respectively. Dexamethasone inhibited the edema by 82% and cytokine contents by over 65%. These results suggest that the three alkylphenols might alleviate CHS-associated inflammatory reaction by reducing the levels of cytokines locally released in the lesioned skin. *Acknowledgements: This work was supported by the Generalitat Valenciana (GVPRE/2008/155) and by the Spanish Ministry of Science and Technology (SAF 2006 – 06726).* **References:** [1] Góngora, L. et al. (2002) Planta Med. 68:561 – 564. [2] Olmos, A. et al. (2007) Br. J. Pharmacol. 152:366 – 373. [3] Martin, S.F. et al. (2008) J. Exp. Med. 205:2151 – 2162.

PJ9 Phytochemical investigations on the composition of phenols and carbohydrates of *Myrothamnus flabellifolia* Welw.

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The homoiochlorophyllous resurrection bush *Myrothamnus flabellifolia* Welw., indigenous to southern parts of Africa is traditionally used for infections of the respiratory and urinary system and well known for its ability to withstand dry seasons up to one year without fatal damages [1]. Besides the occurrence of osmotica, strong antioxidant systems [2] and physical adaption to intense solarisation [3], this species obviously uses condensed tannins and thoroughful galloylation of other substance-classes as a form of natural sunscreen [4]. Within a phytochemical characterisation of the plant, isolation procedures towards purified compounds were performed using Sephadex-LH20, MCI-CHP20P, RP-18 and RP-8 stationary phases in LPLC-, MPLC and HPLC-systems as well as MLCCC and FCPC. Structure elucidation was achieved conducting 1D-/2D-NMR experiments and ESI-MS. Sugar analysis was accomplished using CE [5], HPAEC-PAD and an enzymatic fructan-assay. The flavonoid fraction was shown to be composed of quercetine and kaempferol, with the respective 3-O-β-D-glucosides, 3-O-β-D-galactosides-, 3-O-α-L-rhamnosides- and 3-O-β-D-glucuronides. Additionally, the kaempferol- and quercetine-3-O-β-D-glucoside exist at varying levels of galloylation. Furthermore, different galloylated forms of quinic acid, 2'',3''-di-O-galloyl-arbutine and 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid were identified. To characterise the carbohydrate part of plant material, the fructan content was determined with 2.1%. Dependent on the respective batch of dried material, trehalose content was calculated to be 3.3 – 4.5%, as well as raffinose and stachyose with 0.2 – 0.3% each. Complementally to galloylated and non-galloylated condensed tannins described recently by our group [4], *Myrothamnus flabellifolia* Welw. was shown to contain a number of hydrolysable tannins. **References:** [1] Farrant, J.M. and Kruger, L.A. (2001) Plant Growth Reg. 35:109 – 120. [2] Kranner, I. and Birtic, S. (2005) Integr. Comp. Biol. 45:734 – 740. [3] Bianchi, G. et al. (1993) Phys. Plant. 87:223 – 226. [4] Anke, J. et al. (2008) Nat. Prod. Res. 22:1243 – 1254. [5] Noe, C.R. and Freissmuth, J. (1995) J. Chrom. A 704:503 – 512.

PJ10 Antimalarial and cytotoxic properties of South African *Agathosma* species

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The increasing prevalence and spread of drug resistant *Plasmodium falciparum* malaria parasites has increased efforts in the discovery of new chemically diverse antimalarial agents. Traditional phytomedicines have been the source of quinine (*Cinchona pubescens*) and artemisinin (*Artemisia annua*). Southern African species of *Agathosma* have been used in the treatment of clinical symptoms associated with malaria. The antimalarial activity of 17 indigenous species along with the specificity of their inhibitory action and cytotoxic properties were investigated *in vitro*. As such, the antimalarial activity of 19 acetone extracts were tested against a chloroquine-resistant *Plasmodium falciparum* strain using the [^{3}H]-hypoxanthine incorporation assay to determine the concentration required to inhibit 50% parasite growth (IC_{50} value). The haemolytic properties were assessed using uninfected human erythrocytes. The tetrazolium cell proliferation assay was used to determine the cytotoxicity of the extracts against human erythroleukemia and kidney epithelial cells. All 17 species were highly active with IC_{50} values less than 30 μg/ml. *Agathosma pungens* and *A. ovata* (hook-leaf) were the two most active against malaria and erythroleukemia, but *A. parva* possessed the best safety index. Only *A. ovata* (hook-leaf) displayed minimal haemolytic activity which would not have contributed to the antimalarial activity of this extract. The toxicity profile of the extracts indicated that there was selectivity to protozoa rather to mammalian cells. Thus, the South African indigenous *Agathosma* species show promise as a further source of antimalarial compounds. *Acknowledgements: Faculty of Health Sciences Research Committee, University of the Witwatersrand and the NRF Thuthuka Women in Research.*

PJ11 Antimalarial activity of thirteen South African Menispermaceae species

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Malaria remains a serious problem in most third world countries. In South Africa, the use of traditional remedies plays a significant role in the treatment of malaria and these phytomedicines are a source of novel antimalarial agents. The medicinal plant family, Menispermaceae has reportedly been used in the treatment of malaria and clinical symptoms associated with malaria such as fever. Thus, the 27 methanol extracts from 13 species in the 7 genera found in southern Africa were evaluated for their antimalarial activity against a chloroquine-resistant *Plasmodium falciparum* strain using the [^{3}H]-hypoxanthine incorporation assay. The haemolytic properties were assessed using uninfected human erythrocytes and the tetrazolium cell proliferation assay was used to determine the cytotoxicity of the extracts against human kidney epithelial cells. Six of the 27 extracts displayed high activity at a concentration less than 5 μg/ml, namely *Antizoma miersiana* (rhizomes), *Albertisia delagoensis* (rhizomes and leaves), *Cissampelos capensis* (coastal, rhizomes), *Cissampelos mucronata* (rhizomes) and *Tiliacora funifera* (leaves). The haemolytic activity of *Tinospora fragosa* (leaves) contributed slightly to the antimalarial activity; while the remaining extracts had a direct inhibitory effect on the intra-erythrocytic parasite. The rhizomes of *Antizoma miersiana* and *Cissampelos torulosa* were the most cytotoxic against the human kidney epithelial cells with IC_{50} values less than 25 μg/ml. *Albertisia delagoensis* (rhizomes) yielded a favourable safety index (> 103), while *Cocculus hirsutus* had the lowest safety index (0.67). Select Menispermaceae species have the potential as a source of antimalarial compounds. *Acknowledgements: Faculty of Health Sciences Research Committee, University of the Witwatersrand and the NRF Thuthuka Women in Research.*

PJ12 Anti-inflammatory and anti-asthmatic effects of tiarellic acid in a mouse model of allergic asthma

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Asthma is an inflammatory disease of the airways, and the current focus in managing asthma is the control of inflammation. We investigated anti-inflammatory and anti-asthmatic effects of tiarellic acid isolated from *Tiarella polyphylla* [1] on asthmatic parameters–such as immunoglobulin E (IgE) level, cytokine release, eosinophilia, airway hyperresponsiveness (AHR) and mucus hypersecretion–in an ovalbumin (OVA)-sensitized/challenged mouse model. Tiarellic acid significantly inhibited increases in total immunoglobulin E (IgE), T-helper-2-type cytokines such as interleukin (IL)-4 and IL-13 in bronchoalveolar lavage fluid (BALF), and also effectively suppressed airway hyperresponsiveness, eosinophilia, and mucus hypersecretion, in the asthmatic mouse model. The efficacy of tiarellic acid was comparable to montelukast, an anti-asthmatic drug that is currently available. These results suggest that tiarellic acid could be a major marker in herbal medicines that are used for asthma treatment, and could also act as a lead for the development of anti-inflammatory and anti-asthmatic drugs. **Reference:** [1] Park, S.H. et al. (2002) Arch. Pharm. Res. 25:57 – 60.

PJ13 Antioxidant activities of some endemic *Verbascum* species growing in Turkey

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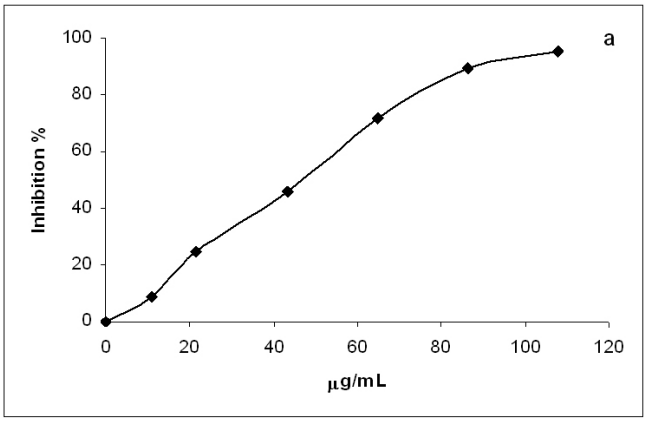
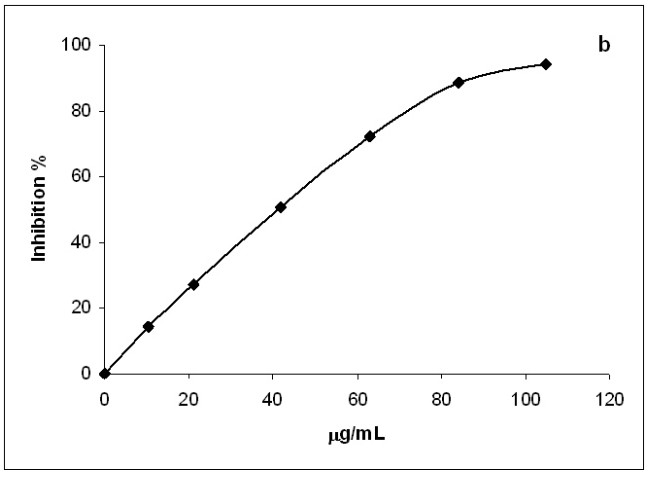
Antioxidant, antimicrobial, antiviral and cytotoxic activities of some members of *Verbascum* genus have been reported [1]. The purpose of this investigation was to determine and characterise the antioxidant activity of the six extracts of the aerial parts from two endemic *Verbascum* L. species growing in Turkey, utilizing the DPPH free radical-scavenging and β-carotene/linoleic acid assays. Accelerated Solvent Extraction (ASE) method was used to prepare the extracts using Dionex ASE 300 accelerated solvent extractor with different solvents namely, ethylacetate, chloroform and methanol at 80°C temperature. The inhibition percentage of the methanol extracts of *V. detersile* (a) *V. pestalozzae (b)* at various concentrations in DPPH test are given in Figure 1.





The methanol extracts of two *Verbascum* species exerted greater antioxidant activity than those of other extracts with an IC_{50} value of 48.0 ± 0.5 µg/ml, and 40.0 ± 0.5 µg/ml, respectively. Ethyl acetate and chloroform extracts were not active in DPPH method. All extracts of both species exhibited no activity in β-carotene/linoleic acid test system. These results indicate that only methanolic extracts of *Verbascum detersile* and *Verbascum pestalozzae* have ability to scavenge free radicals. **Reference:** [1]Tepe, B. et al. (2006) Food Chem. 98:9 – 13.

PJ14 Structure-activity relation of different N-phenylpropenoyl-L-amino acids as antiadhesive compounds against *Helicobacter pylori*

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Recently a new homologous series of secondary products, N-phenylpropenoyl-L-amino acids (NPAs), isolated and structurally elucidated from cocoa seeds, were reported [1]. Hensel et al. demonstrated N-(E)-caffeic acid L-aspartic acid amide as a strong antiadhesive substance with properties against the adhesion of *Helicobacter pylori* (*HP*) to gastric tissue sections [2]. Using a new flow cytometric method, 35 different NPAs were analysed for a better understanding of the structural requirements needed for the antiadhesive effect against *HP*. Within the family of the NPAs, the N-(E)-caffeic acid L-dihydroxyphenylalanine amide and N-(E)-p-coumaric acid L-dihydroxyphenylalanine amide showed the best antiadhesive properties, inhibiting significantly the adhesion of *HP* to gastric epithelial cells (AGS) by almost 20%. This was shown to be a similar range as the positive control 3'sialyllactose, which interacts specifically with the *HP* neuraminyl-lactose-binding hemagglutinin (NLBH). Quantitative structure-activity relations of the NPA homologous serie revealed that the best activity seems to be enhanced by the presence of two vicinal hydroxyl groups within the phenylpropenoyl or amino acid part of the molecule. In addition, the presence of a carboxylic group in the amidic linkage derived from the amino acid also influences positively the activity, as demonstrated with the corresponding compounds N-(E)-caffeic acid L-phenylalanine and N-(E)-caffeic acid L-phenylethylalanine. Higher acidity of the amino acid part increased strongly the inhibitory activity. The described structural properties of NPAs are suggested to be responsible for the antiadhesive effect. **References:** [1] Stark, T. et al. (2005) J. Agr. Food Chem. 53:5419 – 5428. [2] Hensel, A. et al. (2007) Planta Med. 73:142 – 150.

PJ15 The relationship between morphological development and accumulation of saponins in the root of *Polygala tenuifolia* Willd

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The root of *Polygala tenuifolia* Willd. (Polygalaceae) is a well-known Chinese crude drug. The morphological development of roots with their dynamic accumulation of saponins in *Polygala tenuifolia* was investigated by anatomical, histochemical and phytochemical approaches. Histochemical results revealed that the secondary phloem was the main storage region of saponins. We took senegenin as an indicator compound to analyze the regularity of saponin accumulation. HPLC results showed that the average content of senegenin of different-year-old roots in the "skin areas" (1.081%) including periderm and phloem was 16.01 times more than that in the xylem (0.072%). During the growth period from April to October, the percentage of senegenin content of different-year-old roots exhibited a contineous decreasing trend (from 0.899% to 0.836% to 0.667% to 0.651%) and the accumulation of senegenin was opposite to that of biomass accumulation (from1.540 g to 2.865 g to 8.840 g to 11.41 g). The length, diameter, thickness of the "skin areas" and dry weight as well as the total senegenin content of roots increased most quickly from the second to the third growth year. The mid-ten days of August of the third year were the optimal time for collecting the roots (having total senegenin 71.12 mg/plant). The results add data to the relationship between secondary metabolism and plant development, and provide scientific bases for determining the most appropriate period for harvesting the roots.

PJ16 Antibacterial activity of the green alga *Ulva rigida* collected from Tunisian coast: seasonal and geographical variation

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Ulva rigida is one of the most abundant algae in Tunisia with important blooms particularly in warm seasons [1]. We therefore investigated the antibacterial activity of extracts of this alga collected at different seasons and from two different locations: the coastal area of Cap Zebib and the Ghar el Melh lagoon. Antibacterial activity of *Ulva rigida* organic extracts was assayed against both Gram-positive and Gram-negative bacteria. Tests were performed by the diffusion method [2]. The crude extracts of *Ulva rigida* collected in summer were more effective than those of the other seasons. The strongest antibacterial activity was obtained by samples collected from the lagoon. Two gram positive bacteria: *S.aureus* and *Streptococcus sp.* were found to be the most sensitive strains to algal extracts. The bioassay guided fractionation of the dichloromethane/methanol extract for *Ulva rigida* collected in summer from the lagoon, showed at least two different compounds with an antibacterial activity observed for the non polar fractions (eluted with 100% CH_2Cl_2 and 95/5 CH_2Cl_2/MeOH) and another one for moderately polar fraction (85/15 CH_2Cl_2/MeOH). These results highlighted a variable antibacterial activity of *Ulva rigida* related to the bacterial strain, the season of harvest and the geographic location. Previous study on this alga collected from Tunisian coast did not report any significant antibacterial activity [3]. Thereby *Ulva rigida* collected from Ghar El Melh lagoon should be considered as a valuable source, especially for antibacterial activity against pathogenic bacteria. **References:** [1] Shili, A. et al. (2000) J. Cost. Conserv. 8:127 – 134. [2] Rios, J.L. et al. (1988) J. Ethnopharmacol. 23:127 – 149. [3] Ktari, L. et al. (2001) J. Rech. Oceanogr. 26:37 – 41.

PJ17 Analysis of phenolic constituents in Single Malt Scotch Whiskies by UPLC

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Distilled spirits contain numerous phenolic constituents which are extracted, in part, from the wood cask during maturation. Such compounds may provide information on authenticity and quality of the products. Analyses have so far focused on the determination of the volatile constituents by GC, including GC-MS. Few HPLC methods for whisky analysis have been published. However, they are time-consuming [1] or afford only limited resolution [2]. This prompted us to explore the potential of Ultra High Performance Liquid Chromatography (UPLC) for the analysis of whiskies. UPLC is a recent development of HPLC relying on small size particles, which increase significantly resolution and considerably reduce separation time [3]. Using a HSS T3 column (2.1 x 100 mm; 1.8 µm, Waters) and a gradient of methanol in water containing 0.1% TFA over 60 min, more than 15 compounds, including phenolic acids, aldehydes, phenols and furans could be identified from their chromatographic and UV properties, and by comparison with authentic samples. In addition, several lignans were identified after semipreparative isolation and ^{1}H NMR measurement using a 1 mm TXI microprobe (Bruker) [4]. The UPLC assay has been subsequently used for the quantification of the constituents in a selection of Single Malt Scotch Whiskies that have been classified into ten different clusters on the basis of their organoleptic properties collected from hundreds of tasting notes [5]. **References:** [1] Goldberg, D.M. et al. (1999) J. Agr. Food Chem. 47:3978 – 3985. [2] Aylott, R.I. et al. (1994) Analyst 119:1741 – 1746. [3] Swartz, M.E. (2005) J. Liq. Chromatogr. R. T. 28:1253 – 1263. [4] Griffin, J.L. et al. (2002) Analyst 127:582 – 584. [5] Wishart, D. (2006) Whisky classified – choosing single malts by flavour. 2nd ed. Pavilion Books. London.

PJ18 Quantitative determination of lycorine in Amaryllidaceae plants

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Lycorine, a widespread alkaloid in Amaryllidaceae species, has been proven to have antiviral, cytotoxic and antimalarial activities [1 – 3]. A reversed-phase high-performance liquid chromatographic method has been developed and validated for the determination of lycorine in Amaryllidaceae plants. A simple method for the extraction of lycorine in low-mass plant samples was employed utilizing pre-packed columns with diatomaceous earth (Extrelut®) [4]. The chromatographic separation was performed using an isocratic system with a mobile phase trifluoroacetic acid-water-acetonitrile (0.01:90:10) and diode array detector [5]. The linearity of the method was studied by injecting five known concentrations of the standard in the range of 62.5 – 500 µgmL^{-1}. The calibration curve was determined as Y = 23.9788285 X + 62.391901. Validation procedures showed that the method was specific, accurate and precise. The above-mentioned method was applied to the aerial parts and bulbs of *Sternbergia sicula* Tineo ex Guss., *S. lutea* (L.) Ker-Gawl. ex Sprengel and *Pancratium maritimum* L. collected during two different vegetation periods. Considering the botanical parts (aerial parts, bulbs) and vegetation periods (flowering and fruiting), there were significant differences in the content of lycorine. The amount of lycorine in *S. sicula* ranged between 0.097 – 0.529%, and the highest amount was found in the aerial parts collected during flowering period. The lycorine content in *S. lutea* and in *Pancratium maritimum* ranged between 0.195 – 0.399% and 0.048 – 0.145%, respectively. The highest content of lycorine in these two species was detected in the bulbs collected during the fruiting period. **References:** [1] Gabrielsen, B. et al. (1992) J. Nat. Prod. 55:1569 – 1581. [2] Liu, J. et al. (2004), FEBS Lett. 578:245 – 250. [3] Şener, B. et al. (2003) Phytother. Res. 17:1220 – 1223. [4] Berkov, S. et al. (2008) Phytochem. Anal. 19:285 – 293. [5] Mustafa, N.R. et al. (2003) J. Liq. Chromatogr. R. T. 26:3217 – 3233.

PJ19 Prophylactic and curative effect of STW 5 in experimental inflammatory bowel disease

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STW 5 (Iberogast®), a multiherbal preparation, has been shown to be clinically effective in functional dyspepsia and irritable bowel syndrome. Its potent anti-inflammatory effects prompted us to study its efficacy in inflammatory bowel disease (IBD). IBD was induced in male Wistar rats by injecting them intra-colonically with trinitrobenzene sulfonic acid (TNBS) in ethanol under light ether anesthesia. STW 5 was tested both as a prophylactic and as a curative measure. Prophylactically, STW 5 was given orally for 1 week before TNBS and for 4 more consecutive days. Drug effects were assessed macroscopically and substantiated immunohistochemically as well as by measuring relevant biochemical parameters and mediators. In the curative setting, inflammation was first induced, followed after 48 h by STW 5 orally daily for 7 days. Lesions were then assessed and biochemical parameters measured. Prophylactically, STW 5 dose dependently reduced the area of lesions and colonic and spleen mass indices. It prevented changes in colonic myeloperoxidase (MPO) and reduced glutathione (GSH) levels and protected against changes in the level of ICAM-1, TNFα, IL-1β, IL-10, LTB_4 and PGE_2 in blood and colon. The effect was comparable to that of sulfasalazine and confirmed histopathologically. In the curative experiments, the drug reduced the area of lesions and colon mass index and tended to normalize the changes in GSH and MPO activity. The findings lend evidence to both protective and curative effect of STW 5 in colonic inflammation, indicating a potential therapeutic usefulness in inflammatory conditions of the lower gastrointestinal tract.

PJ20 Study on the anti-inflammatory effects of tanshinone IIA on hepatic stellate cells

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Anti-inflammation via inhibition of NF-κB pathways in hepatic stellate cells (HSCs) is one therapeutic approach to hepatic fibrosis. Tanshinone IIA ($C_{19}H_{18}O_3$) and cryptotanshinone ($C_{19}H_{20}O_3$) are two major diterpenes from *Salvia miltiorrhiza* Bunge, with reported anti-inflammatory activity. We tested whether tanshinone IIA and cryptotanshinone could inhibit HSC activation. A cell line of rat hepatic stellate cells (HSC-T6) was stimulated with tumor necrosis factor (TNF)-α (1 ng/ml) or lipopolysaccharide (LPS) (100 ng/ml). Cytotoxicity was assessed by MTT assay. NF-κB activity was assessed by the luciferase reporter gene assay. Western blot analysis was performed to measure NF-κB-p65 and JunD nuclear translocations and MAPK (p38, JNK, ERK) phosphorylations. HSC-T6 cells were pretreated with tanshinone IIA and cryptotanshinone, then induced by TNF-α (1 ng/ml) and LPS (100 ng/ml). One-way analysis of variance was used for comparison of parameters. Both tanshinone IIA (3 and 10 µM) and cryptotanshinone (10 µM) inhibited TNF-α- and LPS-induced NF-κB luciferase activity. Tanshinone IIA (10 µM) also inhibited TNF-α-stimulated p65 and LPS-induced JunD nuclear translocations, but did not modulate phosphorylations of p38, JNK, or ERK. Tanshinone IIA was not cytotoxic at 3 and 10 µM. Our results suggested that tanshinone IIA inhibited TNF-α-stimulated HSC activation with NF-κB inhibiton. **References:** [1] Xu, M. et al. (2009) Eur. J. Pharmacol. 607:194 – 200. [2] Xu, Y. et al. (2008) J. Clin. Immunol. 28:512 – 519. [3] Jang, S.I. et al. (2006) Eur. J. Pharmacol. 542:1 – 7.

PJ21 *Poria cocos* inhibited the activation of hepatic stellate cells

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Activation of hepatic stellate cells (HSCs) plays a central role in hepatic fibrosis. *Poria cocos*, a commonly used Chinese herb, has been shown to exert anti-inflammatory bioactivities. We aimed to study its effect on

the HSC activation. Two HSC cell lines, HSC-T6 (rat) and LX-2 (human), were used. These cells were pre-treated with an ethyl-acetate soluble extract from *Poria cocos* (PC-EA) and then stimulated with tumor necrosis factor-α (TNF-α). Nuclear factor-kappa B (NF-κB) and peroxisome proliferator-activated receptor gamma (PPAR-γ) activities were measured using reporter gene assays. Expressions of α-smooth muscle actin (α-SMA) were detected by Western blotting. Quantitative RT-PCR was used to analyze the mRNA expressions of α-SMA, intercellular adhesion molecule 1 (ICAM-1), and PPAR-γ. Cytotoxicity was accessed using MTT assay. One-way analysis of variance was used for comparison of parameters. The results showed that PC-EA (3.125 – 25 μg/ml) concentration dependently inhibited TNF-α-induced NF-κB activities in both HSC-T6 and LX-2 cells. PC-EA also attenuated TNF-α-induced protein and mRNA expressions of α-SMA. TNF-α-induced ICAM-1 mRNA upregulation was ameliorated by PC-EA whereas TNF-α-suppressed PPAR-γ activities and gene expression were both reversed by PC-EA. PC-EA alone could also enhance the PPAR-γ activities of HSCs. No significant cytotoxicity of PC-EA was observed within the concentration range we used. In conclusion, PC-EA could inhibit the activation of HSCs induced by TNF-α, possibly due to PPAR-γ upregulation.

PJ22 Comparison of the inhibitory potency of curcumin, demethoxycurcumin and bisdemethoxycurcumin on iNOS-derived NO in activated macrophages and on gastric ulcer in rats

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Sustained overproduction of nitric oxide (NO) generated from inducible nitric oxide synthase (iNOS) expressed in activated macrophages can lead to a modulation of leukocyte infiltration and an interaction of NO with leukocyte-derived $O_2^{\cdot-}$ that forms other cytotoxic oxidants [1]. Likewise, the suppression of the excess generation of iNOS-derived NO supports gastric mucosal defence and promotes the onset of gastric ulcer healing [2]. Curcumin (Cur), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) are three main curcuminoids isolated from turmeric (*Curcuma longa* L.). These three curcuminoids have been shown to be strong nitric oxide scavengers each with similar scavenging potency [3]. Our recent study demonstrated that curcumin directly accelerates ulcer healing in an acetic acid induced gastric ulcer model in rats by a mechanism that involves its ability to suppress iNOS expression [4]. In the present study, each compound was evaluated for its potency to inhibit the iNOS-derived NO in the lipopolysaccharide activated macrophage cell line RAW 264.7 and the acetic acid induced gastric ulcer in rats. All three curcuminoids significantly suppressed NO production and iNOS protein expression in activated macrophages and the relative potency was Cur > BDMC > DMC. These three curcuminoids also significantly accelerated gastric ulcer healing 14 days after the induction. Interestingly, the antiulcer potency of curcumin was equal to bisdemethoxycurcumin and was stronger than that of demethoxycurcumin. Thus, the methoxy group was not essential for the nitric oxide scavenging activity. *Acknowledgements: The financial support of Prince of Songkla University of Thailand for some parts of this work is gratefully acknowledged.* **References:** [1] Pavlick, K.P. et al. (2002) Free Radic. Biol. Med. 33:311 – 322. [2] Holzer, P. (2001) Curr. Opin. Gastroenterol. 17:489 – 496. [3] Sreejayan, Rao, M.N.A. (1997) J. Pharm. Pharmacol. 49:105 – 107. [4] Mahattanadul, S. et al. (2006) J. Nat. Med. 60:191 – 197.

PJ23 Bioavailability of dodeca-2*E*,4*E*,8*E*,10*E*/*Z*-tetraenoic acid isobutylamides after oral administration in rats and distribution in various tissues

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The pharmacokinetics and tissue distribution of dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides (tetraens), the main alkamides in *Echinacea* preparations, were investigated in rats after a single oral dose administration of 2.5 mg/kg. Plasma, liver and 4 different brain regions (hippocampus, cerebral cortex, striatum and cerebellum) were collected after 8, 15, 30 minutes and 1, 2, 3 and 6 hours after oral dosing. Plasma and tissue concentrations were determined by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method with benzanilide as internal standard (IS) using the respective $[M-H]^+$ ions, m/z 248/152 for the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides and m/z 198/105 for the IS. The lipophilic constituents were rapidly absorbed and well distributed to the tissues examined. The highest concentration was found in the striatum. The total tetraens amount in plasma (794 min*ng/mL) was calculated as $AUC_{0-\infty}$ and about 13~45% of that found in different brain parts (1764 – 6192 min*ng/mL), and 63% of that in liver tissues (1254 min*ng/g). The C_{max} in plasma was 26.4 ng/mL, while the C_{max} in the different brain regions varied between 33.8 ng/g and 46.0 ng/g. The results demonstrate that the dodeca-2*E*,4*E*,8*Z*,10*E*/*Z*-tetraenoic acid isobutylamides are bioavailable in rats with a rapid passage across the blood-brain barrier. **Reference:** [1] Woelkart, K. et al. (2009) Planta Med. submitted.

PJ24 A double-blind cross over study comparing *Achillea wilhelmsii* with mefenamic acid for the treatment of primary dysmenorrhea

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A. wilhelmsii is used to regulate the menstrual cycle and reduces bleeding and pain in folk medicine [1]. We compared the effect of the powder of flowering aerial parts of *A. wilhelmsii* with mefenamic acid on primary dysmenorrheal pain. Randomized, double-blind and crossover trial was conducted in seventy single and sexually inactive female students (aged 26 ± 2) who had primary dysmenorrhea, regular menstrual cycles, and pain score of 5 or higher on Visual Analog Scale. The study was conducted over 3 menstrual cycles as follows: at cycle 1 (placebo) pain severity was measured in first day of menstruation; at cycle 2, the volunteers were randomly assigned to take *A. wilhelmsii* (1000 mg) or mefenamic acid (250 mg) at recommended doses as needed; at cycle 3, the volunteers switched treatments. The pain intensity was recorded at regular scheduled intervals (1, 2, 3 and 6 h) after taking the medications. Compared with placebo both mefenamic and *A. wilhelmsii* decreased the pain score significantly ($p < 0.001$). But the pain relief induced by *A. wilhelmsii* was high ($p < 0.01$). The menstrual blood loss ($p = 0.02$), signs of dysmenorrhea ($p = 0.001$), the duration of bleeding and pain ($p = 0.001$) in *A. wilhelmsii* treated group were less than of mefenamic acid. The duration of self-medication for mefenamic acid was 167 ± 108 min and for *A. wilhelmsii* 99 ± 82 ($p < 0.0001$). The number of capsules chosen by patients was 1.7 ± 0.8 and 2.1 ± 0.7 ($p < 0.0001$) for the plant and mefenamic acid, respectively. *A. wilhelmsii,* when taken in recommended doses, was more effective in alleviating pain and bleeding associated with primary dysmenorrhea than mefenamic acid. **Reference:** [1] Javidnia, K. et al. (2004) DARU: Journal of the school of pharmacy, Medical Sciences University of Tehran 12:63 – 66.

PJ25 Phytochemical characterisation of the leaves of *Rhododendron ferrugineum* L.

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Rhododendron ferrugineum L. (Ericaceae) is a European alpine plant traditionally used for rheumatic disorders, gout and accumulation of renal calculi. Due to insufficient phytochemical characterisation and a report on animal poisoning after oral ingestion of the plant, in 1990 the Kommission E published a negative monograph. Aim of the present study was the phytochemical characterisation of the main compounds from the leaves of *Rhododendron ferrugineum* L. The essential oil was obtained with 7.35 mL/kg from the lyophilized, powdered herb by steam distillation. α-pinene, *ar*-curcumene, α- and β-selinene were identified as main components, comprising 53.7% of the total oil. The total amount of tannins in the leaves was determined with 3.2% (Ph. Eur. method). An acetone/water extract was fractionated on Sephadex®LH20, MCI®-CHP20P, RP-18 and FCPC which led to the isolation and identification (1D-, 2D-NMR-experiments, ESI-MS) of chlorogenic acid, flavan-3-ols (catechin, epicatechin, gallocatechin, epigallocatechin), procyanidins B1 to B4 and flavonoids (*trans*-taxifolin-3-O-β-L-arabinoside, quercetin-3-O-(6''-O-acetyl)-β-D-glucoside, hyperosid, isoquercitrin, quercetin-3-O-α-L-arabinoside and the until now not described dihydromyricetin-3-O-β-L-arabinoside). Investigation on the carbohydrates contained in the leaves of *Rhododendron ferrugineum* L. yielded a total amount of 1% ethanol-precipitable polysaccharides (mainly arabinogalactans) and AGPs, oligomeric fructan 3.0%, glucose 4.3%, fructose 1.9%, sucrose 2.9%, raffinose 0.8% and stachyose 0.1%.

PJ26 *Quercus ilex* L.: a rich source of polyacylated flavonoid glucosides

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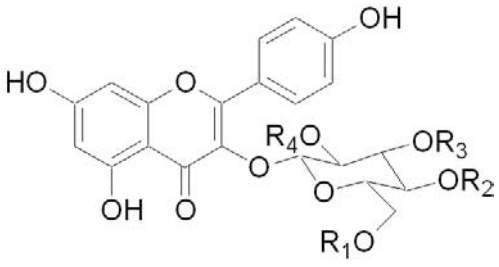
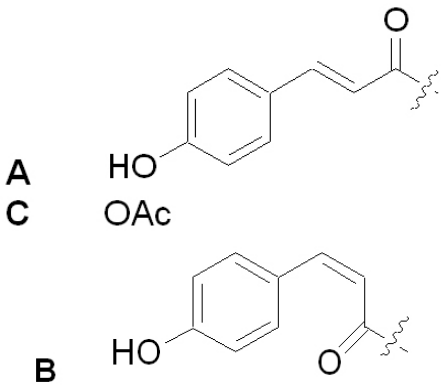
From the methanolic extract of *Quercus ilex* leaves a series of acylated flavonol glucosides (**1-10**) were identified, among them five new naturally occurring compounds. The constituents, which were all *p*-coumaroyl glucosides of kaempferol, were characterized either as pure compounds either as inseparable, complicated mixtures of *cis* and *trans* isomers. Their complete structure elucidation was done by 2D NMR (COSY, HSQC, HMBC, ROESY) and HPLC-DAD-MS analyses. 2D NMR spectral data allowed the discrimination between different isomers. Quantitative analysis of the methanolic extract of the plant revealed that it is a rich source of acylated flavonoid glucosides (1.22%). Under the experimental conditions chosen HPLC-DAD-MS analyses showed that *cis* isomers are less polar than *trans* isomers and their detailed identification, the first in the literature so far, could serve as a tool for the detailed characterization of analogous isomers by HPLC-DAD-MS in other complicated plant extracts.





1	R_1 = A, R_2 = H, R_3 = H, R_4 = H
2	R_1 = B, R_2 = H, R_3 = H, R_4 = H
3	R_1 = A, R_2 = H, R_3 = H, R_4 = A
4	R_1 = A, R_2 = H, R_3 = H, R_4 = B
5	R_1 = B, R_2 = H, R_3 = H, R_4 = B
6	R_1 = A, R_2 = C, R_3 = H, R_4 = A
7	R_1 = A, R_2 = C, R_3 = H, R_4 = B
8	R_1 = B, R_2 = C, R_3 = H, R_4 = B
9	R_1 = A, R_2 = C, R_3 = C, R_4 = A
10	R_1 = B, R_2 = C, R_3 = C, R_4 = B

PJ27 Taxonomic distribution of thymoquinone and related compounds in selected plant species

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Because of their significant biological activities, such as anti-inflammatory [1], anti-microbial [2], or anti-tumor [3] effects, in recent years a growing attention has been devoted to plant-derived quinones dithymoquinone (DTQ), thymohydroquinone (THQ), and thymoquinone (TQ). At present, a well-known natural source of TQ is the oil from the seeds of *Nigella sativa* [4]. In our study, twenty-one plant species were analyzed for the content of DTQ, THQ, and TQ with the aim to compare their quantities and taxonomic distribution in plants. The dried plant material was extracted with *n*-hexane; the analytes were reextracted to methanol and quantified by capillary GC with a flame-ionization detector (identity confirmation by GC/MS). The results of analysis were as following ($mg.kg^{-1}$ of dried matter): *Monarda didyma* 98 (DTQ), 1811 (THQ), 1905 (TQ), *Nigella sativa* (seeds) 38 (DTQ), 76 (THQ), 1597 (TQ), *Satureja hortensis* 16 (THQ), 54 (TQ), *Satureja montana* 88 (THQ), 152 (TQ), *Thymus serpyllum* 15 (THQ), 44 (TQ), and *Thymus vulgaris* 21 (THQ), 143 (TQ). Trace amounts of TQ (< $10\ mg.kg^{-1}$) were found in *Eupatorium cannabinum* and *Juniperus communis*. Thus, we confirmed previously reported presence of TQ in following four families: Asteraceae, Cupressaceae, Lamiaceae, and Ranunculaceae. According to the above presented results, *Monarda didyma* can be recommended as an alternative source of TQ and related compounds, especially in the climatic conditions of moderate zone. *Acknowledgements: Ministry of Education, Youth and Sports of the Czech Republic (research project MSM 6046070901).* **References:** [1] Maršík, P. et al. (2005) Planta Med. 71:739 – 742. [2] Mouhajir, F. et al. (1999) Pharm. Biol. 37:391 – 395. [3] Worthen, D.R. et al. (1998) Anticancer Res. 18:1527 – 1532. [4] Salem, M.L. (2005) Int. Immunopharmacol. 5:1749 – 1770.

PJ28 *In vitro* multiplication of *Glycyrrhiza glabra* L. through somatic embryogenesis

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Licorice (*Glycyrrhiza glabra* L., Fabaceae) is a well known important medicinal plant. The roots and stolons, as well as extracts thereof, are used for the treatment of various disorders. Of predominant importance

are the antiulcus/antiphlogistic, and the spasmolytic activity, which are attributed to triterpene saponins (glycyrrhicinic acid and derivatives) and flavonoids (liquiritin, isoliquiritin and their aglycones), respectively [1]. According to the purposed use, it would be useful to have to disposal different licorice genotypes with a respective composition of the active compounds. Although licorice is routinely cultivated, it is well known that propagation through conventional methods like e.g. cuttings is slow, when compared to in vitro-techniques. With the aim to develop an *in vitro* protocol for the rapid multiplication of selected genotypes, in our study we chose the method of somatic embryogenesis, because of its potential for scale-up [2]. Cotyledon explants of 7 day old seedlings proved to be best suitable to establish callus cultures. As for the formation of embryogenic callus, the growth regulator TDZ was superior to 2,4-D or picloram, and resulted in vigorous growth of embryogenic callus. For embryo maturation, subculture on nutrient medium without growth regulators gave best results of more than 80 embryos per gram of inoculated callus tissue. Within this study, the genotype did not significantly influence the embryogenic potential. Through this protocol, the large scale clonal propagation of selected genotypes of *Glycyrrhiza glabra* is feasible, allowing for the production of plantlets of defined quality for further field culture. **References:** [1] Wichtl, M. (2009) Teedrogen und Phytopharmaka. 5th edition. Wissenschaftliche Verlagsgesellschaft mbH. Stuttgart, Germany. [2] George, E.F. (2008) Plant Propagation by Tissue Culture. 3 rd edition. Springer. Dordrecht, The Netherlands.

PJ29 Comparative effects of a valerian extract and single compounds on sleep and body temperature in mice evaluated by telemetry

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Traditional use of *Valeriana officinalis* L. suggests sleep promoting properties, yet contemporary observations in clinical trials and rodent models using the extract and isolated compounds are contradictory [1,2]. We evaluated locomotor activity and body temperature of mice using telemetry to obtain evidence of sleep promoting effects. This method provides a reduced variable environment which improves upon previous methodologies. A 70% ethanolic extract of *Valeriana officinalis* root (250, 500, and 1000 mg/kg) was administered orally and data recorded for 180 minutes thereafter in male C57BL/6J mice. Oral administration of valerian extract had no effect on locomotor activity and body temperature compared to vehicle. Zolpidem (5 mg/kg, positive control) significantly decreased locomotor activity by 57% (activity counts after 30 min; control: 492.1 ± 41.8, zolpidem: 212.6 ± 44.2; $p < 0.001$) and body temperature by 0.57 °C (ΔT_{max} at 18 minutes, control: 36.53 ± 0.12 °C, zolpidem: 35.96 ± 0.13 °C; $p < 0.01$) whereas caffeine (5 mg/kg, negative control) induced an increase in activity of 47% (activity counts after 30 minutes; control: 492.1 ± 41.8, caffeine: 725.1 ± 76.4; $p < 0.01$) without affecting body temperature. In conclusion, telemetry is a simple, adequate method for the specific measurement of sleep promoting effects. The extract showed no significant difference to vehicle; yet, further studies on single compounds may help substantiate the use of *Valeriana officinalis* as insomnia treatment. **References:** [1]Hattelstohl, M. et al. (2008) Phytomedicine 15:2 – 15. [2] Fernández, S. et al. (2003) Pharmacol. Biochem. Behav. 77:399 – 404.

PJ30 Raunitidine isolated from *Duroia macrophylla* (Rubiaceae)

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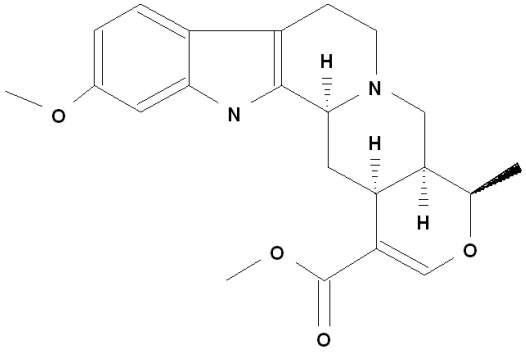
Duroia macrophylla Huber is a tropical tree, known as "puruí", which occurs in the Amazon region. Their fruits can be eaten and, as we know, no chemical study has been performed before. Leaves of *D. macrophylla* were dried at room temperature, ground and extracted with dichloromethane, methanol and later with water, by using ultra-sound for 20 minutes, each twice repeated and concentrated with reduced-pressure evaporator or liophylizer. The methanolic extract was fractioned by using chromatographic techniques and HPLC for further purification. The chemical identification of the indolic alkaloid raunitidine was achieved by NMR and MS data analyses and literature comparison [1].



Acknowledgements: PPBio/INPA/MCT; CNPq; FAPEAM. **References:** [1] Merlini, L. et al. (1976) Helv. Chim. Acta 59:2254 – 2260.

PJ31 Cannabinoid receptor Gα fusion proteins as a highly sensitive model system for characterization of receptor ligands

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So far two human cannabinoid receptors (hCBRs) have been identified [1], both belonging to the family of G-protein coupled receptors (GPCRs): the hCB1R [2] is mainly located in the brain and the hCB2R [3] is predominantly located in the periphery on immune cells. Because of their involvement in many physiological functions, such as movement, metabolic regulation, host defense, analgesia and memory, there is a great interest in targeting these receptors for therapeutic applications. For the search for new CBR ligands, we refined an existing *in vitro* assay [4] that allows for the differentiation of the pharmacological properties of a compound. In the already established steady-state [γ-^{32}P]-GTPase assay *Spodoptera frugiperda* (SF9) cells were used for the co-expression of the CBR, the Gα-subunit and the Gβγ-heterodimer. Because the expression levels and the density of these proteins in the cell membrane influence the efficiency of the interaction between the receptor and the G proteins, we improved this assay using CBR-Gα fusion proteins. With these fusion proteins we can ensure a close proximity and defined stoichiometry of the signalling partners, resulting in higher coupling efficiency than the conventional co-expressing system. This very sensitive test system enabled us to detect an agonist at the CBRs in a matrix of other compounds. Therefore we added Δ^9-THC to a Δ^9-THC-free *Cannabis sativa* extract and found the expected increase of potency (e.g. extract $logEC_{50}$ -6,08 vs. extract enriched with Δ^9-THC $logEC_{50}$ -6,86 at the CB_1R and extract $logEC_{50}$ -5,86 vs. extract enriched with Δ^9-THC $logEC_{50}$ -6,38 at the CB_2R). **References:** [1] Howlett, A.C. et al. (2002) Pharmacol. Rev. 54:161 – 202. [2] Matsuda, L.A. et al. (1990) Nature 346:561 – 564. [3] Munro, S. et al. (1993) Nature 365:61 – 65. [4] Egger, M. et al. (2008) Chem. Eur. J. 14:10978 – 10984.

PJ32 Catechin-derivates affinity for human cannabinoid receptors

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Flavonoids are common secondary plant metabolites and possess manifold health-enhancing effects. In addition to neuroprotective and anti-inflammatory activities, growing evidence suggests that flavonoids may

also possess analgesic properties [1]. These functionalities make a role for cannabinoid receptors (CB) in mediating biological effects possible [2]. The present study examines affinities of five catechin derivates for human CB1 and CB2. Using membrane preparations of recombinant human cannabinoid receptors 1 and 2, the affinities of (-)-epigallocatechin gallate, (-)-epicatechin gallate, (-)-epigallocatechin, (-)-epicatechin and (+)-catechin were studied by radioligand binding assays. Dose dependent binding to CB1 and CB2 was noted for all compounds under study. Catechin derivates differed in their affinity for CB1 by several orders of magnitude, ranging from the mid-micromolar range for (-)-epigallocatechin gallate and (-)-epicatechin gallate (K_i < 50 μM), to the millimolar range for (-)-epicatechin and (+)-catechin (K_i > 2.5 mM). Affinities for CB2 were comparable to CB1 results. Competitive radioligand binding assays identified (-)-epigallocatechin gallate and (-)-epicatechin gallate as ligands with moderate affinity to human CB2 (K_i < 150 μM). Very weak inhibition constants in the millimolar range were obtained for (-)-epigallocatechin, (-)-epicatechin and (+)-catechin. Further characterization of lead compounds is initiated to determine in more detail the structural correlates of CB bioactivity. **References:** [1] Nahrstedt, A. et al. (2007) Wien Med. Wochenschr. 157:348 – 351. [2] Di Marzo, V. et al. (2004) Nat. Rev. Drug Discov. 3:771 – 784.

PJ33 Antibacterial and composition of the essential oil of *Dracocephalum subcapitatum* from Iran

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The chemical composition of the essential oil obtained by hydrodistillation and its antibacterial activity from the aerial flowering parts of *D. subcapitatum* collected from Iran was analyzed for the first time by GC and GC-MS and tested. Monoterpenoids including oxygenated and hydrocarbons comprising 71.8 and 26.1% were the main compound groups of the essential oil, respectively. Totally, 22 components accounting for 98.2% of the total oil were characterized [1]. Perilla aldehyde (64.8%) and limonene (22.9%) were determined as the major constituents. Antibacterial activity of the essential oil of *D. subcapitatum* was performed against six Gram-positive and Gram-negative bacteria (*Bacillus pumilis, Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*) by disc diffusion method and determining minimum inhibitory concentration (MIC) [2,3]. The results of the bioassays showed that the oil exhibited high antibacterial activity against all tested bacteria. Gram-positive bacteria were more sensitive strains with MIC values ranged 0.15 – 0.3 mg/ml. *Pseudomonas aeruginosa* was the most resistant strain with MIC value of 5.0 mg/ml. **References:** [1] Adams, R. (2007) Identification of essential oil components by gas chromatography/quadropole mass spectroscopy. Allured Publishing Corporation, Carol Stream. [2] Baron, M.S. and Finegold, S.M. (1990) Diagnostic Microbiology. Mosby Co., Baltimore. [3] NCCLS (1999) Performance Standards for Antimicrobial Susceptibility Testing. Wayne, Philadelphia.

PJ34 Effects of light and differentiation on gingerol and zingiberene production in cultured cells of *Zingiber officinale*

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Ginger (*Zingiber officinale* Roscoe) is a perennial plant with aromatic odour and a pungent taste. It has not been cultivated in Iran yet. The most important compounds, responsible for Ginger's therapeutic activity including treat nausea due to pregnancy, after surgery or chemotherapy, motion sickness are related to gingerols and zingiberene [1]. A number of chemical and physical factors that could influence secondary metabolism in *Z. officinale* cell cultures have been found [2]. In this study the effect of light and differentiation were evaluated. A sterile *in vitro* plant was obtained on MS medium. Then different explants from the sterile ginger were cut and inoculated on mediums suitable for callus growth. One group stored at 16/8 light cycle and the other at continuous dark environment. Then different samples from both groups in several stage of growth were collected and extracted with dichloromethane and analyzed by TLC. n-Hexan- diethyl ether (40:60) were used as solvent system. The accumulation of 6-gingerol and zingiberene was much higher in culture systems of *Zingiber officinale* in light treated samples where morphological differentiation was apparent as presented in the following table. So, light is a stimulatory factor for this secondary metabolite production.

Stage of Growth Kind of Treatment	Stage 1	Stage 2	Stage 3	Color
Dark	R_1 ~0.35	R_1 ~0.35	None	Light Violet
Light	-	R_1 ~0.2	R_1 ~0.2	Violet
	R_1 ~0.3	R_1 ~0.3	R_1 ~0.3	Light Violet
	R_1 ~0.35	R_1 ~0.35	R_1 ~0.35	Light Violet
	-	R_1 ~0.9	R_1 ~0.9	Dark Purple

References: [1] Mascolo, N. et al. (1989) J. Ethnopharmacol. 27:129 – 140. [2] Dörnenburg, H. (1995) Enzyme Microb. Tech. 17:674 – 684.

PJ35 Formulation of mangosteen (*Garcinia mangostana* L.) peel extract granules for the treatment of diarrhea

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Mangosteen (*Garcinia mangostana* L.) peel is used in Thai primary health care as an astringent to treat noninfectious diarrhea; for this, a beverage is prepared by boiling the dried peel of half a fruit (4 g) in water. Because this procedure is inconvenient and mangosteen is a seasonal fruit, the aim of this study was to formulate ready-to-use antidiarrheal granules from a mangosteen peel extract. Peels were extracted with ethanol: water (1:1) and the extract lyophilized (yield 21.7%). Quantity of (-)-epicatechin in the dried extract was determined by HPLC [1] as 11.38 mg/g. Formulations of granules and effervescent granules, containing 1 g of dried extract per dose, were prepared using cross-linked homopolymer of N-vinyl-2-pyrrolidinone (Crospovidone), polyvinylpyrrolidone K30, aspartame, lactose, sodium bicarbonate, sodium carbonate, citric acid monohydrate and tartaric acid as excipients [2]. Physical appearance, taste and disintegration of all formulations were evaluated. A suitable formulation was the granules consisted of 20% mangosteen peel extract, 3.5% Crospovidone, 2% polyvinylpyrrolidone K30, 0.0018% aspartam and lactose. The granules rapidly disintegrated in water within 60 seconds, showed good appearance as well as good taste. Physical stability and chemical stability in term of (-)-epicatechin quantity were evaluated after storing at room temperature and 45 °C under 75% RH for 1 month. *Acknowledgements: National Research Council of Thailand, Faculty of Pharmaceutical Sciences and Prince of Songkla University, Thailand.* **References:** [1] Tsanova-Savova, S. et al. (2005) J. Food Compos. Anal. 18:691 – 698 [2] Lachman, L. et al. (1986) Theory and Practice of Industrial Pharmacy. 3rd ed. Lea & Febiger, Philadelphia.

PJ36 The protective effect of safranal, a constituent of saffron, on subacute effects of the organophosphate insecticide diazinon in rats

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As reactive oxygen species have been implied in the toxicity of diazinon (DZN) [1], the protective effect of safranal as an antioxidant [2] was evaluated against DZN subacute toxicity. Rats administered intraperitoneally with doses of 0.025, 0.05 and 0.1 ml/kg safranal, were treated with DZN (20 mg/kg/day, orally) for 4 weeks. At a high dose of safranal (0.1 ml/kg), hematological indices such as Hct, Hgb and RBC ($P < 0.05$) were reduced when treated with DZN. Safranal at lower doses (0.025 and 0.05 ml/kg) decreased the higher reticulocyte index induced by DZN ($P < 0.05$). Also safranal at these doses reduced the elevated liver enzyme (SGOT, SGPT and GGT) and uric acid levels which were induced by DZN ($P < 0.05$). Safranal has no effect on histopathological changes in

liver, heart and lung and no effect on acetylcholine esterase enzyme changes in RBC induced by DZN. According to the present study, it is concluded that lower doses of safranal reduce some toxic effects of DZN on hematological and biochemical indices and that this effect is not mediated by acetylcholine esterase activity. **Reference:** [1] Sutcu, R. et al. (2007) Toxicol. Ind. Health 23:13 – 17. 2. Assimopoulou, A.N. et al. (2005) Phytother. Res. 19:997 – 1000.

PJ37 Hepatoprotective activity of *Stachys* extracts against CCl_4-induced hepatotoxicity in rats

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Plant species of the genus *Stachys* L. have been used traditionally in different conditions (headache, neuralgia, nervous conditions, dyspepsia, wounds healing and skin inflammation) and variety of pharmacological effects was proven for some of them [1,2]. Phytochemical studies of *Stachys* species revealed the presence of several secondary plant metabolites: different polyphenols (flavonoids, tannins, phenolic acids, phenylethanoid glycosides), iridoids, terpenoids and sterols [3]. In our continuation in investigating pharmacological activities of MeOH extracts of four endemic Balkan *Stachys* species (*S. beckeana* Dörfler & Hayek, *S. anisochila* Vis. et Pančić, *S. plumosa* Griseb., and *S. alpina* L. subsp. *dinarica* Murb.), their hepatoprotective activity was assayed. Hepatic damage in rats was induced using CCl_4, and monitored using levels of marker enzymes: aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in serum. The biochemical observations were supplemented by pathohistological examination of liver sections (liver damage score (LDS) for degenerative and vascular changes according to the 5-point semiquantitative scale). In rats who received CCl_4 (2.5 ml/kg body weight *s.c.*) a significant increase of serum levels of AST, ALT, and ALP was observed, along with massive lesions in liver tissue (LDS = 4.29). Four-day treatment with investigated *Stachys* extracts (200 and 100 mg/kg body weigh *p.o.*) significantly reduced altered biochemical parameters in intoxicated rats ($p < 0.001$). In addition, pathohistological evaluation of liver sections also pointed out lesser degree of degenerative and vascular changes. Extract of *S. alpina* subsp. *dinarica* showed the best and dose-related hepatoprotective effect. **References:** [1] Kukić, J. et al. (2006) Biol. Pharm. Bull. 29:725 – 729. [2] Kukić, J. et al. (2007) Pharm. Biol. 45:560 – 563. [3] Meremeti, A. et al. (2004) Biochem. Syst. Ecol. 32:139 – 151.

PJ38 Antimicrobial activity of selected extracts and some compounds from *Ruscus aculeatus* L., *R. hypoglossum* L. and *R. alexandrinus* Garsault (Ruscaceae)

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Here we report on the antimicrobial activity of some polar extracts of *Ruscus aculeatus* (MeOH, EtOAc and BuOH herb extracts and MeOH rhizome extract), the MeOH extracts of the *R. hypoglossum* and *R. alexandrinus* herbs, as well as of some compounds previously isolated from these *Ruscus* extracts (rutin, *p*-coumaric, caffeic, and dimethoxycinnamic acid) [1]. The modified microdilution technique [2,3] was used for testing on eight bacterial strains: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (humane isolate), *Listeria monocytogenes* (NCTC 7973), *Bacillus cereus* (humane isolate), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (ATCC 6538), and five fungi: *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6275), *Aspergillus fumigatus* (ATCC 9142), *Penicillium funiculosum* (ATCC 36839), and *Trichoderma viride* (IAM 5061). All tested extracts exhibited antimicrobial activity in a wide range of concentrations (0.1 – 4 mg/ml). Values of MICs and MBCs against bacterial strains were 0.1 to 2 mg/mL and 0.2 to 4 mg/mL, respectively. Antifungal activity was almost in the same range (MICs 0.25 to 2 mg/mL, and MFCs 0.5 to 3 mg/mL). EtOAc extract of *R. aculeatus* herb had the best antimicrobial effect. The four isolated compounds showed much better activity against tested microorganisms (MICs, MBCs and MFCs in a range 0.05 – 0.3 mg/mL, 0.05 – 0.4 mg/mL, and 0.1 – 0.3 mg/mL, respectively). Activity of isolated compounds was comparable with the activity of standard antibiotics: streptomycin (MICs and MBCs in a range 0.05 to 0.3 mg/mL) and ampicillin (MICs and MBCs in a range 0.1 to 0.5 mg/mL) and fungicides: bifonazole (MBCs and MFCs in a range 0.1 to 0.25 mg/mL) and ketoconazole (MICs and MFCs in a range 0.2 to 3 mg/mL, respectively). Results pointed out that these compounds mainly contribute to the obtained antimicrobial effects of the investigated *Ruscus* extracts. **References:** [1] Hadžifejzović, N. (2006) PhD Thesis. University of Münster, Germany. [2] Hanel, H. and Raether, W. (1998) Mycoses 31:148 – 154. [3] Daouk, R.K. et al. (1995) J. Food Protect. 58:1147 – 1149.

PJ39 Antimicrobial, anti-inflammatory and anti-ulcer activities of *Ferula heuffelii* root extracts

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Ferula heuffelii Griseb. ex Heuffel (Apiaceae) is an endemic and rare W. Moesian perennial species, predominantly growing in E. Serbia, and locally in S.E. Romania and E. Bulgaria [1]. Roots of this plant were extracted with $CHCl_3$ and then with MeOH. Antimicrobial activity of $CHCl_3$ and MeOH extracts was tested against 7 standard bacterial strains and two standard strains of yeast *Candida albicans* using the agar diffusion [2] and broth microdilution methods [3]. The best inhibitory effect (MIC = 12.5 µg/ml) $CHCl_3$ extract exhibited against *Staphylococcus aureus*, and MeOH extract against *S. aureus* and *Micrococcus luteus*. In assessing anti-inflammatory activity, the carrageenan-induced rat paw oedema test was used [4]. MeOH and $CHCl_3$ extracts showed significant dose dependant anti-inflammatory effect (in dose of 100 mg/kg p.o. extracts reduced oedema with 84.00% and 64.71%, respectively). These effects were comparable with that of indomethacin (76.00% in dose of 8 mg/kg p.o.). Extracts inhibited ethanol-induced gastric ulcers in rats [4] and their activity was comparable with the activity of ranitidine used as a positive control. Gastric damage score for the animals treated with MeOH and $CHCl_3$ extracts (in dose of 100 mg/kg p.o.) was 0.50 ± 0.55 and 0.25 ± 0.42, respectively, while the score for the animals treated with ranitidine (in dose of 20 mg/kg p.o.) was 0.58 ± 0.49. Statistical analysis was performed by Student's t test for antimicrobial activity and Mann-Whitney *U*-test for anti-inflammatory and anti-ulcer activity. Preliminary LC-MS analysis revealed the presence of several analogs with elemental composition of $C_{24}H_{30}O_4$. Analysis of tandem mass spectra of these metabolites suggested that they are likely sesquiterpene coumarins. **References:** [1] Nikolić, V. (1973): *Ferula* L. In: Josifović, M. (ed.): Flora SR Srbije 5:274 – 276, SANU, Beograd. [2] Acar, J.F. and Glodstein, F.W. (1996) In: Lorian, V. (ed) 4th Ed. Williams & Wilkins. Baltimore. [3] Candan, F. et al. (2003) J. Ethnopharmacol. 87:215 – 220. [4] Đorđević, S. et al. (2006) J. Ethnopharmacol. 109:458 – 463.

PJ40 Chemical investigation and antimicrobial properties of mastic water and its major constituents

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A phytochemical and antimicrobial study of mastic water was carried out for the first time. Mastic water, which is obtained together with mastic essential oil (MEO) during the distillation of mastic gum (the resin of *Pistacia lentiscus* L. var. *Chia*), was passed through XAD4 resin and the adsorbed organic compounds were eluted with diethylether, affording the mastic water extract (MWE). The extract was analysed with chiral GCMS and the major compounds identified were verbenone (12.90%), α-terpineol (12.09%), linalool (7.29%) and *trans*-pinocarveol (5.97%). The antimicrobial activity of MWE, of its major constituents and of MEO was investigated. The antimicrobial effect was tested against Gram+ and Gram- bacterial strains and *Candida* spp. For the experimentations, wild strains resistant to antimicrobials and ATCC strains were used. Also the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined for each compound. Examination of the antimicrobial activity of each compound or extract was performed by the agar diffusion method against three *Escherichia coli* strains (Gram-), three *Staphylococcus aureus* strains (Gram+) and three *Candida* spp. strains (yeast). The MIC and MBC were performed by employment of a microtiter method and subsequent inoculation in Mueller Hinton agar. The MWE, (-)-*trans*-pinocarveol, (-)-linalool, (±)-linalool and α-terpineol exhibited considerable antimicrobial activity in both Gram+ and Gram- bacterial strains. The average inhibition zones measured for *E. coli* and *S. aureus* were 9.2 mm and 10 mm for (+)-α-terpineol, 10.9 mm and 9.8 mm for (±)-linalool. Sufficient antifungal activity was exhibited by MEO (8.3 mm for *C. albicans*). The most potent antimicrobial agent was (±)-linalool followed by α-terpineol. **References:** [1] Koutsoudaki, C. et al. (2005) J. Agric. Food Chem. 53:7681 – 7685. [2] Magiatis, P. et al. (1999). Planta Med. 65:749 – 752.

PJ41 Effects of calcium, W-7, and forskolin on flavonoid accumulation in cell cultures of *Hypericum androsaemum* L.

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Hypericum androsaemum L. is a common European species whose aerial parts have been used in traditional medicine due to its diuretic and hepatoprotective properties [1]. The diverse flavonoids and phenolic acids found in the plant are thought to be responsible for such biological effects. Recently, cell cultures established from hypocotyl-derived callus of *H. androsaemum* were shown to accumulate small amounts of flavonoids, with maximum levels being reached on the 14th day of the growth cycle [2]. Treatment of 11-day-old cultures for 72 h with 15 mM $CaCl_2$ or 5 μM calcium ionophore A23187 increased considerably the accumulation of flavonoids and the activity of phenylalanine ammonia-lyase (PAL), a key regulatory enzyme of phenylpropanoid metabolism assayed according to [3]. On the other hand, pretreatment of cultures with the calmodulin antagonist W-7 (50 μM) suppressed the Ca^{2+} induced rise in flavonoid contents without any accompanying decrease in PAL activity levels. Furthermore, the addition of the adenylate cyclase activator forskolin (20 μM) to control cultures also enhanced the accumulation of flavonoids, but had no significant effect on the activity of PAL. The results point to a possible involvement of Ca2+/calmodulin-dependent and cAMP-dependent processes in the biosynthesis of flavonoids by *H. androsaemum* cell cultures and also indicate that changes in the accumulation of these compounds can occur independently of changes in PAL activity. *Acknowledgements: Center of Pharmaceutical Studies* **References:** [1] Novais, M. et al. (2004) J. Ethnopharmacol. 93:183 – 195. [2] Paranhos, A. (2006) Planta Med. 72:1060 – 1061. [3] Mori, T. et al. (2001) Plant Sci. 160:355 – 360.

PJ42 Levels of the antioxidant melatonin in fruits of edible berry species

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Melatonin (N-acetyl-5-methoxytryptamine) was originally identified as an animal hormone. It was later found also in many plants. It is a free radical scavenger and antioxidant and also activates some antioxidant enzymes. Evidence, mainly from animal models, suggests that melatonin administration may help to prevent or cure diseases associated with oxidative stress, including neurodegenerative diseases, which frequently occur during aging. (See [1] for review.) Melatonin consumed in edible plants may thus have beneficial effects on human health. It has yet to be shown whether consumption of melatonin in plant-based food increases melatonin levels in humans. However, rats fed with walnuts had increased melatonin blood levels and increased total antioxidant capacity of blood serum [2]. Berries are popular fruits but melatonin levels in most of them are unknown. We therefore investigated melatonin levels in several edible berries. For sample preparation, we developed a method based on Tris/HCl buffer extraction (modified from [3]) followed by partitioning with chloroform and solid-phase extraction using C_{18} cartridges. Melatonin was determined by liquid chromatography – tandem mass spectrometry. Melatonin was measured in fresh fruits of 10 species: blueberry (*Vaccinium myrtillus*), goldenberry (*Physalis peruviana*), blackberry, elderberry (*Sambucus nigra*), grape, highbush blueberry (*Vaccinium corymbosum*), raspberry (*Rubus idaeus*), red currant, cranberry (*Vaccinium vitis-idaea*), and strawberry. Levels ranged between 7 and 48 $pg.g^{-1}$ fresh weight, being the highest in *Vaccinium myrtillus* (48 $pg.g^{-1}$), *Physalis peruviana* (41 $pg.g^{-1}$), and blackberry (21 $pg.g^{-1}$). *Acknowledgements: Supported by grant COST OC 154 by MSMT CR.* **References:** [1] Pandi-Perumal, S. R. et al. (2006) FEBS J. 273:2813 – 2838. [2] Reiter, R. J. et al. (2005) Nutrition 21:920 – 924. [3] Poeggeler, B. et al. (1991) Naturwissenschaften 78:268 – 269.

PJ43 High pressure extraction of plant materials with near critical liquid carbon dioxide

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Different plant materials including thyme (*Thymus vulgaris* L.), Kekik (*Thymus longicaulis* C Preisl.) and Savory (*Satureja montana* L.), were extracted by liquid CO_2 under liquid-vapour conditions near its critical conditions. The apparatus used is shown in figure 1. The liquid CO_2 extracts were compared with hydrodistillates using Thin Layer Chromatography as described in British Pharmacopoeia [1]. Some compounds were identified based on the R_f values given in [1]. For each plant was observed, that every compound present in hydrodistillate, was also present in the CO_2 extracts, and the chromatograms of CO_2 extracts showed the presence of other unidentified compounds as well. The quantitatively determined yields of the CO_2 extractions were 2.5 to 4.9 times larger than the yields of hydrodistillations. For the extraction of the plant materials approximately 4 hours were needed and for the hydrodistillation 6 hours. The yield of the extractions as function of time could be described with a simple equation.

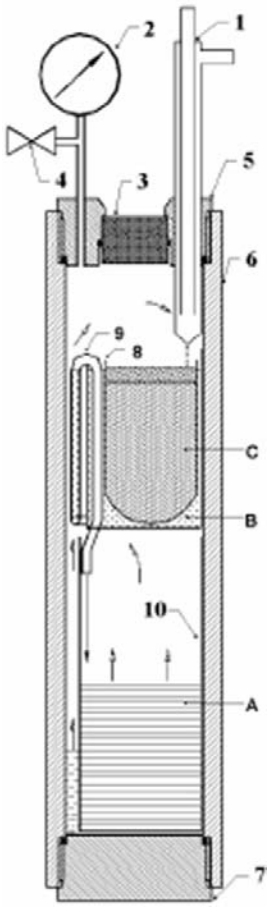

Figure 1: Apparatus for the high pressure extraction with liquid CO_2 under liquid – vapour equilibrium conditions. 1. Cooling finger; 2. Pressure gauge; 3. Sapphire Window; 4. Valve; 5. Upper cover; 6. Steel Cylinder; 7. Bottom cover; 8. Extracting thimble; 9. Syphone; 10. Product droping glass; A. Product; B. Extract solution; C. Plant material

Reference: [1] Bernan, British Pharmacopoeia 2003 & British Pharmacopoeia Veterinary, Bernan Association, Monographs: Medicinal and Pharmaceutical substances Thyme Oil, f.1534.

PJ44 Anti-inhibitory effect of eupatilin and jaceosidin from *Artemisia princeps* Pampini

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Main constituents, eupatilin and jaceosidin from *Artemisia princeps* Pampanini (family Asteraceae), which is widely used as a hepatoprotective, antioxidative, anti-inflammatory, and antibacterial agent, in a herbal medicine, were isolated and their inhibitory effects against carrageenan-induced inflammation in air pouch prepared in the back of mice were investigated [1 – 3]. These constituents significantly reduced inflammatory markers, leukocyte number and protein increased by carrageenan in the exudates of air pouch. These constituents did not only inhibited cyclooxygenase-2 expression and nuclear factor-kappa B activation by immunoblot analysis, but also markedly reduced tumor necrosis factor-α and prostaglandin E2 levels by enzyme-linked immunosorbent assay. Eupatilin and jaceosidin also inhibited hind paw edema induced by carrageenan. These findings suggest that eupatilin and jaceosidin potently express anti-inflammatory effect by the inhibition of transcription factor nuclear factor-kappa B activation. **Reference:** [1] Kim, S.H. et al. (1997) Res. Commun. Mol. Pathol. Pharmacol. 97:165 – 170. [2] Park, S.C. et al. (2006) J. Gastroenterol. 41:772 – 778. [3] Kim, J.Y. et al. (2005) J. Toxicol. Environ. Health A 68:2063 – 2080.

PJ45 A promising method for efficient analysis of secondary metabolites in plant extracts by a matrix-free Desorption/Ionization on self-Assembled Monolayer Surfaces (DIAMS) technique

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Plants are one of the major sources for the biologically active organic compounds and play a key role in medicinal chemistry for the treatment of various diseases [1]. DIAMS method is able to determine the secondary metabolites of complex vegetal extracts. The high throughput analyses of vegetal extracts are relatively difficult to perform in MALDI mass spectrometry, since the preparation of the sample involves the co-crystallization of the matrix with the analyte. Moreover irradiation of the matrix ion produces many low-m/z *vs* high-intensity ions preventing the detection of low molecular weight molecules such as secondary metabolites. We have developed a matrix-free alternative to MALDI analyses by the means of an original *desorption/ionization on self-assembled monolayers surfaces* (DIAMS) technique [2]. Monolayers were formed by using novel thiophene and coumarin-triazole analogues that absorbs the laser beam at 337 nm. We herein disclose our findings with respect to the DIAMS method which is well suitable for the detection and quantification of the low molecular weight compounds that are present in plant extracts. Some of the isoquinoline alkaloids from the root extracts of *Thalictrum flavum* have been detected by the DIAMS method. Indeed, this technique would be promising suitable for the qualitative and quantitative analysis of polar and non-polar organic components that are widely distributed in the plants, without any preliminary chromatographic resolution [3]. *Acknowledgements: Financial support of Agence nationale de la recherche (ANR).* **References:** [1] Newman, D.J. et al. (2007) J. Nat. Prod. 70:461 – 477. [2] Sanguinet, L. et al. (2006) J. Mass Spectrom. 41:830 – 833. [3] Bounichou, B. et al. (2008) J. Mass Spectrom. 43:1618 – 1626.

PJ46 Supercritical fluid fractionation for thymoquinone enrichment in black cumin and savory isolates

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Supercritical fluid extraction (SFE) using carbon dioxide has been widely studied as a modern, environmentally friendly method to separate high-added value compounds from plants that allows dissolving preferably the required compounds by changing extraction pressure and temperature. Thymoquinone (TQ) is a pharmacologically significant compound which has potential use for the treatment of colon cancer and inflammatory and asthma diseases. In recent studies [1,2] we have shown that with help of this "green" technology, it is possible to obtain extracts with TQ concentration and biological activity significantly higher than those isolated by conventional separation methods. This work is focused on the enhancement of TQ concentration in volatile oil using SFE of black cumin (*Nigella sativa* L.) seeds and savory (*Satureja hortensis* L.) aerial parts. Black cumin volatile oil is one of the richest sources of TQ contrary to savory, where TQ is the minor component. For both materials the volatile oil composition in extracts was determined using GC-MS and GC-FID. It was found to be strongly influenced by extraction pressure (12 – 28 MPa) and solvent composition (0 – 4.7 wt. % of acetone in CO_2). The highest concentration of TQ in savory extracts, 1.5 wt. %, was obtained at 28 MPa, 50 °C and 4.7 wt. % of acetone. On the contrary, in case of black cumin, the use of mild conditions (12 MPa, 50 °C and 0 wt. % acetone) allowed us to separate the volatile oil from fatty oil (almost 40% of seeds). A high concentration of TQ in the extract, 20 wt. %, was reached using 3.7 g CO_2/g seeds. *Acknowledgement: The authors thank the Ministry of Education, Youth and Sports (Project No. 2B06049) and the Czech Science Foundation (Project No. 525/08/1179) for financial support.* **References:** [1] Kokoska, L. et al. (2008) J. Food Prot. 71:2475 – 2480. [2] Pavela, R. et al. (2008) Appl. Entomol. Zool. 43:377 – 382.

PJ47 HS-SPME-GC-MS method development of volatile constituents from *Achillea collina*

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A. collina is a tetraploid proazulenes-containing species of the *Achillea millefolium* aggregate (yarrow) cultivated in alpine areas. Yarrow is commonly used as a medicinal plant for its digestive, anti-inflammatory, analgesic and antipyretic properties. Terpenes, especially sesquiterpenes, are considered to be mostly responsible for its bioactivity [1]. The emitted volatile fraction plays a central role in plant-environment interaction, being involved in very important processes in plant life cycle, such as reproduction, defense, communication, etc. [2]. In this work, a valuable method for accurate screening of volatile compound emissions from *A. collina* plants (e.g. leaves, flowers and stems) is presented, and the opportunity to use it to evaluate variations caused by plant-insect interactions (e.g. aphids), is discussed. Headspace Solid-Phase Microextraction (HS-SPME) method was developed and integrated with Gas Chromatography-Mass Spectrometry (GC-MS). Three types of SPME fibers including PDMS, PDMS-DVB and DVB-CAR-PDMS were investigated and a best extraction was achieved with the mixed fiber DVB-CAR-PDMS. Parameters for HS-SPME in term of extraction temperature, extraction time, sample amount and desorption time were also investigated showing that 120 min at room temperature from 2.5 g of sample given the best results, while 240 min at room temperature were chosen as the best for the "in vivo "sampling. As a result, 100 compounds were identified from the plant materials. The main components were camazulene (19%), δ-cadinene (5.21%), followed by β-myrcene (4.5%) and trans-β-caryophyllene (2.9%). The present method is simple, rapid and effective and can be applied for the analysis of volatile compounds not only in herbs derived drugs but also in "in vivo" plants supporting phytochemical and physiological studies. **References:** [1] Benedek, B. et al. (2007). Chem. Biodivers. 4:849 – 857. [2] Pareja, M. et al. (2007). J. Chem. Ecol. 33:695 – 710.

PJ48 Flavonoids and antioxidant activity in flowers of *Crataegus*

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In Mexico, are located 15 species of the genus *Crataegus* (Rosaceae) known locally as '*Tejocote*', but which have not been studied phytochemically. The pharmacological and clinical effects of the leaves, flowers and fruits are attributed to its flavonoids and proanthocyanidins [1]. The aim of this work was to identified the flavonoids and evaluate antioxidant activity of extracts of flowers from *Crataegus.* Flowers were collected from the genetic collection at the experimental station of the Autonomus Chapingo University. Flavonoids were identified by Liquid Chromatography-Mass Spectrometry and the test of antioxidant activity was made by the method of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay [2]. Four flavonols glycosides were identified in the flowers from six accessions of '*tejocote*'. Accessions 6 and 52 presented the major antioxidant activity (93.87 and 94.35% of $DPPH_{(red)}$). In both were identified quercetin 3-O-rhamnoside, this compound was most abundant in the two accessions (98.55 and 72.64%, respectively). Among accessions, the highest antiradical efficiency (AE_{50}= 1.266) was observed in the accession 52, the lowest in the accession 48 (2.1759), however, quercetin presented of AE_{50}= 0.293. The presence of a sugar moiety is important in increasing the rate and extent of absorption in comparison with the aglycon quercetin [3]. These results explain some of the medicinal properties of '*tejocote*' and contribute to chemotaxonomical knowledge of the genus. **References:** [1] Chang, Q. et al. (2002) J. Clin. Pharmacol. 42:605 – 612. [2] Sánchez-Moreno, C. (2002) Food Sci.Tech. Int. 8:121 – 137. [3] Harborne, J.B. and Williams, C.A. (2000) Phytochemistry 55:481 – 504.

PJ49 Antioxidant phenolic compounds from *Sedum dasyphyllum* L.

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Twelve crude extracts from six Swiss plants of the family Crassulaceae were submitted to rapid TLC tests against DPPH and acetylcholinesterase. *Sedum dasyphyllum* L., which showed interesting activities against these two targets, has been studied. The chemical investigation of the methanol extract from the whole plant afforded the new flavonols kaempferol 3-*O*-α-rhamnoside-7-*O*-β-sophoroside, gossypetin 3,7-di-*O*-β-glucoside-8-*O*-β-glucuronide, herbacetin 3,7-di-*O*-β-glucoside-8-*O*-β-glucuronide, herbacetin 3-*O*-β-(3''-acetylglucoside)-7-*O*-β-glucoside-8-*O*-β-glucuronide, herbacetin 3-*O*-β-(3''-acetylglucoside)-8-*O*-β-glucuronide, and hibiscetin 3-*O*-β-glucoside-8-*O*-β-glucuronide, along with thirteen known flavonols, isoflavones, cyanogenic glycoside, caffeic acid and ferrulic acid derivatives. The structures of the products were established by means of spectroscopic data analysis. Among the isolates, seven exhibited strong scavenging activity against DPPH (IC_{50} from 20 to 75 μM).

PJ50 Role of Glutathione-S transferase Pi (GSTπ) during the treatment of doxorubicin and the plant extract *Phyllanthus urinaria* L. in H9c2 cells

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Doxorubicin (DOX) is widely used to treat many types of cancer but frequently causes cardiotoxicity [1]. Our previous study has shown that an extract from *Phyllanthus urinaria* (PU) protected against DOX-induced cytotoxicity in H9c2 cells [2]. In this study, we examined the mechanism of cytoprotection in association with the expression and localization of glutathione-S transferase in H9c2 cells. The expression of antioxidant enzymes including glutamylcysteine synthetase (GCS), MnSOD, and CuZnSOD was also investigated. We found that among three major GST subtypes GST Pi (GSTP) is predominantly expressed in H9c2 cells. While no significant alterations in the enzyme activities, the cytoprotective effect of PU treatment appeared to involve nuclear localization of GSTP. Using RNA interference technique to suppress GSTP expression provided evidence that DOX was highly accumulated in nuclei and apoptosis was increased as evaluated by TUNEL assay. In conclusion, PU may be used as an alternative resource of antioxidants with distinctive mechanisms of action that may be suitable for specific type of oxidative insults. *Acknowledgements: Srinakharinwirot University Research Fund; The Matsumae International Foundation* **References:** [1] Zunino, F. et al. (1990) Anticancer Drug Des 5:307 – 317. [2] Chularojmontri, L. et al. (2005) Bio. Pharm. Bull. 28:1165 – 1171

PJ51 Effects of salvianolic acids on oxidative stress and hepatic fibrosis in rats

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Reactive oxygen species (ROS) is associated with activation of hepatic stellate cells (HSCs) and liver fibrosis *in vivo*. The present study is to investigate the *in vitro* and *in vivo* anti-fibrotic effects of salvianolic acids A (Sal A, $C_{26}H_{22}O_{10}$) and B (Sal B, $C_{36}H_{30}O_{16}$) from *Salvia miltiorrhiza*. A cell line of rat HSCs (HSC-T6) was stimulated with platelet-derived growh factor (PDGF, 10 ng/ml). Intracellular hydrogen peroxide

(H_2O_2), α-smooth muscle actin (α-SMA), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits and phosphorylations of mitogen-activated protein kinases (MAPKs) were measured. Liver fibrosis was induced by intraperitoneal injections of thioacetamide (TAA, 200 mg/kg) twice per week for 6 weeks. Sal A (10 mg/kg), or Sal B (50 mg/kg) was given by gavage twice per day for 1 month starting 2 weeks after TAA injection. PDGF increased the accumulation of hydrogen peroxide in HSCs, which was attenuated by Sal A (10 μM) and Sal B (200 μM). Sal A and B attenuated the PDGF-stimulated expressions of NADPH oxidase subunits $gp91^{phox}$ and $p47^{phox}$ in membrane fractions. Sal B reversed PDGF-stimulated phosphorylations of p38 and JNK. *In vivo* studies showed that the hepatic collagen contents, fibrosis scores and expressions of α-SMA and $gp91^{phox}$ were increased in TAA-intoxicated rats, all of which were attenuated by Sal A and Sal B treatment. Our results showed that Sal A and B attenuated PDGF-induced ROS formation in HSCs, possibly through inhibiton of NADPH oxidase. Sal A and B treatments were also effective against hepatic fibrosis in TAA-intoxicated rats.

PJ52 Protective effect of milk thistle and grape seed extracts on fumonisin B1 induced hepatotoxicity in rats

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Fumonisin B1 (FB1) is a mold metabolite produced by *Fusarium* species that is frequently found in corn worldwide [1]. It is toxic to both liver and kidney [2]. Research: Hepatotoxicity was induced in rats by feeding them FB1 contaminated corn. Evidence of hepatotoxicity was observed after 60 days by an increase in the plasma activity of alanine aminotransferase (ALT), where that elevation reached 78% (p= 0.000), in comparison with the control group. Pretreatment with milk thistle (S), or grape seeds (G) extracts or both (S+G) was found to return the ALT level back to normal. FB1, drastically depleted glutathione peroxidase (GpX) to 48%, while pretreatment with S, G, and S+G could elevate the GpX by 30%, 31% and 50%, respectively. Lipid peroxidation represented by malondialdehyde was elevated significantly to 137%. On the other hand, the pretreated groups (S, G, and S+G) have altered the levels down to 38%, 37%, and 44%, respectively. In addition to the hepatotoxicity of FB1, the kidney function was investigated too, where the creatinine level was elevated to 65%. The pretreatment by S and S+G lowered the level down to 22% and 24%, respectively and the pretreatment with G could successfully return the creatinine level to normal. Serum activity of urea was significantly elevated to 30%, and the pretreatment groups S, G, and S+G could significantly reduce it to 52%, 37% and 46%, respectively. Histological examination of liver sections confirmed the serum analysis, where significant improvements were observed in all pretreated groups in comparison with the liver sections of rats fed on FB1. These improvements might be due to their ability to lower serum total cholesterol and low- density lipoprotein cholesterol levels as well as slowing the lipid peroxidation process by enhancing antioxidant enzyme activity. **References:** [1] Gelderblom, W.C. et al. (1991) Carcinogenesis 12:1247 – 1251. [2] Carlson, D.B. et al. (2001) Toxicol. Appl. Pharmacol. 172:29 – 36.

PJ53 Antioxidant properties of rosemary and clove botanical extracts

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Rosemary leaves and clove buds were extracted with 50% acetone and 70% ethanol, and evaluated for antioxidant properties. Radical scavenging capacity in Trolox equivalent (TE) was determined against 2 – 2-diphenyl-1-picrylhydrazil (DPPH·) and hydroxyl radical (OH·). Oxygen radical absorbance capacity (ORAC) and total phenolic content (TPC) were also measured for each extract. All botanical samples showed antioxidant activity. Clove extract showed the highest activity against OH· (2813.52 μmol TE/g), and also had the highest TPC (171.46 mg gallic acid equivalent/g) and ORAC value (3538.84 μmol TE/g) in both extraction solvents. Acetone extracts were stronger than ethanol extracts in the above assays. The rosemary extract in 50% acetone showed the highest DPPH· scavenging activity (652.27 μmol TE/g dry weight) of the samples. The clove extract in 70% ethanol had a higher DPPH· scavenging value than the 50% acetone extract (477.81 μmol TE/g dry weight vs. 243.02 μmol TE/g dry weight, respectively. The results of this study show that these botanicals may serve as natural dietary sources of radical-scavenging antioxidants. The type of extracting solvent was able to significantly alter the antioxidant property estimation. This study provides background for future research into the health benefits of these botanicals.

PJ54 Effects of total extract from rhizomes of *Cynodon dactylon* (L.) Pers. on compensated right heart failure in rats

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It is now believed that medicinal plants can be a valuable source of assistance for prescription medicines and can be taken to aid recovery from serious disease such as heart failure. The rhizomes of *Cynodon dactylon*, also known as Bermuda grass, are used for the treatment of heart failure in folk medicine [1]. We investigated the effects of hydro-alcoholic extract of *C. dactylon* rhizomes on cardiac contractility in normal hearts and on cardiac functions in rats with compensated right heart failure. To produce the heart failure, wistar rats were injected 50 mg/kg (i.p) monocrotaline. Two weeks later, the animals were treated orally with different doses of the extract for two other weeks. The treated rats showed very less signs of fatigue, peripheral cyanosis and dyspnea. The survival rate was high in the extract treated groups (90%). Administration of *C. dactylon* in monocrotaline-injected rats led to profound improvement in cardiac functions as demonstrated by decreased level of right ventricular end diastolic pressure (RVEDP) and elevated mean arterial pressure. $RVdP/dt_{max}$, and $RVdP/dt/P$ as indices of myocardial contractility were also markedly ($p < 0.001$) increased by the extract. The extract reduced heart and lung congestion by decreasing tissue wet/dry and wet/body weight ratios ($p < 0.01$). In the isolated rat hearts, the extract produced a remarkable ($P < 0.001$) positive inotropic effect concomitant with a parallel decrease in the LVEDP. The results of this study indicated that *C. dactylon* exerted strong protective effects on right heart failure, at least in part by positive inotropic action and improving cardiac functions. **Reference:** [1] Miraldi, E. et al. (2001) J. Ethnopharmacol. 75:77 – 87.

PJ55 Anti-inflammatory activity of Thai traditional medicine preparation called Prasaprohyai

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Thai traditional medicine preparation called Prasaprohyai was commonly used to treat a cold, asthma and as antipyretic drug. It is composed with nineteen plants, *Amomum testaceum*, *Anethum graveolens*, *Angelica dahurica*, *Angelica sinensis*, *Artemisia annua*, *Atractylodes lancea*, *Cuminum cyminum*, *Dracaena loureiri*, *Foeniculum vulgare*, *Kaempferia galanga*, *Lepidium sativum*, *Ligusticum sinense*, *Mammea siamensis*, *Mesua ferrea*, *Mimusops elengi*, *Myristica fragrans*, *Nelumbo nucifera*, *Nigella sativa* and *Syzygium aromaticum* [1]. The objective of this research is to investigate on anti-inflammation activity of this preparation and its components. It and its components were extracted by ethanol, ethanol-water and water which imitated with using in Thai traditional book [1]. These extracts were examined for their inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW 264.7 cell lines. Nitric oxide in the culture supernatant was measured by Griess reaction [2]. The ethanolic extract of Prasaprohyai preparation showed high anti-inflammation with this assay (IC_{50}= 7.291 μg/ml). The ethanolic extract of *Myristica fragrans* (Chan thet) which is an ingredient

of this preparation exhibited the most potent inhibitory activity, with an IC_{50} value of 1.613 µg/ml, followed by the ethanolic extract of *Ligusticum sinense* (Kot hua bua) (IC_{50}= 3.769 µg/ml) and *Nigella sativa* (Thian dam) (IC_{50}= 4.085 µg/ml). The water and ethanol-water extracts of all plants were apparently inactive (IC_{50} > 100 µg/ml). These results can support using Prasaprohyai in Thai traditional medicine for antipyretic caused by inflammation. **References:** [1] Foundation of resuscitate and encourage Thai Traditinal Medicine (2005) Thai Pharmaceutical Book Pikanate Printing Center Cooporation. [2] Tewtrakul, S. and Itharat, A. (2007) J. Ethnopharmacol. 109:412 – 416.

PJ56 Ethnopharmacological study of two plants of Northern Madagascar: bronchodilator activity of *Tetracera madagascariensis* and antispasmodic activity of *Mascarenhasia arborescens*

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Ethnobotanical investigations were conducted with population and traditional healers in several villages in the North of Madagascar. From this field research, two plants have been selected to justify their traditional use. *Tetracera madagascariensis* Willd. ex Schltdl. (Dilleniaceae), a species endemic to Madagascar, is traditionally used to treat respiratory disorders. The bronchial asthma is a widespread disease in Madagascar. Bioassay-guided fractionation using isolated guinea pig trachea pre-contracted with histamine at 2.10^{-5}M led to the identification of methylene chloride extract as the main active fraction. This extract induced a concentration-dependant relaxation with a median effective concentration (EC_{50}) of 53 ± 0.5 µg/ml (n = 6). Subfractions are in analysis process. *Mascarenhasia arborescens* A. DC. (Apocynaceae), a tree growing in the East of Africa and Madagascar, is widely used in Northern of Madagascar to treat intestinal disorders and diarrhoea. It is on account of these data that we investigated this species for antispasmodic activity. Bioassay-guided fractionation using isolated guinea pig ileum pre-contracted with histamine at 3.10^{-6}M to monitor the activity led to the isolation of davidigenin (dihydrochalcone) as the main active constituent from methylene chloride fraction. Effectively, it induced a concentration-dependant relaxation of the histamine pre-contracted guinea pig ileum with an EC_{50} of 11.1 ± 0.7 µg/ml (n = 4). This data is in accordance with the literature underlined an antispasmodic effect of davidigenin on mouse jejunum [1]. *Acknowledgements: A. Rakotozafy (botanist, IMRA), J.P. Nicolas (association "Jardins du Monde"), population and healers surveyed.* **Reference:** [1] Sato, Y. et al. (2007) Biol. Pharm. Bull. 30:145 – 149.

PJ57 Isoflavonoids in tropical and subtropical neglected leguminous species

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Isoflavonoids, bioactive compounds belonging to the phytoestrogens, are known to prevent some kinds of hormone-related cancers and may participate as alternatives to the conventional hormone replacement therapy [1]. Although they have already been identified in about 50 different plant families [2], the quantities were usually in trace amounts and therefore legumes remain the most important source of isoflavonoids. Since the tropical and subtropical regions offer wide range of neglected leguminous species, we decided to analyze 27 of them for the presence of isoflavonoids. Aqueous/methanolic extracts obtained from dried seeds were pretreated on an immunoaffinity column (IAC) [3] and subsequently analyzed by reverse phase HPLC/UV-DAD. Immunosorbents for IAC are characterized by high molecular selectivity so that single group of structurally related compounds can be targeted. UV spectra and retention times were compared with set of standards. As a result, we have identified certain commonly known (e. g. genistein) and high levels of uncommon isoflavonoids in several samples. Genistein was present in *Spartium junceum* L., *Pachyrhizus tuberosus* Spreng. and *Trigonella foenum-graecum* L., in concentrations 13, 7 and 0.8 µg/g of dry seeds, respectively. Interestingly, the extract of *P. tuberosus* contained significantly higher amounts of total isoflavonoids than soybean. Though presence of isoflavonoids in these species has never been published before, the fact itself is basically not striking, but the amounts are hint for their further investigation and possible use in dietary products *Acknowledgements: This research was supported by project GACR 525/09/0994 and MSM 6046070901.* **References:** [1] Adlercreutz, H. et al. (2004) BioFactors 22:229 – 236. [2] Mackova, Z. et al. (2006) Phytochemistry 67:849 – 855. [3] Delaunay, N. et al. (2000) J. Chromatogr. B 745:15 – 37.

PJ58 Antibacterial effects of leaves of *Vaccinium vitis-idaea* L

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This study was realized to investigate the antibacterial activity of extracts from leaves of *Vaccinium vitis-idaea* L. (Ericaceae) against eleven strains of *Escherichia coli*. Leaves of *Vaccinium vitis-idaea* L. collected on Klekovača Mountain (RS, Bosnia and Herzegovina, W. Balkans) were extracted by different solvents (water, ethanol and ethyl acetate). Cultures of bacteria were clinical isolates and standard strain of *E. coli* ATCC 25922. Minimal Inhibitory Concentrations (MICs) and Minimal Bactericidal Concentrations (MBCs) of extracts and antibiotic amoxicillin were determined by tube dilution method. The results revealed that water extract exhibited the highest activity against all strains of *E. coli* (MICs were 5 mg/ml). MICs of ethyl acetate extract were 20 mg/ml for all bacteria strains tested. Ethanol extract exhibited antibacterial activity with MIC values between 20 and 40 mg/ml. In conclusion, water extract from leaves of *Vaccinium vitis-idaea* L. showed high antibacterial activity against *Escherichia coli* with MBCs 5 mg/ml for nine strains and 10 mg/ml for two strains.

PJ59 *In vitro* antibacterial activity of cloves (*Syzygium aromaticum*) against MRSA

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The process of microbial resistance against antibiotics makes it essential to seek for novel drugs. There have been many studies over the last years, in which a lot of plant species have been checked for their antimicrobial activities. Cloves (*Syzygium aromaticum*), which is a commonly used spice worldwide, have antibacterial, antifungal, antiviral, antioxidant, antimutagenic, anaesthetic, insecticidal, anti-inflammatory, antithrombotic, antiparasitic and antiulcerogenic activities [1]. Staphylococci are a well known cause of both hospital and community acquired infections. Isolates that have acquired methicillin resistance pose serious problems for treatment and eradication. After the introduction of the drug, Methicillin Resistant *Staphylococcus aureus* (MRSA) strains were reported in the early 1960 s. Epidemics have occurred around the world and the clones have diversified since then. MRSA is still on the rise. Nowadays, the imminent threat of reduced susceptibility to vancomycin have emerged [2]. The purpose of this study was to evaluate antibacterial activity of cloves against MRSA. All 100 (one hundred) clinical MRSA isolates were screened by using agar dilution method. Both aqueous and ethanol extracts of cloves were obtained. All of the strains were found to

be susceptible to both of the extract forms. At 1000 and 500 mg/mL concentrations all of the isolates were found sensitive. At 250 mg/mL, 11% of the isolates were sensitive. The isolates were multi- drug resistant, mostly against beta-lactams, aminoglycosides, tetracyclines, floroquinolones and macrolide antibiotics. In terms of a practical use of the cloves as an antibacterial drug, clinical studies are urgently needed. **References:** [1] Chaieb, K. et al. (2007) Phytother. Res. 21:501 – 506. [2] Fluit, A.C. and Schmitz, F.J. (2003) MRSA Current Perspectives. Caister Academic Press. Wymondham, UK.

PJ60 *In vitro* and *in vivo* anti-ischemic activities of the stems and leaves of *Vitis amurensis*

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Ischemic stroke results from a transient or permanent reduction in cerebral blood flow caused by occlusion of a cerebral artery via an embolus or local thrombosis. Loss of blood flow results in depletion of metabolic substrates such as oxygen and glucose, leading to hypoxia. During ischemia/reperfusion condition, there is a heavy production of the free radicals such as superoxide, hydroxyl and hydrogen peroxide (H_2O_2). These free radicals inhibit the uptake of glutamate and enhance glutamate release, resulting in excitotoxicity through NMDA receptor overstimulation, which is one of the major pathological factors leading to neuronal death in stroke [1]. *Vitis amurensis* (VA, Vitaceae), a species of *Vitis*, is distributed in Japan, China and Korea. The roots and seeds of VA have been reported to have antioxidant and anti-inflammatory effects [2]. In the present study, we performed *in vitro* and *in vivo* investigations on the neuroprotective effects of an ethanol extract of the stems and leaves of VA. In cultured cortical neurons from rats, VA (10 – 100 μg/ml) inhibited H_2O_2 (100 μM)-, glutamate (0.5 mM)-, and hypoxia-induced neuronal cell death. In rats, VA prevented cerebral ischemic injury induced by 2 h of middle cerebral artery occlusion, followed by 24 h reperfusion. Ischemic infarct and edema volumes were significantly reduced in rats that received VA (25 – 100 mg/kg, orally). These animals exhibited a corresponding improvement in neurological function. Viniferin, an active compound isolated from VA, also inhibited H_2O_2-, glutamate-, and hypoxia-induced neuronal cell death, suggesting that some of the neuroprotective effects of VA may be attributable to this compound. It is possible that the anti-excitotoxic and anti-oxidative activities of VA may be responsible for its neuroprotective effects against focal cerebral ischemic injury. In the future, VA might play a therapeutic role in the prevention and treatment of neurodegeneration in stroke. References: 1. Ikemune, K. et al. (1999) Neurosci. Lett. 275:125 – 128. 2 Lee, E.O. et al. (2006) Carcinogenesis 27:2059 – 2069.

PJ61 Inhibition of TNF-α induced ICAM-1, VCAM-1 and E-selectin expression by *Momordica charantia* L.

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The initiation of an atherosclerotic lesion involves establishment of an endothelial cell pro-inflammatory state that recruits leukocytes and promotes their movement across the endothelium. These processes require endothelial expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial-leukocyte adhesion molecule-1 (E-selectin). Tumor necrosis factor-α (TNF-α) is a powerful inducer of these adhesion molecules [1]. The fruit of *Momordica charantia* L. (MC) is a common vegetable in tropical areas of Asia and Africa. MC also has been traditionally used as a bitter stomachic and an antidiabetic [2]. Experiments were performed to test whether MC alters TNF-α-induced expression of these adhesion molecules. Human umbilical vein endothelial cells (HUVEC) were treated for 18 h with or without an extract and fractions of MC and TNF-α. ICAM-1, VCAM-1 and E-selectin were detected by cell-based ELISA, Western blots and RT-PCR. MC significantly inhibited TNF-α-induced expression of each adhesionα molecule in a dose-dependent manner. The chloroform fraction of MC significantly inhibited TNF-α-induced expression of each adhesion molecule in a dose-dependent manner. Nuclear factor-κB (NF-κB) is required for transcription of ICAM-1, VCAM-1 and E-selectin adhesion molecule genes [3]. Western blot analysis revealed that the chloroform fraction of MC inhibits translocation of the p65 subunit of NF-κB to the nucleus. Thus, the chloroform fraction of MC inhibited TNF-α-mediated induction of ICAM-1, VCAM-1 and E-selectin in HUVEC by inhibiting NF-κB, and lipophilic components of MC may suppress inflammation and modulate the immune response. *Acknowledgements: This work was supported by the Second Stage of Brain Korea21 project in 2008, Oriental Medical Science Center.* **References:** [1] Chen, C.C. et al (1995) Journal of Immunology 155:3533 – 3545. [2] Lii, C.K. et al. (2009) J. Ethnopharmacol. 122:227 – 233. [3] Mo, S.J. et al. (2007) J. Ethnopharmacol. 109:78 – 86.

PJ62 Polyacetylenes and polyenes from *Echinacea pallida* and their anti-inflammatory activity *in vitro*

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Echinacea is among the most popular medicinal plants today and has a long history of use for the treatment of the common cold, upper respiratory infections and inflammatory diseases. Besides *Echinacea purpurea* and *Echinacea angustifolia,* also the roots of *Echinacea pallida* are used medicinally. Polyacetylenes and polyenes are the major lipophilic constituents of *Echinacea pallida* root extracts. They are natural compounds known for their antifungal and antibacterial activity, and have enzyme inhibitory effects [1]. There is some evidence that they might also exhibit antiallergic as well as anti-inflammatory activities, and recently cytotoxic effects have been found [2]. Fractionation of a supercritical CO_2-extract of *Echinacea pallida* roots led to the isolation and structure elucidation of seven polyacetylenes and polyenes, namely 8-hydroxy-tetradeca-(9E)-ene-11,13-diyn-2-one, 8-hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one, tetradeca-8Z-ene-11,13-diyn-2-one, pentadeca-8Z-ene-11,13-diyn-2-one, pentadeca-8Z,13Z-diene-11-yn-2-one, (8Z)-pentadeca-8,11-diene-2-one and (8Z)-pentadeca-8-ene-2-one. The structures of the compounds were determined by UV (DAD-HPLC), NMR (including 1D and 2D NMR experiments) and MS in comparison with data from literature [3,4,5]. The anti-inflammatory activity of various silica gel fractions of the CO_2-extract as well as of the compounds has been evaluated *in vitro* by using an ELISA assay determining the inhibition of leukotriene B4 formation in human granulocytes. Fractions 6, 7 and 8 showed potent inhibitory activity on LT B4 formation (86.5%± 1.17; 67.56%± 8.96; 75.34%± 0.43, respectively). **References:** [1] Binns, S.E. et al. (2000) Planta Med. 66:241 – 244. [2] Chicca, A. et al. (2008) Brit. J. Pharmacol. 153:879 – 885. [3] Pellati, F. et al. (2006) Phytochemistry 67:1359 – 1364. [4] Bauer, R. et al. (1986) Planta Med. 52:424. [5] Morandi, S. et al. (2008), Org. Biomol. Chem. 6:4333 – 4339.

PJ63 Effects of STW 5 and its components on viability of Caco-2 cells

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Herbal preparations like STW 5 (Iberogast ®) are widely used in treatment of dyspepsia and motility-related disorders of the gastrointestinal tract. STW 5 is a fixed combination of nine individual plant extracts, containing 15% *Iberis amara* fresh plant extract (STW 6) and showing a very good efficacy and tolerability in a large number of clinical and preclinical studies [1,2]. In order to characterize the mode of action STW 5, STW 6 as well as cucurbitacines E and I, belonging to the phytochemical constituents of STW 6, were tested on the Caco-2 model to determine cell viability using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. The effects were tested time-dependently, 0.5, 1, 3 and 24 hours, after substance application. Short-term incubation of cells with STW 5 (0.5, 1 h) increased cell viability highly in a concentration-dependent manner, but moderately within 3 hours. After long-term incubation (24 h) the viability was maximum stimulated by 256 μg/ml STW 5, whereas high concentrations reduced cell viability concentration-dependently. STW 6 as well as cucurbitacine I (0.01 – 100 μM) did not influence the cell viability. Cucurbitacin E (0.1 – 100 μM) had no effect after short-term incubation (0.5, 1 h) but reduced the cell viability at high concentration (50 – 100 μM) after long-term

incubation (24 h). The present data indicate: (1) STW 5 is able to increase cell viability of epithelial cells in vitro, suggesting this mechanism may contribute to the protective effect against morphological changes seen in an experimental model of intestinal inflammation; (2) STW 5 did not affect the integrity of epithelial cells at concentrations of 512 µg/ml and below, not even at long-term incubation; (3) cucurbitacin E had no effect in relevant concentrations. Taken together, these results are in accordance to the well-characterized tolerability of STW 5 and in addition give information on the mechanisms of action involved in its mucosa-protective effects. **References:** [1] Rösch, W. et al. (2006) Phytomedicine 13:114 – 121. [2] Michael, S. et al. (2009) Phytomedicine 16:161 – 171.

PJ64 Multi target action of the herbal combination STW 5 in dyspeptic heartburn

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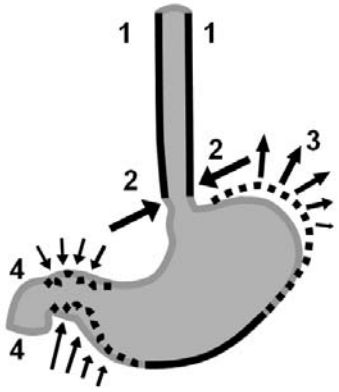
A multi-target action is the key to understanding the effect of herbal medicines in a large number of therapeutic fields. To these belongs also heartburn, frequently occurring in dyspeptic patients, and caused by motility disturbances [1]. As a number of randomized controlled clinical trials have shown, the herbal medicine STW 5 (Iberogast®) is active not only in functional dyspepsia [2], but also in its symptom heartburn [3]. A systematic review, conducted with the aim to elucidate the underlying mode of action, has identified the following mechanisms: 1. Protection of the esophageal mucosa against acidic reflux: STW 5 inhibits mucosal inflammation in an experimental model of acid reflux [4]. 2. Enhancing of muscular tone of the lower esophageal sphincter, contributing to the inhibition of gastric reflux into the esophagus [5]. 3. Relaxation of fundus and corpus: This allows for a better adaptation of the volume to ingested food, thus reducing intragastric pressure [6,7] 4. Prokinetic effect on gastric antrum, improving gastric function. An onset of this effect within 10 min after oral intake of STW 5 has been shown [6,7]. 5. Inhibition of increased acid secretion, thus potentially reducing the exposure of gastric and esophageal mucosa to acid. [8].



Possible mechanisms of action of STW 5, acting on multiple targets (explanation see text).

References: [1] Tack, J. et al. (2006) Gastroenterology 130:1466 – 1479. [2] Schmulson, M.J. (2008) Nat. Clin. Pract. Gastr. 5:136 – 137. [3] Madisch, et al. (2007) Gut 56, SIII:A336. [4] Khayyal, M.T. et al. (2008) Neurogastroent. Motil. 20, S2:98. [5] Schemann, M. et al. (2008) Gastroenterol. 46:1039. [6] Schemann, M. et al. (2006) Phytomedicine 13:90 – 99. [7] Pilichiewicz, A.N. et al. (2006) Neurogastroent. Motil. 18:745. [8] Khayyal, M.T. et al. (2006) Phytomedicine 13:56 – 66.

PJ65 Antibacterial activity of propolis from two sources in the Basque Country

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Propolis has a lot of active ingredients, such as flavonoids and phenolic acids, from different trees and bushes and it shows antifungal, antibacterial and antiviral properties [1]. The aim of this study was to test the antimicrobial activity of ethanol extracted propolis against different *in vitro* bacteria. The propolis was sourced from two places – Urdaibai and Dima – in the Basque Country. The Minimal Inhibitory Concentration (MIC) of propolis to reduce microbial growth as well as the Minimal Bactericidal Concentration (MBC) were determined. As the presence of ethanol in the propolis extracts can have an additional effect, MICs determinations were also calculated for ethanol. The method used to determine MIC and MBC for the bacteria was based on the protocol ISO 20776 – 1:2006 and ISO 20776 – 2:2007. The assays were carried out in three bacterial species: *Streptococcus mutans* (CECT 479), *Streptococcus pyogenes* (CECT 985) and *Staphylococcus aureus* (CECT 240). Results showed that ethanol concentration needed for bacterial inhibition was lower than ethanol concentration present in the MICs values of propolis extracts for the different strains. No difference was found in the properties of the propolis from Dima and Urdaibai. In all cases, MIC and MBC values were quite similar, with differences not higher than 1 dilution. Therefore, our results show that propolis can be used as an inhibitor of bacterial growth, and due to its natural origin it could be an interesting antimicrobial agent. This study is being extended by the use of Supercritical Fluid Techologies which allows enriched fractions of the propolis to be obtained without organic solvents. **Reference:** [1] Ferreira, F.B.D. (2007) Oral Surg. Oral Med. O. 104:709 – 716.

PJ66 Antibacterial activity of *Iris pseudacorus* L.

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Iris pseudacorus L., a species belonging to Iridaceae family, is native to Europe, Great Britain, North Africa and the Mediterranean region. It has been introduced in temperate areas nearly world-wide and occurs throughout the United States. The aim of this study was to establish antibacterial and antifungal activity of methanolic, etherous, acetate and butanolic extracts from rhizomes and roots of *Iris pseudacorus* L. All examined crude extracts were tested *in vitro* against the reference strains of 6 Gram-positive bacteria (*Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240), 4 Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453) and two yeasts (*Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019). The tested extracts had inhibitory activity on the growth of most examined strains. Most active against bacteria and yeasts was methanolic extract from the rhizomes of *I. pseudacorus* L. The Gram-positive bacteria were more sensitive to the extracts than Gram-negative ones (inhibitory zones ranging from 7 – 24.5 mm and 0 – 19 mm, respectively). The minimal inhibitory concentrations (MICs) were determined by the agar dilution method, using two-fold dilutions of examined extracts in Mueller-Hinton agar (for bacteria) or Mueller-Hinton agar supplemented with 2% glucose (for yeasts). Among Gram-negative bacteria only *K. pneumoniae, P. aeruginosa* and *P. mirabilis* were sensitive to methanolic, acetate, butanolic extracts from rhizomes (MIC = 250 mg/l). The growth of yeasts tested was inhibited by methanolic extract from rhizomes and etherous extract from roots of *I. pseudacorus* L. (MICs 500 – 1000 mg/l and 1000 mg/l, respectively).

PJ67 Effectiveness of Cystus 052 in the prophylaxis and treatment of upper and lower respiratory tract infections

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In this prospective, randomised, placebo controlled clinical study, we aimed to investigate the clinical effect of a *Cistus* extract (CYSTUS 052®) in 160 patients with infections of the upper respiratory tract In the placebo group there were 45 viral and 35 bacterial infections, in the treatment group 47 viral and 33 bacterial infections. The Cystus 052 tablets were taken orally. The primary outcome measure was a well-beeing score, which could reach a maximum of 30, and constituted of pain, cough (intensity and frequency), sputum and rhinorrhea. There was no difference in the score at the beginning of the treatment, therefore ensuring the homogenity of the two groups. Starting with day 4, the score differed significantly between the two groups. From the 5th day onwards, the difference was highly significant ($p < 0{,}001$). In the group taking Cystus, 77% of the completers respondes to the treatment in about 60%. In the placebo group, the treatment response was 25%. Among the inflammatory markers investigated the C-reactive protein was most affected by Cystus 052, which decreased significantly in the treatment group. In addition the antiviral effect is independent of the spectrum of pathogens. Bacteries and viruses are both inhibited, therefore the efficiency of Cystus 052® is mainly related to it's physical effects. Especially in the early phase of infection, Cystus 052 is an effective agent in the prevention of a further dissimination of the disease, as the active agents minimize the risk of a re-infection of other cells.

PJ68 Two new isolated epimers seem to contribute to the bitter principle of *Leonurus japonicus* Houtt.

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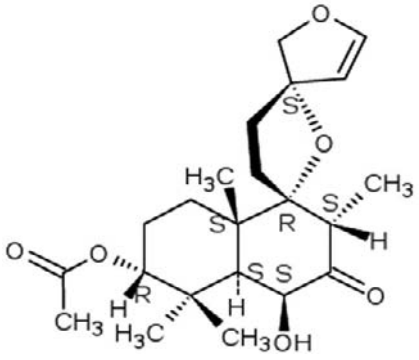
The aerial parts of Chinese motherwort (*Leonurus japonicus* Houtt) (Yimucao) are commonly used in traditional Chinese medicine (TCM) to regulate menses and to treat associated conditions. As the herb is more and more used in Europe, it has been started to cultivate the plant in Germany [1]. Since bitterness is an important sensory feature of the plant, the aim of the investigation was to isolate and identify the chemical constituents responsible for the bitter taste of Yimucao. It was found that the bitter principle can be extracted by n-hexane. Therefore, the n-hexane extract of *Herba Leonuri* has been fractionated using Fast Centrifugal Partition Chromatography (FCPC).This led to a fraction containing a mixture of two compounds which seemed to be responsible for the bitter taste of Yimucao. Structure elucidation by MS and NMR spectroscopy led to two new epimeric diterpenoid compounds **1** and **2** (exact mass 392,2), which are related to furanic labdane type lactones previously found in *Leonurus japonicus*, *Leonurus cardiaca, Leonotis leonurus* and *Marrubium vulgare* [2,3].



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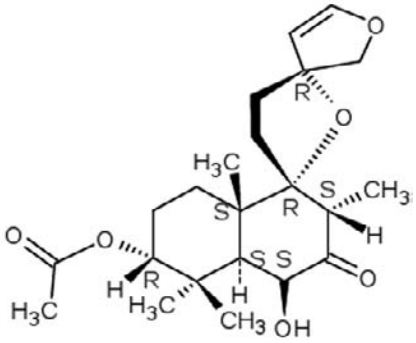

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References: [1] Bomme, U. et al. (2007)J. Altern. Complem. Med. 13:597 – 601. [2] Brieskorn, C. and Broschek, W. (1972) Pharm. Acta Helv. 47:123 – 132. [3] Laonigro, G. et al. (1979) Gaz. Chim. Ital. 109:145 – 150.

PJ69 Frankincense – historical evidence from traditional Chinese literature of beneficial effects on the human physiology

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Frankincense (olibanum), obtained as a white gum resin of the *Boswellia* tree, has been used since ancient times in the Orient and the Occident both for religious and festive purposes, but also for medical applications and as an addictive drug [1,2]. In early history, frankincense was used in the resin-form and also burned as frankincense pyrolysate. It was known to be antiseptic, disinfectant, an efficient drug against catarrh or diarrhoea, and was used in mixtures to initiate abortion [3]. In classical Chinese literature, the most comprehensive work on pharmacology is the Ben Cao Gang Mu (((IMG SRC="pj069_1.jpg"))), compiled by Li Shizhen (((IMG SRC="pj069_2.jpg"))) in the 16th century during the Ming dynasty. This work consists of 52 volumes (juan ((IMG SRC="pj069_3.jpg"))), wherein 1892 drugs are described, and more than 10.000 recipes are given. The present study focuses on a translation of the passages on frankincense to be found in the Ben Cao Gang Mu, most specifically of the respective recipes. Based on these translations, historical pharmacological evidence is briefly compared with today's scientific achievements. *Acknowledgements: We wish to thank Professor H.-C. Langowski for his support of our scientific work.* **References:** [1] Papyrus Ebers 742 (89,6 – 89,7) and 743 (89,7 – 89,8). [2] Mittwede, M. (1977) Ayurvedic text Bhava prakasha Nighantu, Pandita Vishvanathadvivedi Shastri, Motilal Banarsidas, Delhi. [3]Martinetz, D. and Lohs, K. (1981) *Vom geweihten Rauch des Olibanum – zur Kulturgeschichte des Weihrauchs*", Wissenschaft und Fortschritt 31.

PJ70 *In vitro* cytotoxic activity of lichen *Laurera benguelensis*

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The petrol ether, ethyl acetate and methanol extracts of the lichen *Laurera benguelensis* (Trypetheliaceae) from Thailand and its major anthraquinone metabolite were separately tested for cytotoxic activity. 1,8-dihydroxy-6-methoxy-3-methylanthraquinone (physcion) was isolated from the lichen *Laurera benguelensis* as the main pigment by column chromatography. Physcion content in the petrol ether, ethyl acetate and methanol extracts was 1.15%, 0.78% and 0.48%, respectively. The cytotoxic activity of above-mentioned individual extracts and physcion were evaluated *in vitro* using HeLa (human carcinoma of the cervix) cell line [1 – 3]. The lichen extracts and physcion were dissolvent in dimethyl-

sulfoxide and serially diluted with Dulbecco's Modified Eagle Medium to obtain concentrations 0.001, 0.01, 0.1, 10 and 100 mg/ml. HeLA Cells were seeded in 96-well micro plates and routinely cultured for 24 h. After 24 h physcion and the extracts were added in serial concentrations, and re-incubated for 24 and 72 h. The effects of tested samples on the variability of these cells were assayed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. Saponin pure white (Merck) concentration of 0.2 mg/ml and cisplatin concentration of 0.6 µg/ml were used as positive control. All the extracts exhibited moderate cytotoxic activity in a concentration above 0.1 mg/ml while physcion showed activity above 0.01 mg/ml on HeLa cells. This is the first report that describes the potential cytotoxic activity of *L. benguelensis*. . *Acknowledgements: The authors acknowledge financial support by the Ministry of Science and Environmental of Republic of Serbia (Grant No. 142025) and Medical Faculty University of Kragujevac, Serbia (Grant No. JP 5/08).* **References:** [1] Bézivin, C. et al. (2004) Planta Med. 70:874 – 877. [2] Ge, H.M. et al. (2008) Phytochemistry 69:571 – 576. [3] Háznagy-Radnai, E. et al. (2008) Fitoterapia 79:595 – 597.

PJ71 Influence of chlorophyll and tannins in plant extracts on cell-based luciferase reporter gene assays

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Various studies indicate that several ubiquitous plant compounds, like chlorophyll and tannins, possibly interfere with biological in vitro assays. Chlorophyll might interact with fatty acids, whereas tannins can form tight complexes with metal ions, proteins and polysaccharides [1]. The aim of this study was, to examine whether the chlorophyll and/or tannin content of plant extracts leads to false positive or false negative results in several luciferase gene assays. Therefore, two model plants, *Sambucus nigra* and *Urtica dioica,* were extracted with dichloromethane (DCM) and methanol (MeOH) using the Accelerated Solvent Extractor (Dionex ASE200). From the DCM extract chlorophyll was removed, whereas tannins were separated from the MeOH extract. The chlorophyll separation method was based on a liquid-liquid-repartition between DCM and MeOH:H_2O (1:1). For the separation of tannins a liquid-liquid-solvent partition in $CHCl_3$ and 1% NaCl [2] was used. In addition HPLC-MS fingerprints were generated. Samples, with and without the possible interfering substances, were examined for their potential to activate the peroxisome proliferator-activated receptors (PPAR)-α and -γ as well as to inhibit the transcription factor NF-κB. Assays were performed in HEK293 cells transfected with luciferase-reporter constructs for PPAR α/γ or pNF-κB and with green fluorescent protein as internal normalization control. Further on, luciferase activity and fluorescence intensity were quantified. The results indicated that in all three assay systems the purified extracts were more active. Thus, the pure substances chlorophyll a and b, tannic acid, and epicatechin gallate were tested in the different assay formats too. Since, neither the pure chlorophylls nor the pure tannins had any influence in our assay systems, the higher activity of the purified extracts might be due to enrichment of the active compounds in those. *Acknowledgements: This work is funded by the Austrian Science Fund, FWF: S 10704-B037.* **References:** [1] Potterat, O. and Hamburger, M. (2006) Curr. Org. Chem. 10:899 – 920. [2] Wall, M.E. et al. (1996) Phytomedicine 3:281 – 285.

PJ72 Screening of 35 plants used in Austrian folk medicine for PPAR-α and -γ activation and NFkB inhibition

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Austria and its adjacent regions have a great history in traditional folk medicine. Folk-medicinal knowledge was collected over years and transferred to the VOLKSMED database [1] which contains an exact botanical description of each used plant. The aim of this study was to investigate the potential *in vitro* anti-inflammatory activity of plants selected from that database, using luciferase reporter gene assays. Thirty five preselected plants were extracted with dichloromethane (DCM) and methanol (MeOH) using the Accelerated Solvent Extractor (Dionex ASE200). The chlorophyll, if present, was separated from the DCM extract, whereas the tannins were removed from the MeOH extract, in order to avoid possible interferences with the assay formats [2]. Crude and purified extracts were then examined for activation of PPAR-α and -γ and inhibition of NFκB using HEK293 cells transfected with green fluorescence protein plasmid (as internal control). The cells were also accordingly transfected with PPAR-α or -γ plasmids and reporter plasmid pPPRE-tk3x-Luc in the PPAR assay, while a pNFκB-luc transfection and a TNF-α stimulation were used in the NF-kB assay. Luciferase activity and fluorescence intensity were then measured using a GeniosPro plate reader. The extracts of fifteen plants showed no activity in the applied assays, while the other twenty exhibited activity in one or more of the test systems. The three most active ones in both assays were the DCM extract with the chlorophyll separated of *Urtica dioica* leaves, the MeOH extract with the tannin separated of *Sambucus nigra* fruits and the DCM extract with the chlorophyll separated of *Prunella vulgaris* herb. *Acknowledgements: This work is funded by the Austrian Science Fund FWF: S 10704-B037* **References:** [1] Saukel, J. (2006) Sci. Pharm. 74:36. [2] Potterat, O. and Hamburger, M. (2006) Curr. Org. Chem. 10:899 – 920

PJ73 *Couroupita guianensis*: Evaluation of its anxiolytic and antidepressant activity in mice

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Couroupita guianensis Aubl. (Family: Lecythidaceae) popularly known as "cannon ball tree" contains triterpenoids [1] which have been implicated in anxiolytic and antidepressant activity [2]. Therefore the present research was aimed to evaluate the potential anxiolytic and antidepressant activity in methanolic and aqueous extracts of roots of *Couroupita guianensis* in mice. These extracts were administered orally in a dose range of 125, 250 and 500 mg/kg of the body weight. The anxiolytic activity [3] was evaluated using light and dark model; elevated plus maze model; and hole-board test. The results indicate that the methanolic and aqueous extract when administered at the stated doses augment (was able to increase) number of entries in light area; occupancy of open arm and in the number of head dips in the hole-board paradigms which indicates that both extracts posses anxiolytic activity when compared with diazepam (2 mg/kg) as a standard. The antidepressant activity [4] was evaluated using tail suspension test and forced swim test, producing a decrease in the immobility time, similar to that of the imipramine (10 mg/kg) which served as a positive control. These findings indicate significant ($p < 0.05$) anxiolytic and antidepressant activity for methanolic extract as compared to aqueous extract. In conclusion, the results indicate that this plant possesses potential anxiolytic (through its action on benzodiazepine receptors) and antidepressant activity (through noradrenergic mechanisms) and has therapeutic potential in the treatment of CNS disorders. **References:** [1] Lewis, Y.S. (1964) Curr. Sci. India 33:682. [2] Aragão, G.F. et. al. (2006) Pharmacol. Biochem. Be. 85:827 – 834. [3] Herrera-Ruiz, M. et. al. (2006) J. Ethnopharmacol. 107:53 – 58. [4] Sharma, A. et.al (2001) J. Ethnopharmacol. 78:165 – 170.

PJ74 Study of *in vitro* and *in vivo* anti-inflammatory activity of aqueous extract of leaves *Erythrina indica*

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Erythrina indica widely known as 'Indian coral tree' belongs to the family Fabaceae. It is used in the traditional medicine for treatment of inflammation, joint pain, acute and chronic dysentery [1]. Aqueous extract of leaves *Erythrina indica* (EIA) was studied for its anti-inflammatory potential using *in vitro* models viz. albumin denaturation, membrane stabilization and proteinase inhibitory action [2]. *In vivo* anti-inflammatory activity was studied at the dose of 125, 250 and 500 mg/kg using carrageenan, histamine, serotonin induced paw edema [2] and cotton pellet induced granuloma formation in rats [3]. EIA showed significant ($P < 0.05$) protection against denaturation of proteins and heat induced Red Blood Cells (RBCs) damage. It also exhibited significantly

anti-proteinase activity which plays an important role in the development of tissue damage during inflammatory reactions. EIA showed significant decrease in carrageenan induced rat paw edema, a test which has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation. It also effectively inhibited the inflammation induced by histamine, and serotonin which suggest that the anti-inflammatory activity of EIA is possibly mediated by inhibiting the action of these mediators. It showed the significant activity in chronic proliferative phase of the inflammatory process by decrease in the weight of granuloma formation in rats. It is concluded that, aqueous extract of leaves *Erythrina indica* exhibited significant dose dependent *in vitro* and *in vivo* anti-inflammatory activity. **References:** [1] Basu, K. (1984) Indian Medicinal Plants 1:781. [2] Chatterjee, S. et al. (1996) Indian J. Pharmacol. 28:116 – 119. [3] Agrawal, R.B. et al. (2003) Indian J. Exp. Biol. 41:890 – 894. [4] Kale, M. et al. (2007) J. Ethnopharmacol. 112:300 – 304.

PJ75 Pharmacological investigation of an aqueous extract of *Cinnamomum tamala* leaves by various *in vivo* and *in vitro* models of inflammation

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Cinnamomum tamala (Family Lauraceae) was used traditionally in the treatment of inflammation [1] but there is no scientific evidence to validate the folkloric use of the plant. Thus the present work was aimed at investigating the anti-inflammatory effect of the aqueous extract of *Cinnamomum tamala* leaves (CTW) by various *in vivo* and *in vitro* screening methods. CTW at dose of 100, 200 and 400 mg/kg was evaluated in acute inflammation against carrageenan induced paw edema in rats and acetic acid-induced vascular permeability in mice. *In vitro* anti-inflammatory activity of CTW was studied by membrane stabilizing activity i.e. red blood cells (RBC's) exposed to hypotonic solution and inhibition of heat induced albumin denaturation. *In vitro* experiment was performed in triplicate to minimize the errors CTW significantly inhibited edema induced by carrageenan in rats and reduced significantly ($p < 0.05$) acetic acid-induced vascular permeability in mice. When tested *in vitro,* CTW exhibited significant membrane-stabilizing property and inhibited heat induced protein denaturation. Indomethacin was used as a positive control. Results were analyzed by One-way ANOVA followed by Dunnett's test $p < 0.05$ and were considered significant as compared to control. The results indicate that this plant possesses anti-inflammatory activity and has therapeutic potential for the treatment of inflammatory diseases. **References:** [1] Varier, S.V. (1993) Indian Medicinal Plants. First edition, Vol. 1. Madras (India): Orient Longman Ltd.

PJ76 The antimicrobial activity of *Satureja kitaibelii* Wierzb. ex Heuff., Lamiaceae

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The aerial parts of *Satureja kitaibelii* were used in Serbian traditional medicine as herbal remedy for respiratory, digestive and urinary tract diseases, as well for inflammation of skin and mucous membranes [1]. Previous studies concerned the composition of essential oil [2], the presence of acacetin glycosides [3], and antioxidant and antimicrobial activity of different extracts [4]. The aerial parts of *Satureja kitaibelii* were collected in July 2008 on mountain Rtanj (Serbia). The antimicrobial activities of *S. kitaibelii* essential oil and the methanol extract of aerial parts were tested using broth microdilution method [5]. The essential oil was highly active against tested Gram-positive and Gram-negative bacteria (MIC 0,39 – 25 μg/ml) and especially against *Candida albicans* with MIC 0,097 μg/ml. The major compounds in the essential oil were o-cymene (17.09%), limonene (13.56%), γ-terpinene (13.56%), ß-ocimene (7.73%) and thymol (8.21%) determinated by GC-FID and GC/MS analysis. Luteolin and its methoxy derivatives, diosmetin and acacetin were identified using HPLC.

	Methanol extract (μg/mL)	Essential oil (μg/mL)
Staphylococcus aureus (ATCC 25923)	0.39	0.78
Staphylococcus epidermidis (ATCC 12228)	0.78	0.78
Micrococcus luteus (ATCC 10240)	0.78	0.78
Enterococcus faecalis (ATCC 29212)	25	25
Bacillus subtilis (ATCC 6633BB)	0.78	0.78
Escherichia coli (ATCC 25922)	0.78	1.56
Klebsiella pneumoniae (NCIMB 9111)	1.56	1.56
Pseudomonas aeruginosa (ATCC 27853)	25	12.5
Candida albicans (ATCC 10259).	12.5	0.097
Candida albicans (ATCC 24433).	6.25	0.097

References: [1] Tucakov, J. (1973) Lečenje biljem, Beograd. [2] Slavkovska, V. et al. (2001) Phytochemistry 57:71 – 76. [3] Marin, P. et al. (2001) Phytochemistry 58:943 – 947. [4] Četković, G. et al. (2007) Int. J. Mol. Sci. 8:1013 – 1027. [5] Candan, F. et al. (2003) J. Ethnopharmacol. 87:215 – 220.

PJ77 The antimicrobial activity of *Usnea barbata* ethanol extract

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The well-known antimicrobial activity of usnic acid, isolated from different *Usnea sp*., was used in many patents for topical and oral administration [1,2]. Also, usnic acid is used for weight loss, but hepatotoxicity in humans was reported after the ingestion of dietary supplements with usnic acid [3]. Because of economic reasons, as well as reported liver toxicity were the motives for further research of antimicrobial properties of remaining solution after the isolation of crystal usnic acid. The cutted lichen *Usnea barbata* (20 g) was extracted with 96% ethanol under reflux during 15 min. Usnic acid was isolated by crystalization, the solvent was evaporated under reduced pressure at 40°C (total yield 1,54 g). The composition of the extract was monitored by TLC and HPLC. Our results have shown remaining activity of ethanol extract after the isolation of usnic acid, and even higher activity against Gram-positive bacteria *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Bacillus subtilis* with MIC 0.78 μg/ml, and Gram-negative *Klebsiella pneumoniae* (MIC 1,56 μg/ml). Antifungal activity was lower compare to usnic acid.

	Ethanol extract (μg/mL)	Usnic acid (μg/mL)
Staphylococcus aureus (ATCC 25923)	0.78	1.17
Staphylococcus epidermidis (ATCC 12228	0.78	1.17
Micrococcus luteus (ATCC 10240)	0.78	1.17
Enterococcus faecalis (ATCC 29212),	50	2.34
Bacillus subtilis (ATCC 6633BB)	0.78	1.17
Escherichia coli (ATCC 25922)	0.78	0.58
Klebsiella pneumoniae (NCIMB 9111)	1.56	2.34
Pseudomonas aeruginosa (ATCC 27853)	50	4.68
Candida albicans (ATCC 10259).	50	37.5
Candida albicans (ATCC 24433).	25	18.78

References: [1] Stankovic, M. et al. (1989) Yu Pat 43978. [2] Stankovic, S. et al. (2001) Pat WO 01/95900 and EP 1294373. [3] Guo, L. et al. (2008) J. Environ. Sci. Heal. Part C 26:317 – 338.

PJ78 Isolation of metabolites from the wild mushrooms *Helvella lacunosa* and *Helvella crispa*

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Wild mushrooms are good sources of a wide range of metabolites that can exhibit diverse nutritional and medicinal properties. Very few species of wild mushrooms have been extensively investigated for their activities. The present work, in a continuation of our research for the isolation of new metabolites from wild mushrooms, focuses on the investigation of two species of Ascomycetes *Helvella lacunosa* and *Helvella crispa,* which belong to the family *Helvellaceae*. Both species are considered edible, although they are regarded as suspicious for gastrointestinal symptoms to some people. The species *H. lacunosa* and *H. crispa* were collected in Mt. Parnitha from *Abies cephalonica*. In the lab the specimens were lyophilized and finally extracted. *H. lacunosa* was first extracted with a Supercritical Fluid Extractor using CO_2 and then with Accelerated Solvent Extractor (ASE) using EtOH. *H. crispa* was extracted directly with Accelerated Solvent Extractor using EtOH as a solvent. Subsequently, the fractionation and investigation of *H. lacunosa* supercritical extract lead to the isolation and identification of hexadecanoic acid, octadecanoic acid, linoleic acid, oleic acid, hexadecanoic acid ethyl ester, oleic acid methyl ester, oleic acid ethyl ester, linoleic acid ethyl ester, crinosterol and squalene. The extract from the ASE lead to the isolation of hexadecanoic acid, octadecanoic, eicosanoic, docosanoic and tetracosanoid acid. In addition, crinosterol and mannitol were found to be the major compound of this extract. The investigation of the EtOH extract of *Helvella crispa* lead to the isolation of crinosterol, oleic acid butyl ester, octadecanoic acid butyl ester and mannitol. Those findings indicate that both species *H. lacunosa* and *H. crispa* are good sources of some essential fatty acids and may support to their nutritional value.

PJ79 *Salvia miniata* Fernald (Lamiaceae): characterization of a new clerodane diterpenoid and phytotoxic activity of previously isolated diterpenes

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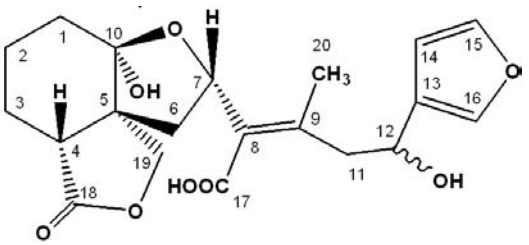
In a preliminary test, the aerial part exudate of *Salvia miniata* Fernald [1] showed anti-germinative activity against *Papaver rhoeas* L. and *Avena sativa* L. In this work, the antigerminative activity of previously isolated diterpenes [2] has been evaluated and the phytotoxicity (total germination inhibition at 60 mg/L against *Papaver* and at 80 mg/L against *Avena*) of one of these is described. Moreover, we report a new compound obtained from the chromatographic separation of a not previously considered exudate fraction. The surface exudate, obtained by rinsing the plant material with CH_2Cl_2, and subjected to repeated column chromatography on Sephadex LH-20 and silica gel and to semi-preparative reversed-phase HPLC, yielded a new clerodane diterpenoid (1), identified by IR and NMR analysis, including TOCSY, COSY, HSQC, HMBC and ROESY experiments.



(**1**)

References: [1] Epling, C. (1940) A Revision of *Salvia*, subgenus Calosphace. In: Repertorium Specierum Novarum Regni Vegetabilis. Vol.110. Fedde F. Berkley, California: University of California Press. [2] Bisio, A. et al. (2008) Planta Med. 74:1041 – 1041

PJ80 Investigation of bioactive compounds in the genus *Garcinia* (Guttiferae) of Cameroon

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The very high cost of imported drugs coupled with the inadequacy of modern health care personnel and infrastructures excludes a very large majority of the Third World population from any modern health care program. Thus, traditional medicine remains and will remain for a long time, the main source and method of health care for most developing countries. The genus *Garcinia* (Guttiferae) which comprises 200 species is widespread in the tropical regions and 21 species of *Garcinia* are found in Cameroon [1]. Three of these, *G. epunctata*, *G. brevipedicellata* and *G. preussii*, are indigenous medicinal plants of Cameroon [1], and were screened for their activities in simple benchtop tests. Biologically active compounds belonging to the triterpenoid, xanthone and flavonoid classes have been found in the genus *Garcinia* [2]. Some of these exhibit a wide large of biological and pharmacological properties such as antimicrobial and antioxidant action [2]. As no phytochemical investigation of *G. preussii* has been reported so far, this specie was selected for further study. Antioxidant activity was investigated by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging on TLC plates. A TLC bioautographic method for the detection of acetylcholinesterase inhibitory activity was also performed. The screening test was able to detect inhibition of acethylcholinesterase (AChE) and positive antioxidant activity by compounds present in the hexane extract of the fruit. Thus, several compounds responsible for these activities were isolated from this part, using usual chromatographic methods. With the aid of spectroscopic methods (IR, RMN ^{1}H, RMN ^{13}C, SM, HMQC, HMBC, COSY) and by comparaison with information available in the literature, three of these compounds have been identified respectively as Garcinol, isogarcinol, and guttiferone E **References:** [1] Guedje, N.M. et al. » Le genre *Garcinia* au Cameroun: diversité et utilisations traditionnelles « [on line] available on: http://carpe.umd.edu/resources/Documents/report-guedje_-chaungueu.pdf. [2] Waffo, A.F.K. et al. (2006) Chem. Pharm. Bull. 54:448-451.

PJ81 Evaluation of the anti-inflammatory efficacy of *Glycyrrhiza uralensis* according to extracting solvents

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Glycyrrhiza uralensis (Leguminosae) is a well-known herbal medicine that has long been valued as a demulcent to relieve inflammatory disorders [1]. To compare the influence of different extracting solvents on the anti-inflammatory efficacy of *G. uralensis*, we measured the inhibition of pro-inflammatory mediators such as NO, TNF-α, and PGE_2 in lipopolysaccharide (LPS)-stimulated mouse macrophage RAW 264.7 cells by extracts produced using different solvents (water, ethanol, methanol, or *n*-hexane) [2]. The results showed that methanol was the most effective extracting solvent for the inhibition of both NO and PGE_2 production in RAW 264.7 cells. However, there was no difference among the extracts for inhibition of TNF-α, and the extract from *n*-hexane had no detectable activity. Further study must be performed for the analysis of correlation between the anti-inflammatory activity of extracts produced using different solvents and the content of major bioactive compounds in *G. uralensis*, such as glycyrrhizin and liquiritin [3]. The present study suggests that methanol may be a more appropriate extracting solvent of *G. uralensis* for yielding the greatest anti-inflammatory activity for food

additives and medicine, as compared with other solvents (water, ethanol, and *n*-hexane). **References:** [1] Cheng, A. et al. (2008) Int. Immunopharmacol. 8:43 – 50. [2] Fujiwara, N. et al. (2005) Curr. Drug Targets Inflamm. Allergy 4:281 – 286. [3] Sun, C. et al. (2008) Phytochem. Anal. 19:160 – 163.

PJ82 Pomelo fruit juice increased cell survival and enhanced Glutathione-S transferase (GST) activity in doxorubicin-induced rat cardiac cell cytotoxicity

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Pomelo (*Citrus Maxima*, CM) is a tropical fruit native to South East Asia. Broadly proven by scientific evidence, fruits in the citrus family provide antioxidative effects and enhance detoxification metabolism of cytotoxic agents [1,2]. In this study, the amounts of five major antioxidants/common constituents of citrus fruits were evaluated by HPLC. We investigated the cytoprotective effect of CM against DOX-induced cytotoxicity. Enzyme activity and mRNA expression of GST in rat cardiac cell (H9c2) treated with the cytotoxic agent doxorubicin (DOX, 100 nM) were also evaluated. Cell survival was significantly decreased to 69.32%± 6.26% (% Control) in cells treated with DOX while CM dose-dependently protected cells as assessed by crystal violet cell staining assay. DOX significantly decreased GST activity and CM reversed DOX effect by increasing enzyme activity. Despite the apparent effect of CM on GST activity there was no significant alteration in GST-Pi mRNA level. In summary, CM fruit juice can be promoted as functional fruit to protect cells from cytotoxic agent, enhance phase II enzyme activity, and expedite metabolism of potential cytotoxic/carcinogenic agents. *Acknowledgements: Srinakharinwirot University Research Fund; Thammasat University Travel Award; Assoc. Prof. Dr. Ampaiwan Paradornuwat, Faculty of Agriculture, Kasetsart University (botanical identification of Citrus Maxima).* **References:** [1] Prince, M. et al. (20089) Toxicol. Lett. 185:180 – 186. [2] Pisoschi, A.M. et al. (2009) Molecules. 14:480 – 493.

PJ83 Twenty five years of pharmacognostic research on Panamanian flora (1984 – 2009)

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Panamanian flora is one of the richest in the world. Its medicinal and economic potential has not been fully explored. The results of ethnobotanical surveys among the Kuna, Ngöbe-Buglé and Naso Amerindians of Panama will be presented. Results of bioassay-guided fractionation of plants within the framework of multinational collaborative projects executed during the last 25 years 1984 – 2009, supported by the Organization of American States, National Secretariat of Science, Technology and Innovation of Panama, European Union, Convenio Andres Bello, ICBG, and International Foundation for Science will be presented. Examples of successful collaborations with the University of Lausanne-Geneva will also be highlighted. *Acknowledgement: SENACYT and OAS*

PJ84 Effects of extracts from *Myrothamnus flabellifolia* Welw. on *Streptococcus mutans* induced biofilm formation and *Porphyromonas gingivalis* induced inflammation parameters in KB cells

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Streptococcus mutans (ATCC 25175), a Gram-positive, facultatively anaerobic bacterium is a significant contributor to dental caries as well as periodontitis. It is one of the early colonizers of the human oral cavity and involved in plaque formation and accumulation. An acetone/water extract from *Myrothamnus flabellifolia* Welw. was tested on its influence on biofilm formation by *S. mutans*. 10 µg/mL of the extract were incubated with *S. mutans* over 72 hours and biofilm formation decreased to 70%, compared to an untreated control. A polyphenol-enriched extract from *M. flabellifolia* Welw., which has shown antiadhesive effects on the adhesion of *P. gingivalis* on KB cells (investigated via FACS assay), was tested on its influence on inflammation effects caused by *P. gingivalis*. Oral epithelial cells (KB-cells ATCC CCL 17) were treated with *Porphyromonas gingivalis* (ATCC 33277) for RT-PCR investigation of the influence of this Gram-negative bacterium on the gene expression of cyclooxygenase-2 (COX-2), tumor necrosis factor alpha (TNFα) and interleukins IL-1β, IL-6 and IL-8. The expression of ILs and TNFα were stimulated up to 10 times by *P. gingivalis*. Incubation of KB cells with only the extract from *M. flabellifolia* Welw. (10, 50,100 µg/mL, 6 hours) resulted in significant stimulation up to twenty times of inflammation related genes. Coincubation of KB cells with the extract and *P. gingivalis* led to 10- to 25-fold increase of COX-2, IL-6 and IL-8. These results lead to the assumption that polyphenols can stimulate inflammation parameters.

PJ85 Development of a fast method for the isolation of triterpene saponins from *Actaea racemosa*

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Extracts of the sub-aerial parts of *Actaea racemosa* L. (Ranunculaceae), commonly known as black cohosh, belong to the most selling herbal supplements, mainly used to treat mild climacteric symptoms. Although the plant is phyto-chemically well investigated the mechanism of action still is unknown and discussed controversially [1,2]. One discussed group of active principles are cylcoartane glycosides which are also used for standardization of extracts. Due to the fact that only a few reference substances of *A. racemosa* are commercially available and prices for those substances are very high, a fast and simple method for isolation and purification of triterpenes from black cohosh was developed. Accelerated solvent extraction (ASE) was used for defatting and extracting the sub-aerial parts. The obtained extract was subjected to Sephadex LH-20 CC leading to three highly enriched fractions. One fraction mainly contained actein, the second fraction 23-epi-26-deoxyactein, while the third fraction was a mixture of additional derivatives. The most complex third fraction was used for optimization of a high-speed counter-current chromatography system, an established technique for the separation of saponins. Separation parameters were first optimized on analytical scale, using a hyphenated HSCCC-ELSD setup, before the system was scaled up to preparative size. The optimized two-phase solvent system, consisting of *n*-hexane-acetone-ethyl acetate-2-propanol-ethanol-water (3.5:1:2:1:0.5:2, v/v/v/v/v/v), enabled the isolation of the aglycone cimigenol (purity of 98.4%) and three triterpene glycosides (purities of 96.8%, 96.2% and 97.9%). The same method was suitable for the purification of actein (97.0%) and 23-epi-26-deoxyactein (98.3%). **References:** [1] Palacio, C. et al. (2008) Pharmacol. Res. 58:8 – 14. [2] Borelli, F. and Ernst, E. (2002) Eur. J. Clin. Pharmacol. 58:235 – 241.

PJ86 Lamiaceae essential oils and alcoholic extracts and their effects on zoonotic multi rug- resistant bacteria

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In the last decade, an increasing incidence of multi-drug resistant bacterial strains, both in human and animal medicine, has been reported. Furthermore, the phenomenon of drug resistance was encountered mainly in zoonotic bacteria. Vegetal extracts and essential oils, widely used in folk medicine and well-known for their bioactive potential, were suggested by numerous researches to represent a therapeutic alternative. It is only recently that a number of findings have emerged on the chemistry and biological activity of plants in Lamiaceae family, some of them referring to bioactive compounds able to inhibit bacterial growth [1,2]. This study aimed to evaluate and compare antimicrobial properties of essential oils and ethanolic extracts from *Thymus vulgaris*, *Salvia offi-*

cinalis, *Lavandula officinalis*, *Mentha piperita*, *Rosmarinus officinalis*, *Ocimum basilicum*, *Melissa officinalis* and *Origanum vulgare* against multi-drug-resistant strains of *Staphylococcus spp*., *Salmonella spp*., *E. coli* and *Pseudomonas aeruginosa*, isolated from diseased animals. The antimicrobial potential was assessed by disc diffusion test, while minimal inhibitory (MIC) and bactericidal (MBC) concentrations were determined by a broth microdilution method. Synergistic interactions between the tested extracts and two antibiotics (enrofloxacin and amoxicillin-clavulanic acid) were screened by Etest method. The antibacterial effects, dependent on type of vegetal product and on bacterial species, were statistically significant ($p < 0.001 – 0.05$) for all screened Lamiaceae species, more pronounced against Gram-positive than Gram-negative bacteria. *Melissa officinalis* essential oils demonstrated the strongest antimicrobial efficacy against all bacterial strains. *Thymus vulgaris* and *Salvia officinalis* essential oils possessed promising antibacterial properties against tested animal pathogens, displaying synergism with enrofloxacin. **References:** [1] Pasqua, R. et al. (2005) Ann. Microbiol. 55:139 – 143. [2] Delamare, A. et al. (2007) Food Chem. 100:603 – 608.

PJ87 Immunostimulatory activity of *Rhus verniciflua* stokes *in vitro*

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Rhus verniciflua stokes (RVS) have been used as a traditional food and medicine to enhance immune response against infectious agents and to treat cancers [1]. Unfortunately, there is little scientific evidence to support efficacy of this widely used botanical, and little information about potential mechanism of action. In this study, the methanol extract of RVS and its successive *n*-butanol, ethyl acetate and aqueous extracts have been screened on immune response of goat neutrophils and murine RAW 264.7 cells. Freshly isolated neutrophils from healthy goats were incubated fractions of the RVS, and then they were tested for migration and superoxide production induced opsonized zymosan. And also the immunostimulatory effects of its fractions assessed by *in vitro* spleen lymphocyte proliferation and nitric oxide (NO) production. The *n*-butanol fraction stimulated spleen lymphocyte proliferation, NO production, goat neutrohphil migration and superoxide production and also ethyl acetate fraction exhibited some immnostimulatory activity ($p < 0.05$).

Dose µg/ml	Spleen lymphocyte proliferation (OD value/ 1 x 10^4cell ml^{-1})			
	Methanolic extracts	Aqueous extracts	Ethyl acetate extracts	n-butanol extracts
1	0.513 ± 0.056	0.555 ± 0.015	1.094 ± 0.026*	1.108 ± 0.050*
10	0.565 ± 0.007	0.604 ± 0.064	1.184 ± 0.002*	1.222 ± 0.103*
100	0.449 ± 0.020	0.523 ± 0.013	1.189 ± 0.026*	0.806 ± 0.106
Control	0.661 ± 0.027			

These results suggest that RVS *n*-butanol extracts examined here, exhibit immunostimulatory activity, which implicates that RVS *n*-butanol extract may serve as a potential source of natural immunostimulants for treatment of some animal diseases. **References:** [1] Lee, J.H. et al. (2009) Phytomedicine 16:188 – 197.

PJ88 Mechanisms involved in the prophylactic effect of STW 5 in an acute model of esophagitis in rats

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Gastro-esophageal reflux is a gastrointestinal complaint associated with a variety of functional disorders of the stomach, including functional dyspepsia, where STW5 (Iberogast®) has been successfully used to alleviate symptoms including heartburn. The present study was aimed at investigating the effect of this drug in an experimental model of esophagitis. Esophagitis was induced in male Wistar rats by gastric ligation between fore-stomach and corpus as well as between stomach and pylorus. The pH of the lower third of the esophagus was measured 3 h later. Rats were sacrificed 5 h from surgery. The gastric ligations led to marked esophagitis, measured as the ulcerative area of the esophageal mucosa. To test the activity of STW5, the rats were treated with the drug daily for 5 successive days at different dose levels (0.2 – 2 ml/kg) by oral gavage. On day 5, animals were anesthetized 3 h after the last dose, and esophagitis was induced as described above. STW 5 led to a significant dose-dependent reduction of the ulcerative area, but had no effect on lower esophageal pH. Measurement of myeloperoxidase activity and lipid peroxidation as well as mediators, including TNF-α and IL-1ß confirmed the anti-inflammatory activity of the drug. Pantoprazole (5 mg/kg) was used as a reference standard. The findings were confirmed by histopathological examination of the lower esophagus. The results indicate that the beneficial effect of STW 5 (Iberogast®) in heartburn as a symptom of functional dyspepsia could in part result from an anti-inflammatory effect on the mucosa of the esophagus.

PJ89 Selective antimicrobial activity of biochanin A

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Biologically active components such as isoflavonoids are common part of human and animal diets. Isoflavonoids are plant secondary metabolites known to possess various biological activities [1]. These compounds play important roles in growth, development and defence against microorganisms and pests. In this study, we decided to test the selective inhibitory activity of the isoflavonoid biochanin A against potential bacterial pathogens of the human digestive system represented by several species of the genus *Clostridium* and simultaneously towards beneficial human microbiota. In this study, biochanin A has shown significant selective antimicrobial activity. *Clostridium tertium* and *C. clostridioforme* were the most sensitive species with a minimum inhibitory concentration (MIC) of 0.13 mM, followed by *C. ramosum*, *C. paraputrificum* and *C.butyricum* (MIC 0.26 – 0.51 mM). Interestingly, biochanin A did not affect the growth of any strain of *Lactobacillus* spp. or of bifidobacteria even at concentration of 4 mM. Our results suggest the potent selective antimicrobial properties of biochanin A. *Acknowledgements: This research was supported by Czech Science Foundation (Project No. 525/08/H060)* **Reference:** [1] Dastidar, S.G. et al. (2004) Int. J. Antimicrob. Agents 23:99 – 102.

PJ90 Determination of the carvacrol concentration in the essential oil of *Thymus kotschyanus*

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Carvacrol is used as oral bactericidal, anti-fungal and breath freshening compound but there exist some reports on its toxicity [1]. Therfore the amount of carvacrol in oral products should be detected carefully. *Thymus kotschyanus* was collected in the city of Yazd in Iran and the essential oil obtained by hydrodistillation (HD) or microwave oven distillation (MD) was analyzed by GC and GC/MS. Major constituents in the oil isolated by HD were carvacrol (80.66%), 1,8-cineol (2.98%), borneol (1.49%) and thymol (1.47%), whereas in that obtained by MD it were carvacrol (65.99%), thymol (4.4%), borneol (4.19%), and 1, 8-cineol (2.44%). In another study on *T. kotschyanus,* the maximum amount of carvacrol was 65.94% [2]. In order to determine an accurate carvacrol concentration, diphenylamine was used as internal standard. A calibration curve was established by addition of diphenylamine to 2, 4, 6 and 8 mg/ml carvacrol and AUC determination of carvacrol and diphenylamine. The carvacrol concentration of *T. kotschyanus* can be calculated to be 0.196% in MD-method or 1.02% according to the HD-method due to different yield of essential oil compounds in dependence of the isolation method. *Acknowledgments: We would like to thank the authorities of Tehran University for its financial support*. **References:** [1] Sefidkon, F. (2002) J. Essent. Oil Res. 14:116 – 117. [2] Stammati, A. et al. (1999) J. Food Chem.Tox. 37:813 – 823.

PJ91 Chemical composition of Iranian *Artemisia annua* L. essential oil and its antibacterial, antifungal and antioxidant effects

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Artemisia annua essential oil has potential to be used in perfumery, cosmetics and aromatherapy. Since the oil composition and effects varies in different climate, our objective was to investigate the *A. annua* oil from Rasht region in Iran. The *A. annua* were gathered and subjected to hydrodistillation method. The achieved essential oil analyzed by GC/MS and 48 compounds were identified. Major constituents were β-selinene (16.16%), camphor (12.12%) and β-caryophyllene (7.43%).These identified components and their percentages were different from previous studies [1,2]. Antimicrobial and antifungal tests carried out by Agar dilution method, showed inhibition against *S. aureus*, *S. epidermidis*, *P. aeroginosa*, *B. cereus*, *B. subtilis*, *C.albicans* and *A. niger* (MIC = 2.5, 2.5, 2.5, 1.25, 0.156, 2.5, 2.5 $\mu l ml^{-1}$ respectively) and did not show significant activity on E. coli. In antioxidant test, the oil was able to reduce the stable free radical DPPH to the yellow colored with IC_{50} of 1.6 $\mu l ml^{-1}$ which was comparable with α-tocopherol and BHA. It represented that *A. annua* oil can be used in food and other allied industries as a rich source of antioxidant and antimicrobial agents. *Acknowledgments: we would like to thank the authorities of Tehran University for its financial support*. **References:** [1] Verdian, M. (2008) African J. Plant Science 2:16 – 18. [2] Fabien, J. et al. (2002) J. Fitoterapia 73(6):532 – 535.

PJ92 Insecticide activity of peppermint and lavender extracts isolated by different methods

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Increased pest resistance against traditional synthetic insecticides is the present problem of agriculture worldwide. In addition, components of these insecticides are not easily environmentally degradable and have negative effects on human health. Thus, botanical insecticides, i.e. the agents containing natural plant compounds, are expected to be applied in the future as selective, efficacious and toxicologically-safe insecticides [1,2]. Biologically active components were isolated from peppermint (*Mentha piperita* L.) and lavender (*Lavandula angustifolia* L.) using the supercritical fluid extraction (SFE). Three types of extracts were prepared using the benefit of variable solvent power of supercritical carbon dioxide under different experimental conditions, and compared in terms of chemical composition and biological activity with the products of hydrodistillation and Soxhlet extraction with ethanol and hexane. The composition of volatile oil in all extracts was determined using GC-MS and GC-FID. Insecticide activity of all isolates was determined in terms of toxicologic and antifeedant effects against model kinds of insects (*Spodoptera litoralis*, *Musca domestica* and *Leptinotarsa decemlineata*). Strong insecticidal effects of all isolates were observed, but significant differences between the particular isolates and plants were found. The efficiency of CO_2 extracts was comparable with that of hydrodistillate and higher than the efficiency of other extracts. On the basis of LD_{50} values, the peppermint isolates showed specific effect on adults of *Leptinotarsa decemlineata* in contrary to lavender extracts which were more effective against *Musca domestica* adults and *Spodoptera litoralis* larvae. *Acknowledgement* The authors thank the Ministry of Education, Youth and Sports (project No. 2B06049) for financial support. **References:** [1] Isman, M.B. (2000) Crop. Prot. 19:603 – 608. [2] Pavela, R. (2007) Pest. Technol. 1(1):47 – 52.

PJ93 Evaluation of phenolic composition and biological activity of honey

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Honey is a natural food largely known as a sweetener, produced by *Apis mellifera* bees by collecting nectar from flowers. The use of honey in the treatment and prevention of numerous diseases such as respiratory disorders has been known since ancient times. It has been well documented the role of oxidative stress in many diseases and several studies demonstrated that honey contain a great number of metabolites that can scavenge free radicals. In this study, we investigated and compared phenolic content, antioxidant capacity and *in vitro* biological activities of multifloral commercial and artisanal Italian honey samples. Total phenolic content and radical-scavenging activity were analysed by Folin-Ciocalteu reagent and by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays, respectively. Phenolic compounds were extracted using Amberlite XAD-2 resin and a RP-HPLC method involving gradient elution and UV detection was applied to their separation [1]. The antimicrobial activity of the phenolic extracts was tested by means of a luminescence bacterial biosensor [2]. A strain of *Streptococcus pyogenes* isolated from human throat containing a firefly luciferase reporter gene was incubated with the honey phenolic fractions. A luminometer was used to quantify bioluminescence, which is directly connected to the cell metabolic state. The results demonstrated remarkable difference in bioactivity properties of honey from different sources. Total phenolic content, antioxidant and antibacterial activities were higher in artisanal honey samples, suggesting their richness in bioactive compounds that might exert potential health-promoting effects and increase its nutraceutical value. Acknowledgements: *Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università di Padova, AGRIPOLIS, Viale dell'Università n. 16, 35020 Legnaro (PD), Italia, Prof. Stefano Bona, Dr.ssa Sara Sandrini.* **References:** [1] Küçük, M. et al. (2007) Food Chem. 100:526 – 534. [2] Wilson, T. et al. (1998) Annu. Rev. Cell Dev. Bi. 14:197 – 230.

PJ94 New cycloartane-type glycosides from *Astragalus icmadophilus* Hand.-Mazz.

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Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey [1]. The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant, diuretic and tonic drug [2]. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins [2]. Our earlier investigations performed on *Astragalus species* resulted in the isolation of a series of cycloartane-type triterpenic saponins [3,4]. In our continuing search on Turkish *Astragalus* species, we have isolated four new and five known triterpene glycosides from methanolic extract of *A. icmadophilus* by combined chromatografies on reverse phase C-18 and silica gel. The structures of the new compounds were determined as 3-*O*-[α-L-arabinopyranosyl(1→2)-3′-O-acetyl-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(S),25-pentahydroxy-cycloartane, 3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24(S),25-pentahydroxy-cycloartane, 20(R),25-epoxy-3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-cycloartane, 20(R),25-epoxy-3-*O*-[α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-xylopyranosyl]-6-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-cycloartane by a combination of one- and two-dimensional NMR techniques, and mass spectrometry. **References:** [1] Davis,

P.H. (1978) "Flora of Turkey and East Aegean Islands" Vol.3, University Press: Edinburgh. [2] Tang, W., Eisenbrand, G. (1992) "Chinese Drugs of Plant Origin" Springer-Verlag, Berlin. [3] Bedir, E. et al. (1999) Phytochem. 51:1017 – 1020. [4] Bedir, E. et al. (1999) J. Nat. Prod. 62:563 – 568.

PJ95 Thymoquinone is active *in vitro* against *P. larvae* and exhibits low toxicity against adult honey bees

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American foulbrood (AFB) is a serious worldwide spreading disease of honeybees caused by the spore-forming, Gram-positive bacterium *Paenibacillus larvae*. Antimicrobial natural products may provide a safe and acceptable alternative in prevention and treatment of AFB. The inhibiting action of thymoquinone (TQ) on *P. larvae* was previously studied *in vitro* [1] (MIC 8 – 16 μg/mL). Laboratory and field trials were conducted to evaluate the acute oral toxicity and transfer of TQ to royal jelly or to honey. We determined *in vivo* acute oral toxicity on adult honey bees by technique ICBR (1993) [2], expressed as LD_{50}. The defined amounts of TQ were dissolved in a feeding solution (50% v/v sucrose in distilled water). The second experiment transfer to royal jelly was conducted from September to October. Non-toxic dose of TQ was fed on honey bee colony. Amount of TQ was given with the aid of GC-MS methods. LD_{50} value of thymoquinone was higher than 50 μg per bee and would be classifying according to results as slightly or non-toxic compound. TQ occurred in royal jelly in concentrations potentially useful for application against *P. larvae*. *Acknowledgements: This research was supported by grants NAZV QH72144, CIGA 20082012 and GACR (No. 525/08/H060).* **References:** [1] Flesar, J. et al. (2008) Planta Med. 74:1135. [2] ICPBR (1993) Hazard of Pesticides to Bees. Wageningen, The Netherlands.

PJ96 Phytochemical studies on *Astragalus wiedemannianus* Fischer

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Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey [1]. The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant, diuretic and tonic drug [2]. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins [2]. Our earlier investigations performed on *Astragalus species* resulted in the isolation of a series of cycloartane-type triterpenic saponins [3,4]. In our continuing search on Turkish *Astragalus* species, we have isolated a new and four known triterpene glycosides from methanolic extract of *A. wiedemannianus* by combined chromatografies on reverse phase C-18 and silica gel. The structure of the new compound was determined as 20(*R*),24(*S*)-3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl]-25-*O*-β-D-glucuronopyranosyl-3β,6α,16β,25-tetrahydroxy-cycloartane by a combination of one- and two-dimensional NMR techniques, and mass spectrometry. The known saponins were identified as cycloastragenol, astragaloside IV, astragaloside VIII, and cycloascauloside B by spectral data and comparison of their physical properties with those reported previously for these compounds [5 – 7]. **References:** [1] Davis, P.H. (1978) "Flora of Turkey and East Aegean Islands" Vol.3, University Press: Edinburgh . [2] Tang W., Eisenbrand G. (1992) "Chinese Drugs of Plant Origin" Springer-Verlag, Berlin. [3] Bedir, E. et al. (1999) Phytochem. 51:1017 – 1020. [4] Bedir, E. et al. (1999) J. Nat. Prod. 62:563 – 568. [5] Kitagawa, I. et al. (1983) Chem. Pharm. Bull. 31:698 – 708. [6] Kitagawa, I. et al. (1983) Chem. Pharm. Bull. 31:716 – 722. [7] Alaniya, M.D. et al. (2008) Chem. Nat. Comp. 44:324 – 325.

PJ97 A new cycloartane-type glycoside from *Astragalus schottianus* Boiss.

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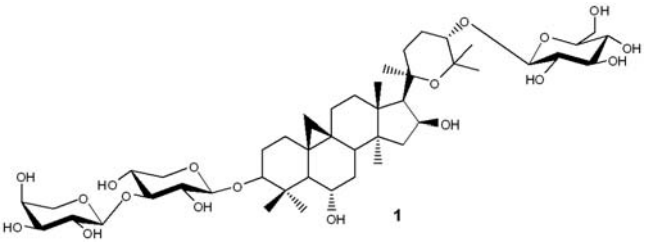
Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey [1]. The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant, diuretic and tonic drug [2]. In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins [2]. In our continuing search on Turkish *Astragalus* species, we have isolated a new cycloartane-type triterpene glycoside from methanolic extract of *A. schottianus* together with three known compounds by combined chromatografies on reverse phase C-18 and silica gel. The structure of the new compound (**1**) was determined as 20(R),25-epoxy-3-*O*-[α-L-arabinopyranosyl(1→2)-β-D-xylopyranosyl]-24-*O*-β-D-glucopyranosyl-3β,6α,16β,24α-tetrahydroxy-cycloartane by a combination of one- and two-dimensional NMR techniques.



References: [1] Davis, P.H. (1978) "*Flora of Turkey and East Aegean Islands*" Vol.3, University Press: Edinburgh. [2] Tang, W., Eisenbrand, G. (1992), "*Chinese Drugs of Plant Origin*" Springer-Verlag, Berlin.

PJ98 Phytochemical investigations of *Centaurea urvillei* DC. subsp. *urvillei*

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The large genus *Centaurea* (Asteracea-Carduae) comprises about 500 species, which are predominately distributed around the Mediteranean area and in W. Asia [1]. There are 180 *Centaurea* species, 109 of them being endemic and distributed almost all over the Anatolian peninsula as aggressively invading weeds [2,3]. *Centaurea* species have been used for their antidandruff, antidiarrhoic, antirhevmatic, antiinflammatory, antibacterial properties in folk medicine [4]. Chemical investigations of various *Centaurea* species have revealed mainly sesquiterpene lactones, flavanoids, alkaloids and lignans [5,6]. In this study, a new and six known compounds (3,5-Dihydroxyphenethyl alcohol-3-O-β-D-glucopyranoside, salidroside, luteolin, apigenin, kaempferol, syringing) were isolated from the MeOH extract of the aerial parts of *Centaurea urvillei* DC. subsp. *urvillei* by using preparative chromatographic methods. The structure of the new compound was determined as 6-hydroxykaempferol-7-O-β-D-glucuronopyranoside by means of 1D and 2D NMR studies. This is the first phytochemical report on *Centaurea urvillei* DC. subsp. *urvillei*. *Acknowledgement: This study was supported by Turkish Scientific and Technological Research Council of Turkey (Project No: SBAG-3445).* **References:** [1] Djeddi, S. et al. (2008) Biochem. System. Ecol. 36:336 – 339. [2] Flamini, G. et al. (2002) Phytochem. 61:433 – 437. [3] Yesilada, E. et al. (2004) J. Ethnopharmacol. 95:213 – 219. [4] Köse, Y. et al. (2007) Fitoterapia 78:253 – 254. [5] Karamenderes, C. et al. (2007) Phytochem. 68:609 – 615. [6] Shoeb, M., Jaspars, M. (2005) Tetrahedron 61:9001 – 9006.

PJ99 Saponins from *Agrostemma gracilis* BOISS

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The genus *Agrostemma*, belonging to the Caryophyllaceae family, is represented by two species in Turkey [1]. Although the genus is known to be toxic, some species has been used for the treatment of cancer in European folk medicine [2]. Saponins, flavonoid glycosides and anthocyanins were isolated from the genus *Agrostemma* previously [3,4]. It has been reported that extracts of some *Agrostemma* species exhibited cytotoxic [5], antihypercholesterolaemic and antioxidant activities [6]. In this study, dried and ground plant material (whole plant) was extracted with MeOH, and then treated with hexane. The residue was fractionated over Sephadex LH-20. The saponin-rich fractions were purified by various chromatographic techniques and gave four new oleanane-type saponins. The structures of the compounds were determined as 3-O-β-D-xylopyranosyloleanolic acid 28-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyloleanolic acid 28-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-O-β-D-xylopyranosylechinocystic acid 28-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-O-β-D-xylopyranosylechinocystic acid 28-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester by a combination of one- and two-dimensional NMR techniques, and mass spectrometry. This is the first phytochemical report on *Agrostemma gracilis* BOISS. **References:** [1] Davis, PH. (1967) Flora of Turkey and East Aegean Islands, Edinburgh University Press., Great Britain. [2] Duke, J.A. (1985) Handbook of Medicinal Herbs, CRC Press, Boca Raton, FL. [3] Siepmann, C. et al. (1998) Planta Med. 64:159 – 164. [4] Ferry, S., Darbour, N. (1980) Plant. Med. Phyt. 14:148 – 154. [5] Hebestreit, P., Melzig, M.F. (2003) Planta Med. 69:921 – 925. [6] Avcı, G. et al. (2006) J. Ethnopharmacol. 107:418 – 423.

PJ100 Additive antimicrobial effects of the active components of the essential oil of *Thymus vulgaris* – chemotype carvacrol

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Herbal remedies are multi component mixtures by their nature as well as by pharmaceutical definition. Being a multi component mixture is a pre-condition for interactions such as synergism or antagonism. Antibiotic activity of thyme oil and single active components were tested against six different strains of microorganisms. The degree of the detected interactions corresponded with the demarcating FICI-measure of 0.5, which separates the additive from the over additive (synergistic) effects [1]. Therefore, the observed effect was called "partial synergism". Partial synergism was observed only in the presence of *Klebsiella pneumoniae*. Additive antimicrobial activity was observed for the combination of the two monosubstances carvacrol plus linalool and thymol plus linalool as well as with the combination of the two essential oils of the carvacrol and linalool chemotypes. An increase of the carvacrol-oil concentration from one to two times the MIC resulted in a considerable acceleration of the kill-rate. Carvacrol-oil (Cve) caused a faster kill-rate than the artificial combination (Ac), which was composed of the two main active monosubstances carvacrol and thymol. **References:** [1] Odds, F.C. (2003) J. Antimicrob. Chemother. 52(1):1.

PJ101 Preliminary determination of biochemical activity of the three plants of the *Echium* genus

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The present study was undertaken to evaluate the total phenolic, flavonoid and nonflavonoid content, reducing power, chelating ability and *in vitro* antimicrobial activity of methanol extracts of three plants of the *Echium* genus (family *Boraginaceae*): *Echium vulgare, Echium italicum* and *Echium rubrum. Echium vulgare* L. (Viper's bugloss) is popular folk medicine in form of extracts for cleaning up the blood and healing wounds, in the treatment of snake bites [1]. *Echium italicum* L. (Pale Viper's-bugloss, Italian Viper's bugloss) flowers have been used as an "anti-stress", tranquilizer, and energizer drink, fighting common cold and bronchitis particularly during pregnancy [2] and also for snake bites [3]. *Echium rubrum* L. (Red Viper's bugloss) is the rarest of the three examined plants. The genus *Echium*, is especially rich in pyrrolizidine alkaloids [4]. *E. vulgare* is reported to contain pyrrolizidine alkaloids, flavonoids, phenolcarboxylic acids, sterones and naphthoquinones [5]. Shikonin pigments were isolated from the root of *E. italicum* [6]. There is very little information on chemical properties of *E. rubrum*. Total phenolic content ranged between 47.55 mg for *E. italicum*, 73.74 mg for *E. rubrum* and for *E. vulgare* 79.71 mg (per gram of methanol extract). *E. vulgare* extract proved to have the highest reducing ability, followed by *E. rubrum* and *E. italicum. E.italicum* had the highest chelating effect ranging from 42.41% to the 91.70%. All of the three methanol extracts exibited antimicrobial activity against all seven tested microorganisms. Methanol extract of *Echium vulgare* showed the strongest antimicrobial activity in general, especially against *Bacillus subtilis* and *Mycrococcus lysodeikticus* with MIC values of 1.25 mg/mL. **References:** [1] Grieve, M. (1984) A Modern Herbal, Middlesex, Penguin Books Ltd. [2] Moallem, SA. et al. (2008) J. Ethnopharmacol. 117(1):108 – 114. [3] Al-Qur, S. (2008) J. Nat. Prod. 1:10 – 26. [4] Robins, DJ. (1982) Fortschr. Chem. Org. Naturst. 41:115 – 203. [5] Kuruüzüm-Uz, A. et al. (2004) Biochem. Syst. Ecol. 32: 833 – 836. [6] Albreht, A. et al. (2009) J. Chromatogr. A 1216:3156 – 3162

PJ102 Antiulcer activity of *Feijoa sellowiana* L. (Mirtaceae): morphological study

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In a project, financed by Regione Sicilia, several studies on tropical fruits, obtained from experimental cultivations located in Sicily (Milazzo-MESSINA), were carried out. This project aims at implementing the cultivation of tropical fruit in Sicily through phytochemical and biological studies. This paper reports the preliminary results of the study about the effect of *Feijoa sellowiana* L. var. *coolidge* fruit, on gastric mucosa. The activity of methanolic extract of the fruit was studied on experimental ethanol-induced ulcer in rat. 2 g/Kg of extract were administered to the rats and, 1 h later, the ulcer was induced by administration 0.5 ml of ethanol (90%). Samples of gastric mucosa, stained by PAS and Hematoxilin/Eosin, have been observed by light microscopy. The results verify that preventive treatment with *F. sellowiana* fruit methanolic extract inhibits the ulcerogenic effect of ethanol. Actually the mucosal surface appears undamaged and the microscopic evaluations show an increase of mucus, mostly in glandular pits. These fruits contain many antioxidant compounds, including terpenes, flavonoids, steroidal saponin, ascorbic acid and minerals [1]. Besides, our study in progress shows in the fruit the presence of pectins and mucilages The protective effect on gastric mucosa could depend on the flavonoids which can stimulate prostaglandins synthesis and therefore favour mucus and bicarbonate secretion and increase mucosal blood flow. Certainly, the antioxidant potential of the fruit plays a protective role by removing damaging agents from the gastric mucosa, but it is possible to suppose a synergistic activity of all the active principles. **References:** [1] Mantas, A. et al. (2000) J. Mol. Struct. (Theochem) 504:77 – 103.

PJ103 Constituents of *Pterocephalus pinardii* Boiss

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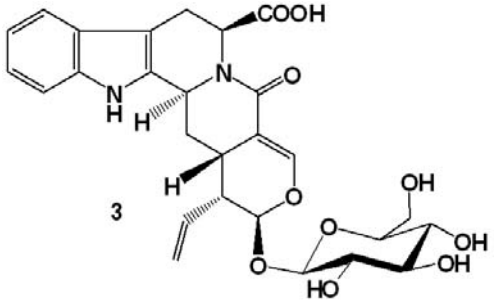
In this study, we have examined chemical constituents of the above-ground parts of *Pterocephalus pinardii* Boiss. which is an endemic plant belonging to the family Dipsacaceae. Hydroxycinnamic acid esters, iridoids, phenolic glucosides, lignans [1], triterpenoid saponins [2,3] and flavonoid C-glycosides [4] were isolated from the genus *Pterocephalus* previously. This is the first phytochemical investigation on *Pterocephalus pinardii*. The MeOH extract of the plant material was suspended in H_2O, and then partitioned in turn with CH_2Cl_2 and *n*-BuOH. Butanolic extract was fractionated over VLC (RP-18). The fractions repeatedly subjected to normal phase silica gel CC and MPLC (RP-18) and four compounds were isolated (**1 – 4**). Structures of **1 – 3** were determined as α-morroniside, β-morroniside and oxayohimban-5-carboxylic acid, respectively, by using spectral methods. Oxayohimban-5-carboxylic acid (**3**) is reported for the second time from nature.



References: [1] Graikou, K. et al. (2006) Biochem. Syst. Ecol. 34:438 – 441. [2] Tian, J. et al. (1993) Phytochemistry 32:1535 – 1538. [3] Tian, J. et al. (1993) Phytochemistry 32:1539 – 1542. [4] Ahmed, F.A., Shahat A.A. (2006) Nat. Prod. Comm. 1:457 – 459.

PJ104 Fatty acid constituents and antimicrobial activity of *Peucedanum alsaticum* L. fruits

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For the first time, the total fatty acid composition of the fruits of *Peucedanum alsaticum* collected in Poland has been examined by gas chromatography combined with mass spectroscopy. After extraction with n-hexane and methyl-esterification, fourteen compounds were identified, among which oleic and linoleic acids were predominant. Furthermore, palmitic, stearic, α-linolenic, n-eicosanoic, palmitoleic, eicosenoic, arachidonic, lignoceric, bohenic, myristic, capric, and nervonic acid were observed. Minimum inhibitory concentrations (MICs) for a panel of reference bacterial and yeast strains were performed by the micro-dilution broth method, using serial two-fold dilutions in Mueller-Hinton broth and Mueller-Hinton agar supplemented with 2% glucose for bacteria and yeasts, respectively. MIC values were between 125 and 1000 μg/ml.

PJ105 Essential oil composition and antimicrobial, antioxidant activities of some Mongolian endemic plants from species *Apiaceae*

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Essential oil plants have been used for a long time in traditional medicine. The *Apiaceae or Umbelliferae* is a cosmopolitan family. The family has about 35 species, 73 kibds in Mongolian region. The chemical composition of essential oil from *Foeniculum vulgare Mill, Coriandrum sativum L., Anethum graveolens L., Cicuta virosa L.* were determined by GC/MS-analysis. Among the 31 components obtained from *Foeniculum vulgare Mill*, α-phellandrene (9.56%), limonene (13.42%), anethol (51.32%) were found as major components. Fifteen components from the oil of *Coriandrum sativum L.* were identified and of those components, linalool, γ-terpinene and α-pinene as major constituents were obtained in yields of 60.70%, 14.50% and 10.39%, respectively. The oil from *Anethum graveolens L.* was characterized by its richness and it contains carvone (40.60%), α-phellandrene (21.03%) and limonene (10.39%) among the 13 components comprising of the total oil. β-Pinene (15.71%), geranyl acetate (15.48%), (Z)-β-ocimene (9.20%) among the 30 constituents found in the oil of *Cicuta virosa L.* were found to be the main components. Essential oils were investigated for activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtitilis by* using an agar dilution method. Interestingly, *Coriandrum sativum L.* oil even showed more high antimicrobial activity against methicilin-resistant *Staphylococcus aureus* (MRSA4) strains. Antioxidative activities (IC_{50}) of ethanol extracts from four *Umbelliferae* species have been studied by the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging test. All the studied extracts showed antioxidant capability and *Anethum graveolens L.* extract exhibited the strongest activity. The scavenging activity of the extracts in decreasing order was: *Anethum graveolens L. > Foeniculum vulgare Mill > Coriandrum sativum L. > Cicuta virosa L.*

PJ106 Quantification of the chemical profile induced in healthy *Citrus sinensis* and *C. limonia* by inoculation with *Xylella fastidiosa*

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Xylella fastidiosa is transmitted by xylem-feeding leafhoppers (Cicadellidae), and colonizes the xylem of plants, causing diseases such as citrus variegated chlorosis (CVC). Rooted *Citrus limonia* trees, however, may be grown in orchards in the presence of high disease and insect pressure without showing foliar symptoms of the disease. This information encouraged us to undertake phytochemical studies of *C. sinensis* L. Osbeck grafted onto *C. limonia*. The graft produces a considerable number of compounds that are not common in either of the seedling plants. The chemical profiles of scion and rootstock differ notably for the absence, in the former, of flavonoids and coumarins containing C_5 prenyl groups attached to the carbon atoms of aromatic and heterocyclic systems or to oxygen [1]. *C. sinensis* grafted onto *C. limonia*, symptomatic and asymptomatic for *X. fastidiosa*, was examined to determine if secondary metabolites in the plant were produced or accumulated as a chemical defense. This study revealed that the flavonoid hesperidin could be present at higher concentrations in symptomatic plants than in asymptomatic ones. Thus, we used HPLC and HPLC-ESI-MS/MS by the selected reaction monitoring (SRM) mode, to develop a rapid and sensitive method for detecting hesperidin and rutin, and the coumarins xanthyletin and seselin in all the aerial parts of plants. The amount of hesperidin was 52% higher in the leaves, and 25% higher in the scion stem of the symptomatic plant than in the asymptomatic one, strongly suggesting that hesperidin plays a role in plant-pathogen interaction, probably as a phytoanticipin. On the other hand, the amount of the coumarin xanthyletin in leaves decreased by 80% and unvaryingly occurred in only small amounts in scion stem, strongly indicating that xanthyletin is recycled within the plant enabling cinnamic acid to be incorporated into flava-

none hesperidin. **References:** [1] Ribeiro, A.B. et al. (2008) J. Agric. Food Chem. 56:7815 – 7822.

PJ107 Essential oil of *Bupleurum pauciradiatum* Fenzl flowers

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Bupleurum is a genus of the family Umbelliferae (Apiaceae), comprising about 50 taxon, including 20 endemic in Turkey. Bupleurum (*Bupleurum chinense*) is a well-known Chinese herb ('Chai Hu') with an affinity for liver [1,2]. Bupleurum root disperses liver energy, clears heat, and relieves congestion [3,4]. The essential oil of *Bupleurum pauciradiatum* Fenzl, grown in Konya, Turkey, has not been studied. The aim of the study was to determine the essential oil composition of the plant. The essential oil of *Bupleurum pauciradiatum* Fenzl flowers was obtained using both hydrodistillation and microdistillation techniques [5,6] and their chemical compositions were analyzed using both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) [7]. Overall, more than 60 compounds were identified representing 93% and 84% for hydrodistillation and microdistillation, respectively. The main components (by hydrodistillation and microdistillation, respectively) found were germacrene-D (46% and 12%) and β-caryophyllene (18% and 10%) in the analyzed essential oils. The microdistillation technique proved to be a useful tool and compliant alternative when compared to hydrodistillation. **References:** [1] A. Barefoot Doctor's Manual (1994). (translation of a Chinese instruction to certain Chinese health personnel). Omnigraphics, Detroit, Michigan. [2] Beinfield, H., Korngold, E. (1991) Between Heaven and Earth: A Guide to Chinese Medicine., Ballantine Books, New York. [3] Lu, H.C. (1991) Legendary Chinese Healing Herbs, Sterling, New York. [4] Tierra, L. (1992) The Herbs of Life: Health and Healing Using Western & Chinese Techniques., Crossing Press, Freedom, CA. [5] Briechle, R. et al. (1997) GIT Lab. Fachz. 41:749 – 753. [6] Baser, K.H.C. et al. (2001) Khim Prir Soedin. 4:282 – 284. (Also in (2001) Chem. Nat. Comp. 37:332 – 335). [7] Adams, R.P. (2001) Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publishing, 362 South Schmale Road, Carol Stream, IL 60188 – 2787, USA.

PJ108 Essential oil of *Bupleurum rotundifolium* L. flowers

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Bupleurum is a genus of the family Umbelliferae (Apiaceae), comprising about 50 taxon, including 20 endemic in Turkey. Bupleurum root is widely used in traditional medicine for the treatment of fever, pain and inflammation associated with influenza or the common cold [1,2]. The essential oil of *Bupleurum rotundifolium* L., grown in Konya, Turkey, has not been studied. The aim of the study was to determine the composition of the plant. The essential oil of *Bupleurum rotundifolium* L. flowers was obtained using both hydrodistillation and microdistillation techniques [3,4] and their chemical compositions were analyzed using both gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) [5]. Overall, more than 60 compounds were identified representing 82% and 95% for hydrodistillation and microdistillation, respectively. The main components (by hydrodistillation and microdistillation, respectively) found were α-pinene (9% and 28%) and β-phellandrene (7% and 19%) in the analyzed essential oils. The microdistillation technique proved to be a useful tool and compliant alternative when compared to hydrodistillation. **References:** [1] "Pharmacopoeia of the People' s Republic of China" (1992) Guangdong Science and Technology Pres. Guangzhou. 5th ed., Vol. I. [2] Chang, H.M., But, P.H.H. (1987) Pharmacology and Application of Chinese Materia Medica. World Scientific Publishing, 1st ed., Vol. II, Singapore. [3] Briechle, R. et al. (1997) GIT Lab. Fachz. 41:749 – 753. [4] Baser, K.H.C. et al. (2001) Khim Prir Soedin. 4:282 – 284. (Also in (2001) Chem. Nat. Comp. 37:332 – 335). [5] Adams, R.P. (2001) Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publishing, 362 South Schmale Road, Carol Stream, IL 60188 – 2787, USA.

PJ109 Study on essential oil composition and antimicrobial, antioxidant activities of *Bupleurum multinerve L.*

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Bupleurum has been widely used for over 2,000 years in Asia. *Bupleurum multinerve* is one of the endemic plants of Mongolia, which is a popular plant used in Mongolian traditional medicine. A number of essential oils from Mongolian aromatic plants are claimed to have antimicrobial and antioxidant activities. The chemical composition of essential oil from *Bupleurum multinerve was determined by GC/MS-analysis.* Among the 36 components germacrene-D (19.40%), trans-beta-ocimene (18.63%), beta-myrcene (9.13%) and limonene (7.81%) were found to be the major ones. Essential oil was investigated for activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtitilis by* using an agar dilution method. Interestingly, *Bupleurum multinerve* oil even showed antimicrobial activity against methicilin-resistant *Staphylococcus aureus* (MRSA4) strains. Antioxidant activity of ethanolic extract from *B.multinerve* was evaluated by using DPPH (1,1-diphenyl-2-picrylhydrazine) radical scavenging assay with an IC_{50} value of 63.83μg/ml.

PJ110 Isolation and purification of new minor dihydropyranochromone and furanocoumarin from fruits of *Peucedanum alsaticum* L. by high-speed counter-current chromatography

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Genus *Peucedanum* is a large group comprising more than 120 species, widely distributed in Europe, Asia and Africa. *Peucedanum alsaticum* L. is species occurring naturally in Poland but also in central and eastern Europe. However the chemical composition of examined plant is poorly known, although some flavonoids, coumarins (peucedanin, imperatorin), phenolic acids and essential oils have been investigated [1 – 6]. A preparative high-speed counter-current chromatography (HSCCC) method was successfully used for isolation of two new minor compounds – namely alsaticol (dihydropyranochromone) and alsaticocoumarin A (5-substituted furanocoumarin) – never identified in nature before. Compounds were obtained from dichloromethane extract of *Peucedanum alsaticum* fruits and their identification was performed with NMR and MS methods. This is first time when these kind of natural compounds, occurring as minor constituents, were isolated using high-speed counter-current chromatography method (HSCCC) with application of preparative 900 ml coil. This development makes an opportunity towards large scale isolation of these novel minor constituents from *P. alsaticum* or other plant species abundant in furanocoumarins and pyranochromones. *Acknowledgment: This work was financially supported by grant no. N N405 3513 33 from the Polish Ministry of Science and Higher Education.* **References:** [1] Cisowski, W. (1975) Roczniki Chem. 49:1823 – 1830. [2] Crowden, R.K. et al. (1969) Phytochemistry 8:1963 – 1974. [3] Murray, R.D.H. (1982) The natural coumarins. Occurance, chemistry and biochemistry. Wiley & Sons, Chichester. New York, Weinheim, Brisbone, Singapore, Toronto. [4] Kapetanos, C. et al. (2008) Chem. Biodivers. 5:101 – 119. [5] Skalicka-Woźniak, K. (2008) Acta Chromatogr. 20:119 – 133. [6] Skalicka-Woźniak, K. (2008) Chromatographia 68:85 – 90.

PJ111 Additive growth inhibitory effect of epigallocatechin-gallate and baicalin with doxycycline, oxytetracycline and cefamandole against various strains of *Staphylococcus aureus*

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Antimicrobial activity of epigallocatechin-gallate (EGCG), the chief flavan-type compound of *Camellia sinensis* Kuntze and baicalin, the flavon constituent of *Scutellaria sp.* as well as their ability to inhibit multidrug resistance in *Staphylococcus aureus* have previously been reported [1 – 5]. In this study we examined *in-vitro* effect of EGCG and baicalin in combination with doxycycline, oxytetracycline and cefamandole on growth of methicillin-sensitive *S. aureus* (MSSA) ATCC 29213, ATCC 25923 and methicillin-resistant ATCC 43300 strains. The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method and the effect of combinations was evaluated according to the sum of fractional inhibitory concentration (ΣFIC) indices as follows: additive effects were observed in combinations of baicalin with doxycycline, oxytetracycline and cefamandole and of EGCG with oxytetracycline and cefamandole, indifferent effect showed combinations of baicalin and EGCG with cefamandole against MSSA strains and of EGCG with doxycycline against all strains tested. The strongest activity exhibited combination of baicalin and oxytetracycline (MICs 853 and 0.33 mg/L alone and 128 and 0.125 mg/L in combination respectively) against *S. aureus* ATTC 29213 (ΣFIC = 0.53). Although the data on additive effect with tetracyclines have been published [3,4], according to our best knowledge, this is the first report on additive effect of EGCG and baicalin with doxycycline and oxytetracycline and the first report on additive effect of EGCG and baicalin with representative of cefalosporine antibiotics – cefamandole. *Acknowledgements: project MSM 604607090.* **References:** [1] Zhao, W.H. et al. (2001) Antimicrob. Agents Chemother. 45:1737 – 1742. [2] Hu, Z.Q. et al. (2002) Antimicrob. Agents Chemother. 46:558 – 560. [3] Hu, Z.Q. et al. (2002) J. Antimicrob. Chemother. 50:1051 – 1054. [4] Roccaro, A.S. et al. (2004) Antimicrob. Agents Chemother. 48:1968 – 1973. [5] Liu, I.X. et al. (2000) J. Pharm. Pharmacol. 52:361 – 366.

PJ112 Antiviral activity of ethanol extracts of *Ficus binjamina* and *Lilium candidum in vitro*

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The aim of the present study was to evaluate the antiviral activity of ethanol extracts from *Ficus binjamina* and *Lilium candidum in vitro. Ficus binjamina* is known to be resistant to various plant viruses, while *Lilium candidum* has a high susceptibility to plant viruses [1]. Vero cells and Herpes Simplex Virus -1 and 2 (HSV-1, HSV-2) and Varicella-Zoster Virus (VZV) were used. The toxicity of the extracts was tested by three methods: direct account, observation of morphological changes by optical inverted microscope and MTT assay. Evaluation of viral infection was performed by cytopathic effect examination and plaque assay. No cytotoxic effect was observed at concentrations over 100 μg/ml in all examined extracts. Extract of *F. binjamina* fruits had no effect on HSV-1 and HSV-2 infection, while significantly inhibited VZV infection with IC_{50} of 10 μg/ml ($p < 0.001$). Stems of *F. binjamina,* bulbs and petals of *L. candidum* didn't show significant antiviral properties. Leaf extracts of *F. binjamina* inhibited all studied viruses. *L. candidum* leaf extracts had no effect on VZV but strongly inhibited HSV-1 and slightly HSV-2. There was an indirect evidence for strong interactions between the plant extracts and the viruses and weak interactions with the cell surface. It is suggested that plant extracts exerted their anti-herpetic effect mainly by blocking the virus access to the host cells. **References:** [1] Petrov, D.B. et al. (1974) Journal of Botany 4:23 – 26.

PJ113 Antifungal and antioxidant activities of extracts from *Drosophyllum lusitanicum*

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Drosophyllum lusitanicum (L.) Link is an insectivorous plant of the family *Drosophyllaceae* native to the western Iberian Peninsula and northwest Morocco. Leaves of this species contain flavonoids, phenolic compounds and higher amounts of the naphthoquinone plumbagin [1,2,3]. The antimicrobial (against bacteria and yeasts) and insecticidal activities of the hexane extract from this species were previously demonstrated by our group [4,5]. The purpose of this study was to study the antifungal and the antioxidant activities of aqueous, methanol and hexane extracts from *D. lusitanicum*. Antifungal activity was tested against several mycotoxigenic fungi using the agar diffusion method followed by the determination of minimum inhibitory concentrations in liquid medium (MIC). The antioxidant activity was determined by Folin-Ciocalteau (F-C), trolox equivalent antioxidant capacity (TEAC) and oxygen radical antioxidant capacity (ORAC) assays. Results demonstrate that hexane extract is the most effective in inhibiting fungi growth, with inhibition zones ranging from 14.00 to 49.00 mm and with MIC values ranging from 15.6 to 62.5 μg ml^{-1}, which may be related with its higher content in plumbagin [3]. Moreover, the most susceptible fungus to all the extracts was *Aspergillus fumigatus*. The results show that the methanol extract has the highest antioxidant activity in all the assays (F-C: 1188.06 ± 52.96 $\mu mol_{GAE}/mg_{extract}$; TEAC: 432.23 ± 6.71 $\mu mol_{TE}/mg_{extract}$; ORAC: 764.18 ± 61.18 $\mu mol_{TE}/mg_{extract}$), possibly due to its higher phenolic content. These results indicate that *D. lusitanicum* extracts have strong antioxidant and antifungal activities, and thus could be used as sources of agents for the food and pharmaceutical industries. *S. Gonçalves acknowledges a grant from Portuguese Science and Technology Foundation (FCT, Grant SFRH/BPD/31534/2006).* **References:** [1] Nahálka, J. et al. (1998) Biotechnol. Lett. 20:841 – 845. [2] Budzianowski, J. et al. (2002). Phytochemistry 61:421 – 425. [3] Grevenstuk, T. et al. (2008). Phytochem. Analysis 19:229 – 235. [4] Gonçalves, S. et al. (2008) Nat. Prod. Res. 23:119 – 229. [5] Gonçalves, S. et al. (2008) J. Hortic. Sci. Biotech. 83:653 – 657.

PJ114 Antibacterial activity of phenolic compounds from Mongolian plants

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Fourty seven pure compounds such as flavonol derivatives (20), simple penolics (9), xanthone derivatives (13), luteolin, cynaroside, prunasin, euscaphic acid and (*z*)-hexenyl-O-α-rhamnopyranosyl-(1 – 6)-β-D-glucopyranoside isolated from Mongolian some medicinal plants have been tested against bacterial strains as *Staphylococcus aureus (Sa)*, *Micrococcus luteus (Ml)*, *Enterococcus faecalis (Ef)*, *Pseudomonas aeruginosa (Pa)* and *Escherichia coli (Ec)* by the disk diffusion method, respectively. Kanamycin was used as a standard antibiotic. From tested compounds only kaempferol, quercetin, ethylgallate, gallic acid and desmethylbellidifolin exhibited at the dose of 200 μg/disk a significant inhibition of the growth of *Sa,* while luteolin was active only at the high dose 750 μg/disk. Ethylgallate demonstrated a great activity against the growth of *Ml* at the dose of 20 μg/disk, while desmethylbellidifolin was active at 200 μg/disk. Quercetin showed activity against *Ml* only at the high dose of 750 μg/disk. Moreover, ethylgallate significantly inhibited the growth of *Ef* at 200 μg/disk and was faint against *Pa* only at the high dose of 1000 μg/disk. Whereas, desmethylbellidifolin was active against the

growth of *Ef* and *Ec* only at the high dose 750 µg/disk. It has been determined that kaempferol, quercetin, gallic acid and luteolin did not show any activity against the growth of *Ef, Ec* and *Pa* even at the highest dose 1500 µg/disk. However, all other compounds including flavonol, flavone and xanthone glycosides even at the highest dose 1500 µg/disk were found not active against the growth of all bacterial strains. This evidence confirmed that antibacterial activity of aglycones of phenolic compounds are much higher than the related glycosides [1,2]. In particular, ethylgallate, the simple phenol, exhibited more prominent activity than all other tested compounds. **References:** [1] Rigano, D. et al (2007) Phytoter. Res. 21:395 – 397. [2] Gatto, M.T. et al (2002) Bioorg. Med. Chem. 10:269 – 272.

J115 COX-2 inhibition by ethanol extracts obtained from roots of certain Ranunculaceae species

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Cyclooxygenase-2 (COX-2) is one of the key enzymes of arachidonic acid metabolism involved in the production of important mediators of inflammation. It has been believed that inhibition of COX-2 derived prostanoids such as prostaglandin E_2 (PGE_2) are responsible for anti-inflammatory, analgesic and antipyretic effects of therapeutic preparations [1]. The plant family of Ranunculaceae comprehends various species of worldwide traditionally used species for medicinal purposes, whose marked biological activities (including anti-inflammatory) have previously been published in a number of studies [2,3]. Thus we decided to evaluate the *in vitro* inhibitory activity against COX-2 of 20 samples of ethanol extracts obtained from roots of various species of the genera *Aconitum, Actaea*, *Anemone*, *Aquilegia*, *Cimicifuga*, *Eranthis*, *Ficaria*, *Helleborus, Ranunculus*, *Thalictrum* and *Trollius* using method previously described by Reininger and Bauer [4]. The ethanol samples were tested at final concentrations of 128, 64, 32 and 16 µg/ml in the assay mixture. Indomethacin was used as a standard reference material. Among all samples tested, the *H. purpurascens* root extract possessed the strongest inhibitory effect against PGE_2 formation at a concentration of 16 µg/ml. At 32 µg/ml, enhanced effect of extracts from *C. racemosa*, *F. bulbifera*, *Trollius. altissimus* and *T. europaeus* could be also determined. Other samples had relatively weak or no effect on PGE_2 production even at 128 µg/ml. Our results show a considerable inhibitory activity of extract of *H. purpurascens* against COX-2, suggesting these species might be a potential source of effective plant-derived anti-inflammatory substances. *Acknowledgements: This research was supported by Czech Science Foundation (Project No. 525/08/1179).* **References:** [1] Smith, W.L. et al. (2000) Annu. Rev. Biochem. 69:145 – 182. [2] Li, R.W. et al. (2003) J. Ethnopharmacol. 85:25 – 32. [3] Jodynis-Liebert, J. et al. (2005): J. Ethnopharmacol. 97:351 – 358. [4] Reininger, E.A., Bauer, R. (2006) Phytomedicine 13:164 – 169.

PJ116 The growth-inhibitory effect of thymohydroquinone and thymoquinone on oral pathogenic bacteria *in vitro*

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Nigella sativa L. is annual herb used in folk medicine all over the world for the treatment of a number of health disorders [1]. Its seed extracts have previously been observed to possessed broad spectrum of antimicrobial activity [2], including inhibitory effect on microorganisms isolated from oral cavity [3]. Although thymohydroquinone (THQ) and thymoquinone (TQ) have previously been identified as main antimicrobially effective quinone constituents of *N. sativa* seeds [4,5] their growth inhibitory effect against oral microorganisms has not been previously described. Thus we decided to evaluated THQ and TQ for their potential antibacterial activity against oral bacteria involved in the pathogenesis of dental caries and periodontal diseases using the broth microdilution method [6]. TQ was purchased from Sigma-Aldrich (Prague, CZ) and THQ was prepared by reduction of thymoquinone, as described by El-Dakhakhny [7]. The results showed that both quinone compounds tested in this study possessed significant effect against oral pathogenic microorganisms, whereas *Streptococcus mutans* and *S. sobrinus* were found to be the most sensitive species with minimum inhibitory concentrations ranging from 8 to 16 µg/ml. *Acknowledgements: Czech Science Foundation (project no. 525/08/1179).* **References:** [1] Ali, B.H., Blunden, G. (2003) Phytother. Res. 17:299 – 305. [2] Hanafy, M.S.M., Hatem, M.E. (1991) J. Ethnopharmacol. 34:275 – 278. [3] Chaudhry, N.M.A. et al. (2008) Pak. J. Bot., 40:461 – 467. [4] El-Alfy, T.S. et al. (1975) Pharmazie 30:109 – 111. [5] Kokoska, L. et al. (2008) J. Food Prot. 71:2475 – 2480. [6] Jorgensen, J.H. et al. (1999). In: Murray P.R. (ed.) Manual of Clinical Microbiology. ASM Press. Washington, DC. [7] El-Dakhakhny, M. (1963) Planta Med. 11:465 – 470.

PJ117 *In vitro* inhibition of cyclooxygenase-2 catalyzed prostaglandin E_2 production by plant-derived quinone compounds

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It was already shown that plant quinones are able to inhibit production of proinflammatory prostaglandin E_2 (PGE_2) catalyzed by cyclooxygenase-1 and -2 (COX-1, COX-2) [1]. Because there are still many compounds left to be explored, in this study we aimed to screening of natural quinones and their derivates as potential COX-2 inhibitors. We investigated 7-methyljuglone (1), diospyrin (2), lawsone (3), aloe-emodin (4), arbutin (5), lapachol (6), plumbagin (7), embelin (8), barbaloin (9), rhein (10), purpurin (11) and carminic acid (12) by enzymatic *in vitro* assay with enzyme immunoassay evaluation described previously by [2]. At highest concentration tested (128 µM) compounds 1, 2 and 7 were the most active while substances 8 and 11 showed relatively weak efficiency when compared with standard inhibitor indomethacin. Remaining compounds had no effect on PGE_2 production. At concentration 64 µM only quinones 1 and 2 prevent significantly PGE_2 formation whereas compounds 7, 8 and 11 showed only slight effect. Substances 1 and 2 were further tested at concentrations 32, 16, 8, 4, 1 and 0.25 µM and possessed efficiency comparable with indomethacin at all corresponding concentrations. *Acknowledgement: This study was supported by Czech Science Foundation project 525/09/P528.* **References:** [1] Marsik, P. et al. (2005) Planta Med. 71:739 – 742. [2] Reininger, E.A., Bauer, R. (2006) Phytomedicine 13:164 – 169.

PJ118 Anti-inflammatory and anti-oxidative activity of *Vaccinium bracteatum*

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Vaccinium bracteatum Thunb. is a shrub growing in undulating countries of China, Japan and Korea [1]. It is used in the traditional Chinese medicine for its anti-tumor properties [2]. We tested extracts from leaves of *V. bracteatum* obtained by successive maceration by *n*-hexane (HE), dichloromethane (DE), methanol (ME) and water (WE) for its anti-inflammatory and anti-oxidative potentials. Anti-inflammatory effect was as-

sessed *in vitro* as an ability to reduce production of prostaglandin E_2 by cycloxygenase-1 and -2 (COX-1, COX-2) detected by PGE_2 EIA Kit [3]. Anti-oxidative activity was evaluated *in vitro* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. We observed that HE was the most potent inhibitor of COX-1 (100% inhibition of blank) and WE of COX-2 (98% inhibition of blank) with activities comparable to the standard inhibitor indomethacin tested in a concentration 300 µg/ml (100% inhibition of blank). Only ME and WE exhibited significant anti-oxidative activity with EC_{50} values 32.7 and 123.2 µg/ml, respectively. The reference compounds trolox and ascorbic acid had an EC_{50} of 2.9 and 0.5 µg/ml, respectively. These preliminary results show good potential of *V. bracteatum* as a source of anti-inflammatory compounds. *Acknowledgement: This study was supported by project ME08070.* **References:** [1] Tu, P. et al. (1997) Zhong Yao. Cai. 22:423 – 448. [2] Duke, J.A., Ayensu, E.S. (1985) Medicinal Plants of China, Reference Publ., Inc., Algonac, US. [3] Knodler, M. et al. (2008) Phytochemistry 69:988 – 993.

PJ119 Portuguese propolis: The effect of collection time and localization on anti-Helicobacter activity

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Propolis or bee glue is a resinous substance collected by bees mixing their own waxes with resins from plant sources. It has been used as a folk medicine from ancient times. Nowadays, it was found to have a wide range of biological activities, namely antibacterial, anti-inflammatory, antioxidative, hepatoprotective effects and anti-tumoral activities. In this work, aqueous, ethanolic and methanolic extracts of propolis harvested at two different times from several locations of the Algarve region were tested for their antibacterial activity against *Helicobacter pylori*. The propolis samples collected at springtime showed significant higher anti-helicobacter activity, in comparison with samples harvested at winter time. The majority of the extract samples showed a dose dependent activity. Statistical differences were obtained between samples collected from different locations. These differences may be linked to a different chemical composition of propolis which in turn can be due to the plant source from which the product is done. *Acknowledgment: This study was partially funded by* Cruz Alta Agricultura, *Lda. Loulé*

PJ120 Antibacterial activity comparison of several Portuguese *Thymus* essential oils

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Antimicrobial activity of Thyme (*Thymus* spp) has been related to its chemical composition. Chemical polymorphism is frequent in Portuguese *Thymus* species [1]. The aim of this work was to compare the antibacterial activity of the essential oil from several Portuguese *Thymus* species collected in the mainland, Madeira and Azores archipelagos (Portugal). The antibacterial activity was determined by agar diffusion against *Helicobacter pylori, Listeria monocytogenes, Staphylococcus aureus* and *Salmonella enteritidis*. The tested bacteria demonstrated different susceptibilities to the *Thymus* essential oils. Essential oils from the same species, collected in different geographical regions, showed significant differences in their antibacterial activity. The best results against *H. pylori* and *L. monocytogenes* were observed with *T. caespititus* essential oil, obtained from plants grown in Pico and Planalto Central (Azores, Portugal) and *T. zygis* from Bragança (mainland, Portugal). *S. aureus* was not susceptible to any of the tested *Thymus* essential oils. *T. zygis* essential oil from plants grown in Bragança (mainland, Portugal) showed the highest activity against *S. enteritidis*. The existence of different chemotypes within each species can partly explain the different antibacterial activities observed. *Acknowledgment: Partially funded by the FCT under research contract PTDC/AGR-AAM/70136/2006.* **References:** Figueiredo, A.C. et al. (2008) Curr. Pharm. Des. 14:3120 – 3140.

PJ121 Micromorphology, anatomy and volatile constituents of leaf in some Lamiaceae species

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The objective of this study is to determine the range of variation in certain micromorphological, anatomical and biochemical characters of leaf within three spontaneous Lamiaceae species: *Hyssopus officinalis* L. (from Montenegro Republic), *Thymus comosus* Heuff. and *Ocimum basilicum* L. (both from Romania) The research material was collected in anthesis stage of plant development. Anatomical characters of leaf epidermis were examined by light microscopy. Scanning electron microscopy was used to examine leaf surface and trichomes. The qualitative analysis of the volatile oils has been carried out using GC-MS. All examined species had bifacial heterofacial and amphistomatous leaf. The stomata are diacytic. The glandular hairs consist of one epidermic basal cell, a uni- or multicellular stalk and a uni- or multicellular secretory head. The non-glandular trichomes are simple, short or long, multicellular uniseriate. The variable micromorphological and anatomical characters of leaf are rather quantitative than qualitative, as follows: 1. the mesophyll thickness; 2. the density of stomata and trichomes; 3. the number and size of intercellular air spaces of spongy mesophyll. The volatile oils produced by the investigated species differ in their composition. The aromatic value and therapeutical efficiency of these products strictly depend on the moment of plant development, metabolic transformations and climatic conditions. Among the analyzed species, the main constituents of volatile oils are: thymol, carvacrol, β-cariophylene, germacrene D, δ-cadinol, linalool and methylchavicol. **References:** [1] Burzo, I., Mihăiescu, D. E. (2004) Contribution to the data concerning physiological and biochemical processes of *Ocimum basilicum* L. An. Şt. Univ. "Al. I. Cuza" Iaşi. XXXVII. [2] Fahn, A. (1988) Secretory tissues in vascular plants. New. Phytol. 108.

PJ122 Chemical composition and antimicrobial activity of the essential oil of the underground parts of *Gentiana asclepiadea*

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The aim of this work was to examine the chemical composition and the *in vitro* antimicrobial activity of the essential oil of *Gentiana asclepiadea*. *G. asclepiadea* is a member of the large genus *Gentiana* in the family Gentianaceae [1]. *Gentiana* species are distributed in Europe, Asia, North America and South America [2]. The underground parts of several *Gentiana* species are widely used throughout the world as potent stomachic and hepatoprotective agents, because they contain bitter principles [3]. The essential oil from the underground parts (roots and rhizomes) of *Gentiana asclepiadea* (*Gentianaceae*) from Southwest Serbia (locality Stranjani, the mountain Jadovnik) was investigated by GC-MC analyses. One hundred and thirty four components were characterized in the oil. Oxygenated monoterpenes (16.18%) and oxygenated sesquiterpenes (30.15%) were the dominating chemical classes in the sample analyzed. The main constituents were the oxygenated sesquiterpenes caryophyllene oxide (7.56%) and τ-cadinol (5.56%). The inhibitory effect of the essential oil was tested against one fungus and six bacterial species by the macrodilution method. Minimum inhibitory concentration (MIC) was tested in the concentration range of 5.00 – 0.078 µL/mL. The oil showed activity with MIC values ranging from 2.5 – 5.0 µL/mL. The most sensitive microorganisms were *Bacillus subtilis, Klebsiella pneumoniae* and *Staphylococcus aureus* (FSB 30) with MIC values of 2.5 L/mL. *Micrococcus lysodeikticus* and *Staphylococcus aureus* (ATCC 25923) showed a higher resistance than other microorganisms in test. The commercial antibiotics, amracin (for bacteria) and nystatin (for fungus), showed stronger antimicrobial activity than the essential oil. **References:** [1] Jiang, R.W. et al. (2005) Phytochem. J. 66:2674 – 2680. [2] Georgieva, E. et al. (2005) Biochem. Syst. and Ecol. J. 33:938 – 974. [3] Szücs, Z. et al. (2002) Chromat. Suppl. J. 56:S19-S23.

PJ123 Impaired sperm parameters of Balb/c mice fed plumbagin

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Plumbagin (2-methyl-5-hydroxy, 1:4naphthoquinone), the active ingredient isolated from the root of *Plumbago zeylanica*, have significant antioxidant abilities. The roots of *Plumabago zeylanica* were boiled as concoction and taken orally by women in the rural areas of Malaysia for contraception, however there were no reports of men consuming the same concoction to reduce fertility. As *Plumbago zeylanica* have high antioxidant activities and NO acts as an antioxidant in lipid peroxidation, this study was therefore undertaken to examine the effects of feeding plumbagin on various sperm parameters (sperm count, sperm motility and nitric oxide NO levels in blood plasma) of mature male Balb/C mice as well as the relationship between NO levels with sperm count and motility. Twenty-one sexually mature male mice (Balb/C) were randomly divided into three groups (initial control, positive control and low dose groups). The treatment group (low dose) was forced-fed with 0.34 mg/ml of plumbagin for four weeks, whereas the initial control group with water and control group 1 ml olive oil. Caudal epididymides and cardiac blood from initial control group was collected one week before starting treatment while for control and low dose groups, is after four weeks. Sperm analyses such as sperm motility, sperm count and NO level in plasma were carried out. Pre-treatment and post-treatment results were compared. The results obtained showed oral consumption of plumbagin exerted a significant ($p < 0.05$) effect in decreasing sperm motility and sperm count in low dose group (0.34 mg/ml). This experiment also showed a significant ($p < 0.05$) increase in NO levels with plumbagin consumption. The levels of NO were significantly correlated ($r = 0.586$) to plumbagin consumption. There was also a significant ($p < 0.05$) negative correlation ($r = -0.632$) between NO levels with sperm count and sperm motility. Thus, data suggests that oral consumption of plumbagin increases NO levels, which in turn decrease sperm count and motility. **References:** [1] Tilak, J.C. et al. (2004) Redox Report. 9(4):219 – 227. [2] Hiramoto, K. et al. (2003) Chem. Pharm. Bull. 51(9):1046 – 1050.

PJ124 The study of some *Ajuga* and *Salvia* species from Romania

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Lamiaceae family, well represented in the Mediterranean area, contains some valuable medicinal species in Romanian flora. Our previous research target it well known species used in traditional medicine, such as *Betonica officinalis, Glechoma hederacea, Ajuga genevensis* and *Teucrium chamaedrys* [1]. Following that study we analyzed four *Salvia* (*S. austriaca, S. nemorosa, S. pratensis, S. aethiopis*) and *Ajuga* species (*A. reptans, A. genevensis, A. laxmanii, A. chamaepytis*). We have researched the presence of rosmarinic, ursolic and oleanolic acids, as well as the presence of phytoecdysones in the above mentioned species by TLC and HPLC. For TLC we used SiO_2 and toluen-ethyl formate-formic acid (50:40:10), ethyl acetate-benzene (25:10) with detection NP/PEG 400 and UV 365 nm, respectively H_2SO_4 50%. HPLC for rosmarinic acid: Thermo Finigan Surveyor Plus, detection UV 260 nm, Hypersil BDS RP-18 column (250 x 4.6 mm) (5 µm). An elution gradient of 0.1% acetic acid and methanol was used as mobile phase. Flow rate: 1 ml/min, injection volume 20 µl, column temperature 48ô¤C [2,3]. HPLC conditions for ursolic and oleanolic acids: Agilent1100 apparatus, detection UV 210 nm, Phenomenex RP 18 column (250 x 4 mm) (5 µm). Mobile phase: mixture of methanol and pH = 2.8 phosphate buffer (85:15). Flow rate: 1 ml/min, injection volume 10 µl. We notice significant differences between *Salvia* and *Ajuga* species; the latter are obviously poorer in ursolic and oleanolic acids content. Although in literature is cited the presence of rosmarinic acid in *Ajuga* species, we didn't find it in the tested *Ajuga* species. **References:** [1] Ionescu, A. et al. (1996) Conferinta Nationala de Fitoterapie, Iasi. [2] Wagner, H., Bladt, S. (1995) Plant Drug Analysis. [3] Tamas, M. et al. (2006) 4th Conference on Medicine and Aromatic plants of South East European Countries, Iasi.

PJ125 Antioxidant and preservative properties of *Millettia leucantha* Kurz var. *latifolia* extract

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The study was conducted to evaluate the antioxidant activity and antimicrobial property as natural food preservative of Sathon leaf (*Millettia leucantha* Kurz var. *latifolia*) water extract. The antioxidant and antimicrobial activities were evaluated using free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and agar well diffusion methods. The extract concentration provided 50% inhibition (IC_{50}) at 9.22 mg/ml while vitamin C and Trolox at 9.34 µg/ml and 11.69 µg/ml, respectively. The minimum inhibitory concentrations of Sathon leaf extract against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311 and *Candida albicans* ATCC 10231 were 250, 125, 250, and more than 2,000 mg/ml, respectively. The MICs of chloramphenicol as positive control were 7.81, 15.63 and 15.63 µg/ml for *S. typhimurium, S. aureus* and *E. coli*, respectively. The Sathon leaf extract was added to the chilli paste, Thai favorite food, in order to determine the preservative property for 6 months. Total bacterial count in Thai chilli paste preserved with 13.7% w/w Sathon leaf extract was reduced at 81.35%± 11.78 (mean ± SD). **Reference:** [1] Kê Lôc, P., Vidal, J.E. (2001) Flore du Cambodge du Laos et du Vietnam. 2. Ferraro, M.J. et al. (2000) Methods of dilution antimicrobial susceptibility test for bacteria that grow aerobically; Approved standard-fifth edition; NCCLS document M7-A5. Pennsylvania, USA. 3. Chen, C.W., Ho, C.T. (1995) Journal of Food Lipids 2:35 – 46.

PJ126 Anticariogenic effects of extracts and fractions of *Potentilla recta* and their antioxidant activity

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The genus *Potentilla* (Rosaceae) has been known since ancient times for its curative properties. Extracts of the aerial and/or underground parts have been applied in traditional medicine for the treatment of inflammations, wounds, certain forms of cancer, infections, diarrhoea, diabetes mellitus and other ailments. Most of the pharmacological effects can be explained by the high amount of tannins and to a lesser extent by flavonoids as well as triterpenes, present in all plants parts. However, future efforts should concentrate more on *in vitro* and *in vivo* studies and also on clinical trials in order to confirm traditional wisdom in the light of a rational phytotherapy [1,2]. The purpose of this study was to investigate *in vitro* the antibacterial activity (by agar well diffusion method and by determination of the MICs) of different *Potentilla recta* extracts and fractions of different polarity (aqueous, 50% ethanolic, diethyl ether, ethyl acetate and *n*-butanolic) against cariogenic *Streptococcus* spp. strains (*S. mutans* CAPM 6067, *S. sobrinus* CAPM 6070, *S. sobrinus* GCM 20381, *S. sobrinus*/*downei* CCUG 21020 and *S. sanguis* ATCC 10556) and their inhibitory effects on insoluble glucan (mutan) and artificial dental plaque formation [3]. In addition, the antioxidative capacity in all extracts and fractions against stable radical DPPH was also examined. *Acknowledgements: This study is financially supported by Medical University of Białystok (grant No. 4 – 12820 F).* **References:** [1] Tomczyk, M., Latté, KP. (2009) J. Ethnopharmacol. 122:184 – 204. [2] Tomczyk, M. (2006) Biochem. Syst. Ecol. 34:770 – 773. [3] Wiater, A. et al. (2008) Herba Polon. 54:41 – 45.

PJ127 Antibacterial properties of certain essential oils against different strains of *Staphylococcus aureus*

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Antibiotics introduce very narrow group of substances. On the other hand natural active substances are chemically very diverse [1]. There have been more and more bacteria which are resistant against same type of antibiotics, for example the large problem introduce Methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals [2]. The aim of this study was to identify antimicrobial properties of the 7 essential oils (EOs) in vapour phase against 2 collection strains of MSSA, 1 collection strains of MRSA and 6 clinical isolates of *S. aureus,* obtained from two hospitals. All these strains have been tested against 10 species of antibiotics in standard doses. Tested EOs were obtained by hydro-distillation and tests of their antimicrobial properties were carried out by the modified diffusion method for testing of EOs in vapour phase [3] in concentrations 0.0083 – 0.53 µl/cm^3 of air. The ampicillin and erythromycin were tested as reference antibiotics by standard diffusion method in direct contact. The best MICs were shown by *Armoracia rusticana* (0.0083 – 0.017 µl/cm^3), followed by *Majorana syriaca* (0.0083 – 0.13 µl/cm^3) > *Allium sativum* (0.0083 – 0.26 µl/cm^3) > *Satureja hortensis* (0.017 – 0.13 µl/cm^3) > *Satureja montana* (0.033 – 0.26 µl/cm^3) > *Thymus vulgaris* (0.033 – 0.26 µl/cm^3) > *Thymus serpyllum* (0.033 – 0.53 µl/cm^3). Based on the results, EOs mentioned above could be considered as effective anti-Staphylococcal natural products. *Acknowledgements: Ministry of Educacion of the Czech republic MSM 6046070901.* **References:** [1] Kloucek, P. et al. (2005) Ziva 65:246 – 248. [2] Hashimoto, M. et. al. (2008) Transpl. Infect, Dis. 10:110 – 116. [3] Lopez, P. et al (2005) J. Agr. Food Chem. 53:6939 – 6946.

PJ128 Quorum quenching activity of the secondary metabolites from *in vitro* cultures of *Dionaea muscipula*

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Extracts from carnivorous plant *Dionaea muscipula* J. Ellis (Droseraceae) were shown to possess antimicrobial properties [1]. Inhibition of bacterial quorum sensing (QS) mechanism is a promising strategy of fighting infections caused by gram-negative pathogens applying minimal selective pressure [2]. The objective of this research was to evaluate quorum quenching (QQ) activity of the secondary metabolites obtained from *D. muscipula* plants grown *in vitro*. Preliminary tests were performed with the use of two indicator strains: *Agrobacterium tumefaciens* NT1 and *Chromobacterium violaceum* CVO17 which respond to QS signal molecules: acyl-homoserine lactones (AHLs), by expression of gene coding β-galactosidase or biosynthesis of violacein, respectively. Water, methanol and chloroform extracts of *D. muscipula* as well as compounds present in this plant: quercetin, myricetin, plumbagin, ellagic acid and rutoside were tested. The QQ activity was observed only in case of chloroform extract and plumbagin. Next a set of *Escherichia coli* indicator strains having different intracellular AHL receptors was employed -pSB1075 and pSB536 with LasR and CviR receptors, respectively as well as pSB401 and JB534 both with LuxR receptor. These strains respond to AHLs by the expression of *luxBCDAE* or *gfp* genes which is measured in the form of chemiluminescence or fluorescence. Chloroform extract from *D. muscipula* inhibited response to AHLs with IC_{50} from 16 to 29 µg/ml, depending on the indicator strain used. The IC_{50} values for plumbagin and chloroform extract were compared with those of known QS inhibitors: patulin (PAT), penicillic acid (PA), 4-nitropyridine-*N*-oxide (4-NPO) and halogenated furanone C30 [3]. C30 and PAT showed IC_{50} values from 3 to 9 µM and from 20 to 56 µM respectively. QQ activity of plumbagin (IC_{50} 89 – 122 µM corresponding to 17 – 23 µg/ml) was lower than C30 and PAT but significantly higher than 4-NPO (IC_{50} 336,2 – 1051,2 µM) and PA (IC_{50} 119 – 380 µM) except for pSB401 strain (IC_{50} for PA – 43 µM). These results indicate the presence of other than plumbagin active metabolite or a synergistic effect between compounds in the chloroform extract. *Acknowledgements: This research was supported by the European Union within the European Social Fund in the framework of the project "InnoDoktorant – Scholarships for PhD students, I edition" and Gdansk University Grant BW/BO51 – 5-0024 – 9.* **References:** [1] Krolicka, A. et al. (2008) Enzyme Microb. Technol. 42:216 – 221. [2] Rasmussen, T.B., Givskov, M. (2006) Int. J. Medical Microbiol. 296:149 – 161. [3] Bjarnsholt, T., Givskov, M. (2007) Phil. Trans. R. Soc. B 362:1213 – 1222.

PJ129 Determination of total anthocyanins and anthocyanine glycosides in the fruit of lingonberry, *Vaccinium vitis-idaea* L. (Ericaceae)

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The aim of this study was to determine total anthocyanins and anthocyanine glycosides in fruits of lingonberry, *Vaccinium vitis-idaea* L. (Ericaceae). Total anthocyanins were estimated with spectrophotometric pH differential method. Cyanidin -3- galactoside served as a standard. Anthocyanins were measured by High Pressure Liquid Chromatography with dioda array detection (HPLC-DAD). HPLC-DAD was performed with a Zorbax StableBond-C18 column (250 x 4.6 mm, 5 µm). Mobile phase was A: acetonitril 100% and B: 10% (v/v) acetic acid and 1% phosphoric acid in water. Supernatants of fresh fruits were hydrolysed with 2 M HCl. As standards for HPLC-DAD were used pelargonidin chloride ($C_{15}H_{11}O_5Cl$), malvidin chloride ($C_{17}H_{15}ClO_7$), delfinidin chloride ($C_{15}H_{11}O_7Cl$), cyanidin-3-galactoside chloride ($C_{21}H_{21}ClO_{11}$), naringenin-7-glycoside ($C_{21}H_{22}O_{10}$), peonidin chloride ($C_{16}H_{13}O_6Cl$), peonidine-3-o-glycoside chloride ($C_{22}H_{23}ClO_{11}$), petunidin chloride ($C_{16}H_{13}ClO_7$) and malvidin-3-o-galactoside chloride ($C_{23}H_{25}ClO_{12}$). Detection was at 517/525 nm and 220/254 nm. Total content of anthocyanine glycosides was 0.80 mg/g of fresh fruit of lingonberry. Using HPLC-DAD only peonidine as aglucone in quantity of 0.025 µg/g in lingonberry was found.

PJ130 Determination of arbutin, rutin, total content of phenols and antioxidant capacity in fruits and leaves of lingonberry, *Vaccinium vitis-idaea* L. (Ericaceae)

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The aim of this study was to determine arbutin, rutin, total phenols content (TCP) and total antioxidant capacity (AC) in the fruits and leaves of lingonberry, *Vaccinium vitis-idaea* L. (Ericaceae). Dry fruits and leaves of lingonberry were collected in Bosnia. For determination of AC was used Oxygen Radical Absorbance Capacity (ORAC) assay with trolox, a water soluble analogue of vitamin E, as a standard. The TCP was estimated by photometric method with Folin-Ciocalteau reagent at 765 nm, with gallic acid as a standard. Arbutin and rutin determined using High Pressure Liquid Chromatography with electrochemical detection (HPLC-ED). As mobile phase it was used a mixture of water, methanol, acetonitrile, formic acid and isopropyl alcohol in proper proportion: 75.5: 12:6:5:1.5. Performance was done at 25 ° C with flow rate of 0.5 ml/min and potential of 0.750 V. The TCP, expressed in milligramms of phenols per gramme of dry weight (mg/$g_{d.w.}$) was 12.7 in fruits, and 164.4 in leaves. The content of arbutin was 0.51 mg/$g_{d.w.}$ in leaves, and 0.04 mg/$g_{d.w.}$ in fruits. The content of rutin was 2.54 mg/$g_{d.w.}$ in leaves, and 0.06 mg/$g_{d.w.}$ in fruits. The values of AC, expressed as mmol trolox equivalents per gramme of dry weight ($mM_{TE}/g_{d.w.}$), were 46.45 $mM_{TE}/g_{d.w.}$ in fruits, and 235.23 $mM_{TE}/g_{d.w.}$ in leaves. The analysis has shown the higher content of phenols, arbutin and rutin in the leaves of lingonberry in comparison with fruits and higher AC in leaves. The higher AC in leaves correspond to the higher values of phenolic compounds.

PJ131 Gastric healing effect of methanolic and alkaloidic fraction from *Strychnos pseudoquina*

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Strychnos pseudoquina St. Hill (Loganiaceae) is used in folk medicine to treat malaria, gastric ulcer and gastritis. In the present studies a methanolic extract (ME) and alkaloidic fraction (AF) from *S. pseudoquina* leaves were investigated for their ability to heal gastric ulcer by the method of TAKAGI *et al*., (1969) [1]. Male Wistar rats (n = 6) were treated orally with saline (vehicle), cimetidine (100 mg/Kg), ME (250 mg/kg) or AF (250 mg/kg) for 14 consecutive days after gastric lesion induction by acetic acid. Vital organs and body weight were also analyzed to evaluate the subacute toxicity. Samples of gastric tissue were collected for histological and immunohistochemical analysis. We also evaluated the *in vitro* anti-*Helicobacter pylori* action of AF from *S. pseudoquina.* Macroscopic analysis after 14-day ME treatment showed no significant healing action in relation to vehicle-treated rats. However, the gastric lesion of animals treated with EAF, at the same dose, induced significant reduction ($p < 0.05$) in internal (42%) and external (38%) lesion area (mm^2). The regenerative areas of AF ($1611.7 \pm 28.14\,\mu m$) and ME ($1516.7 \pm 36.45\,\mu m$) were significantly increased ($p < 0.01$) when compared to animals treated with vehicle ($1462.0 \pm 25.2\,\mu m$) or cimetidine ($1489.5 \pm 17.56\,\mu m$). Immunochemical staining for PCNA showed that AF treatment stimulates cellular proliferation and increases angiogenesis in the region of gastric mucosa regeneration. We also observed anti-*Helicobacter pylori* with MIC of 75 µg/ml. We concluded that EAF from *S. pseudoquina* presents expressive gastric healing action by increasing cell proliferation in gastric mucosa, while augmenting angiogenesis and antibacterial action against H. pylori with absence of toxicity for 14 consecutive days. *Acknowledgements:* Biota/FAPESP and CNPq **References:** [1] Takagi, K., Okabe, S. (1969) Jpn J. Pharmacol. 19:418 – 426.

PJ132 Antimicrobial activity of some isolated triterpene substances from birch bark, *Betula pendula* Roth.

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Antimicrobial activity of several triterpene substances, isolated from birch bark, Betula pendula, Roth., was performed in this study. Using method of dry column chromatography as well as preparative thin layer chromatography, triterpene compounds, betulin, betulinic acid, oleanolic acid and lupeol, were isolated from external part of birch bark. Pharmacopoeian methods of diffusion and dilution were used for investigation of antimicrobial activity of isolated triterpene derivatives. Antimicrobial screening was carried out against selected Gram-positive bacteria, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P and Gram-negative bacteria, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* ATCC 14028. Antifungal activity against *Candida albicans* ATCC 10231 was investigated to. Triterpene substances were dissolved in dimethylsulfoxid which did not show antimicrobial activity by itself in range of concentrations used for investigation. Using both, diffusion and dilution method the most prominent antimicrobial activity expressed oleanolic acid against bacterial species *Staphylococcus aureus* (MIC: 1.25%) and *Bacillus subtilis* (MIC: 0.625%).

Inhibition of Bacillus subtilis growth by different concentration of olenolic acid (o.k.)

Inhibition of Staphylococcus aureus growth by different concentration of olenolic acid (o.k.)

Escherichia coli and *Salmonella typhimurium,* showed their resistance on all investigated samples. Oleanolic acid showed a small antifungal activity against *Candida albicans* by diffusion method (inhibition area 10 mm). **References:** Kovac-Besovic, E. et al. (2003) Bosnian Journal of Basic Sciences III (4):16 – 22.

PJ133 Identification and isolation of betulin, betulinic acid and lupeol from birch bark

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Betulae cortex, Betula pendula Roth., Betulaceae, comprise triterpene substances which are confirmed to possess very important pharmacological activities such as anti-inflammatory, anticancer and antiviral. In this study, acetone-methanol extraction of triterpene substances from both, inner and external birch bark was carried out. Insolubility in water of triterpenes betulin, betulinic acid, lupeol and oleanolic acid, is used for precipitation of triterpene substances from methanolic extracts. Qualitative analysis of triterpenes in precipitated raw extracts was performed by method of thin layer chromatography, applying system for development, benzene – ethyl acetate – formic acid (36:12:5). Separated spots of betulin (Rf 0.62), betulinic acid (Rf 0.59) and lupeol (Rf 0.75) were colored in shadows of violet color by reagents anisaldehyde sulphuric acid and did not show fluorescency (UV lamp 254 and 366 nm). Dry column chromatography and preparative thin layer chromatography were used to isolate triterpene substances from row triterpene mixture. The study include all obtained IC specters and interpretations on the basis of which can be concluded that triterpene substances, betulin, betulin acid and lupeol isolated from external birch bark give identical characteristic signals and absorbance as those referent. *Betulinic acid – IR (KBr) (ν, cm^{-1}):* 3484 (OH); 2870 (CH_2); 1686 (C = O); 1432 (OH); 1362 (CH_2= CH-CH_3); 1156 (C-O), *Betulin – IR (KBr) (ν, cm^{-1}):* 3448 (OH); 2868 (CH_2 i CH_3); 1638 (C = C); 1432 (OH); 1388 (CH_2= CH-CH_3), *Lupeol – IR (KBr) (ν, cm^{-1}):* 3308 (OH); 2872 (CH_2); 1630 (C = C); 1468 (CH); 1380 (CH_3) "Umbrella"; 920 (CH alkenes). Method of dry column chromatography has resulted as simple, efficient, repeatable and economical for laboratory conditions. **References:** [1] Krasutsky, P.A. (2006) Nat. Prod. Rep. 23:919 – 942. [2] Dzubak, P. et al. (2006) Nat. Prod. Rep. 23:394 – 411.

PJ134 Investigation of irritation properties of some extracts and isolated triterpenes from birch, *Betula pendula*, Roth.

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Interest for medicinal plants has increased all over the world, starting from usage of herbal products in cosmetics going beyond their application in auto medication by great number of patients. This effect emphasizes toxicology and clinical pharmacology of herbal preparations, so as to provide high quality information about pharmacological and toxicological properties of herbal drugs which are in every day usage. Very important triterpene derivatives were identified and isolated from different parts of plant species birch. *Betulae folium*, birch leaf, *Betula pendula* Roth., Betulaceae, is official birch drug. Other birch parts are also in large use in pharmaceutical industry as well as in cosmetic and perfume industry. Pharmacological investigations were carried out using methods of irritation and sensibilization on eye, ear and skin of experimental animals, mouse, rats and rabbits. To those effects were tested samples of methanolic extracts and decocts of leaf and external birch bark as well as betulin and betulinic acid isolated from eternal birch bark. Grade of irritation or corrosion was evaluated in determinate time intervals, scores were determinate as well and effects were described completely in order to obtain overall analyses of effects of investigated samples. According to the results obtained on rabbit eye after a on a one-time basis application of samples, with a great probability it is possible to deduce that investigated samples should not cause irritation on a human skin, respectively irritations should be present in a small number of

users of preparations with different birch extracts. During investigation of sensibilization of all tested samples, no presence of erythema or edema or any other sign has been noted which should point to intolerance of experimental animals. **References:** [1] Tolstikova, T.G. et al. (2006) Bioorg. Khim. 32:42 – 55. [2] Magalhaes, A.F. et al. (2003) Mem. Inst. Oswaldo Cruz 98:713 – 718. [3] Flekhter, O.B. et al. (2002) Pharm. Chem. J. USSR 36:29 – 32.

PJ135 Antioxidant activity of three polyphenol-enriched cocoa products obtained on an industrial scale

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Using different protocols we have obtained three cocoa extracts with a high content in polyphenols: A (167 mg/g), B (374 mg/g) and C (787 mg/g). The scavenging capacity of the extracts was measured as the ability to bleach the stable radicals $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, and the antioxidant effect of them by the FRAP assay [1]. The results in the DPPH test were 0.2, 1.4 and 3.0 (expressed as Trolox equivalent, μmol/mg dry weight of each extract), and in the ABTS test were 1.0, 4.7 and 9.8, for A, B and C, respectively. The antioxidant capacity expressed as ascorbic acid equivalent were 17.2, 76.1 and 207.7, respectively. The scavenging properties of cocoa powder against the superoxide anion, H_2O_2, HClO [1], and peroxynitrite [2] were determined and the IC_{50} (μg/mL) values were 77.5, 12.3 and 10.3, for A, B and C, respectively, in the hypoxanthine/xanthine oxidase test, and 225.4, 73.2 and 21.5 as HOCl scavenger, respectively. Only extract C gave relevant effect as scavenger of peroxynitrite anion, with IC_{50} (μg/mL) of 76.1 and 110.0 in absence or presence of bicarbonate. None of the tested extract was active in the hydrogen peroxide test, but B and C significantly increased the deoxyribose degradation in the absence of ascorbate. None of the extracts inhibited the ferrous or copper chelating activity at 100 μg/mL, but they inhibited the lipid peroxidation in brain homogenates and human plasma using non-enzymatic generation systems [3], giving the extract C the best IC_{50} (μg/mL) values, 17.4 and 8.1 against to lipid peroxidation in brain homogenates and human plasma, respectively. We conclude that cocoa may constitute a source of polyphenols which could serve for enriching foods, nutraceuticals and alimentary supplements. **References:** [1] Schinella, G. et al. (2004) Nuevas Fuentes de Antioxidantes Naturales. (A Rosas ed.) CYTED-MCT, Caracas. [2] Koppenol, W.H. et al. (1996) Methods Enzymol. 269:296 – 302. [3] Liu, F. et al. (2000) Life Sci. 66:709 – 723. [4] Liu, F., Ng, T.B. (2000) Life Sci. 66:725 – 735.

PJ136 Shikonin (5,8-dihydroxy-2-[(1R)-1-hydroxy-4-methyl-3-pentenyl]-1,4-naphthoquinone) inhibits 12-*O*-tetradecanoyl-phorbol-13-acetate-induced mouse ear acute inflammation through blocking of mitogen-activated protein kinases

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In our search for anti-inflammatory agents from natural products we found that shikonin, a naphthoquinone major component of the root of *Lithospermum erythrorhyzon* Sieb. et Zucc., topically applied *in vivo* reduced the acute inflammation in mouse ear oedema induced by TPA (ED_{50}= 1 mg/ear) [1]. Mitogen-activated protein kinases (MAPKs), such as ERK, p38 and JNK and protein kinase C (PKC) are signal transducers which activate the transcription of NF-kB in mouse skin after topical application of TPA (12-*O*-tetradecanoyl-phorbol-13-acetate) [2,3]. The aim of this work is to study the *in vivo* topical anti-inflammatory activity of shikonin in TPA-induced skin inflammation through the inhibition of protein kinases activation. Experiments conducted using female Swiss mice (25 – 30 g) were approved by the Institutional Ethics Committee of the University of Valencia. Skin inflammation was induced by topical application of 2.5 μg/ear of TPA in 20 μl of acetone. Shikonin (1.0 and 2.0 mg/ear) was dissolved in 20 μl of acetone, and applied topically in conjunction with TPA. Control received acetone only. Animals were sacrificed by cervical dislocation 1 h after TPA treatment and ear punches of 7 mm in diameter were taken from each mouse. Protein extraction from skin was performed as previously described [4] and the targeted proteins (ERK, p38, JNK, and PKC) were analyzed by Western blot assay. Our results show that the topical application of shikonin in mouse ear resulted in a dose-dependent inhibition of TPA-induced protein kinase activation. At the dose of 1 and 2 mg, the expression of ERK 1/2 was inhibited, without affecting that of p38 nor JNK. PKC translation was reduced by 80% at the same dose. Based on these results and on our previous investigations [5], we might infer that shikonin exerts its anti-inflammatory properties through regulation of PKC and MAPKs activation probably by inhibiting the activity of NFκB. *Acknowledgements: This study was supported by grants from the Spanish government (grant no. SAF2006 – 06726) and from the Generalitat Valenciana (grant no. GVPRE/2008/387).* **References:** [1] Recio, M.C. et al. (2007) Meth. Find. Exp. Clin. Pharmacol 28(1):118. [2] Karin, M. (2005) Proc. Am. Thorac. Soc. 2:386 – 390. [3] Garg, R. et al. (2008) Carcinogenesis 29(6):1249 – 1257. [4] Lai, C.S. et al. (2007) Carcinogenesis 28(12):2581 – 2588. [5] Andújar, I. (2008) Meth. Find. Exp. Clin. Pharmacol 29(supl.):86.

PJ137 *Teucrium cubense* induces glucose-uptake in insulin-sensitive and insulin-resistant murine and human adipocytes

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We investigated the anti-diabetic mechanisms of *Teucrium cubense* Jacq (Lamiaceae) assaying non-toxic concentrations of an aqueous extract of this plant (TE) on the 2-NBDglucose uptake [1] and adipogenesis [2] in 3T3-F442A murine and normal human subcutaneous adipocytes. In insulin-sensitive 3T3 adipocytes, TE stimulated the 2-NBDG uptake by 112% whereas in human adipocytes induced the 2-NBDG uptake by 54% respect to the 2-NBDG uptake stimulated by insulin. TE also induced 2-NBDG uptake in insulin-resistant murine and human adipocytes by 69% and 31% respectively. TE (70 μg/mL) added to murine adipogenic medium increased 3T3 adipogenesis by 167% whereas added to human adipogenic medium induced human adipogenesis by 138%. Under non adipogenic conditions, TE only marginally increased adipogenesis by 13% in 3T3 preadipocytes and by 9% in human preadipose cells, suggesting that this preparation lacks of pro-adipogenic effects. Our results suggest that *Teucrium cubense* exerts its anti-diabetic effects stimulating glucose uptake in both insulin-sensitive and insulin-resistant murine and human adipocytes without affecting triglyceride accumulation. *Acknowledgements:* RZB and AJAC were endowed with graduate fellowships from CONACYT (211445 and 210841, respectively). AJAC also received special support from IPICYT (SA-157/2008). **References:** [1] Alonso-Castro, A.J., Salazar-Olivo, L.A. (2008). J. Ethnopharmacol. 118:252 – 256. [2] Ramírez-Zacarías, J.L. et al. (1992) Histochem. 97:493 – 497.

PJ138 *Opuntia leucotricha* possesses insulin-like activities inducing glucose uptake in murine and human normal and diabetic-like adipocytes

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We investigated the anti-diabetic mechanisms of *Opuntia leucotricha* assaying the effects of non-toxic concentrations of aqueous extracts of fruits (OFE) and seeds (OSE) of this plant on the 2-NBDglucose uptake [1] and adipogenesis [2] in 3T3-F442A murine and human normal subcutaneous adipocytes. In insulin-sensitive 3T3 adipocytes, OFE and OSE (50 μg/ml) stimulated the 2-NBDG uptake by 144% and 152%, respectively whereas in human adipocytes induced the 2-NBDG uptake by 123% and 67%. Tested in the presence of insulin, none of the extracts showed an additive induction of 2-NBDG uptake. Both extracts induced 2-NBDG uptake by 78% in 3T3 insulin-resistant adipocytes whereas in insulin-resistant human adipocytes OFE and OSE stimulated the glucose

analog uptake by 82% and 51% respectively. On the other hand, OFE and OSE moderately increased murine (138% and 153%, respectively) and human (132% and 138%, respectively) adipogenesis, mainly by means of their insulin-like activities. Our results suggest that *Opuntia leucotricha* exerts its anti-diabetic effects stimulating glucose uptake in both insulin-sensitive and insulin-resistant murine and human adipocytes moderately inducing its adipogenesis. *Acknowledgements:* RZB and AJAC were endowed with graduate fellowships from CONACYT (211445 and 210841, respectively). AJAC also received special support from IPICYT (SA-157/2008). **References:** [1] Alonso-Castro, A.J., Salazar-Olivo, L.A. (2008) J. Ethnopharmacol. 118:252 – 256. [2] Ramírez-Zacarías, J.L. et al. (1992). Histochem. 97:493 – 497.

PJ139 Adipocyte culture: an optimal model system for screening new promissory drugs for type 2 diabetes mellitus treatment

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Nowadays the screening for new anti-diabetic compounds is done using experimental animals treated with alloxan or streptozotocin to destroy or at least inactivate pancreatic cells producers of insulin [1]. Nevertheless such experimental models in which animals fail to produce insulin or produce it in insufficient quantities correspond more to type 1 diabetes mellitus than to type 2 diabetes (T2-D), the prevailing form of this disease worldwide. Hypoglycemic oral agents can exert their effects by any of three principal mechanisms of action: decreasing sugar intestinal absorption, stimulating insulin secretion, or stimulating glucose uptake by peripheral insulin-target tissues. This latter mechanism represents the most promissory therapeutic target for T2-D. Here we present the use of an adipocyte culture system for screening new compounds that stimulate glucose uptake and the characterization of their molecular action mechanisms. The proposed model system has enabled us to characterize the mechanisms of the anti-diabetic properties of *Guazuma ulmifolia* [2] and *Cecropia obtusifolia* [3] as well as their lack of proadipogenic effects. *Acknowledgements: RZB and AJAC were endowed with graduate fellowships from CONACYT (211445 and 210841, respectively). AJAC also received special support from IPICYT (SA-157/2008).* **References:** [1] Matteucci, E., Giampietro, O. (2008) J. Ethnopharmacol. 115:163 – 172. [2] Alonso-Castro, A.J., Salazar-Olivo, L.A. (2008) J. Ethnopharmacol. 118:252 – 256. [3] Alonso-Castro, A.J. et al. (2008) J. Ethnopharmacol. 120:458 – 464.

PJ140 Total phenol content and aromatic compounds variation among Latvian medical plants depending on vegetative stage

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The use of medical plants in the form of crude extracts, infusions has been applied as a common practice to treat different diseases. Latvian flora is rich in wide range of medical plants and most popular are chamomile, yarrow, marigold etc. The aim of this research was to determine total phenols, aroma compounds in Latvian medical plants depending on vegetative stage. Plant material chamomile *Matricaria chamomilla* L., St. John's wort *Hypericum perforatum* L., marigold *Calendula officinalis* L., motherwort *Leonurus cardiaca* L., yarrow *Achillea millefolium* L. were harvested in 2008 in Latvia at different vegetative stages. Volatile aroma compounds from dried leaves and flowers were extracted using headspace autosampler Turbomatrix (PerkinElmer) and for the analysis of compounds, a PerkinElmer Clarus 500 GC/MS was used. Total phenols were determined in teas prepared from previous mentioned plants (1% w/w) using Folin-Ciocalteu assay. For comparison commercially available green and black tea were analyzed. Total phenols varied among medical plants. The highest phenol content was detected in St. John's wort tea, and it even was higher then in the green and black tea. The content of total phenols in tea samples significantly depend on plant vegetative stage, and mainly higher content were detected in plants collected in budding stage. In the headspace of medical plants aroma compounds belonging to different chemical classes were detected: monoterpenes, oxygenated monoterpenes, sesquiterpenes, alcohols, aromatic compounds etc.. The highest amount of total identified aroma compounds were detected in St. John's wort and marigold.

PJ141 Phytoestrogenic activity of *Morinda citrifolia* L. *(Noni)* leaf

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Decreasing estrogene blood levels in postmenopausal women can exert a variety of adverse symptoms. One of the most critical side effects of the lack of estrogene is an imbalance in the bone-mineral turnover, leading to osteoporosis. We investigated the effect of extracts prepared from the leaves of the noni plant to increase the activity of alkaline phosphatase (AP), the key enzyme in osteosynthesis, in Ishikawa (endometrial cancer) cells and in U2OS (osteosrcoma) cells. In both cells, AP is regulated via the estrogene receptor complex. Compared to 17-ß-estradiol (E2), an alcoholic extract of noni leaf showed a weak increase in AP-expression. In U2OS cells, however, an aqueous extract of noni leaf was very active in the induction of AP and even stronger than E2. Aqueous, alcoholic and hexane extracts of noni leaf were all active in the estrogene receptor replacement assay. Further experiments including animal studies and human trials are warranted to examine whether noni leaf extracts can be used to antagonize osteoporosis caused by a lack of estrogene.

PJ142 Lemon balm (*Melissa officinalis* L.): Effects of gibberellic acid and dry yeast on growth and essential oil yield and composition

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In field experiments during two successive seasons (2005 – 2006 and 2006 – 2007), the effect of gibberellic acid (GA_3) and active dry yeast on growth, yield, and essential oil (EO) of lemon balm plants was investigated. Application of GA_3 and/or active dry yeast increased vegetative characters (i.e. plant height, number of branches, and herb fresh and dry weight per plant) compared to control (sprayed with water only). The maximum mean values of growth characters were obtained as a result of spraying with 6 g l^{-1} yeast + 300 ppm GA_3. The lowest fresh and dry weights of plants were observed with the treatment of 2 g l^{-1} yeast + 0 ppm GA_3 in the first harvest. EO content in the lemon balm herb increased due to the application of GA_3 and/or active dry yeast compared to control. The highest EO yield per plant was observed with the treatment of 6 g l^{-1} yeast + 300 ppm GA_3. The lowest amount of EO yield was obtained with the control treatment. The highest geranial in lemon balm EO occurred with the treatment of 6 g l^{-1} yeast + 300 ppm GA_3.

PJ143 Phenolic metabolites from *Acacia nilotica* flowers and evaluation of antihyperglycaemic effect of aqueous extract

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Diabetes mellitus is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by pancreas, or by the ineffectiveness of the insulin produced. The present study deals with the isolation and identification of the phenolic constituents from *Acacia nilotica* flowers and evaluation of antihyperglycaemic effect of aqueous alcoholic extract. The aqueous alcoholic extract (MeOH:H_2O, 7:3) of *Acacia nilotic* flowers was subjected to extensive repeated Column chromatography on polyamide, cellulose and Sephadex LH-20 resulted in catechin, catechin 7-*O* – gallate, gallic acid, methyl gallate, naringenin 7-*O*-β- glucopyranoside, quercetin 3-*O*- β- glucoside (2→1) glucopyranoside, quercetin 3-*O*- β-glucopyranoside, chalconaringenin 4'-*O*-β-glucopyranoside, naringenin and quercetin. The structure of the isolated compounds was elucidated on the basis of spectral analysis. The effect of

the oral treatment with dry aqueous alcoholic extract of *Acacia nilotic* flowers (25 mg/Kg for 21 days) on serum glucose in normal and alloxan-induced diabetic rats is reported. Fasting blood glucose levels of diabetic rats were significantly ($P < 0.01$) higher than those in normal rats. A significant decrease in blood glucose level was observed in diabetic rats treated with the extract of *Acacia nilotic* flowers from an initial level of (258.6 ± 22.8) to (118.8 ± 10 mg/dl). The extract failed to produce hyperglycemic activity in normal treated rats. The chemical constituents of plant especially phenolics and other compounds present in the plant may be involved in the observed hypoglycemic effect of the plant extract [1]. The results show that the oral administration of *Acacia nilotic* flowers extract on the diabetic state reducing hyperglycemia. **References:** [1] Resurreccion-Mago, M.H. et al. (2005) Phytother. Res. 19:246 – 251.

PJ144 Antitumor and antibacterial activities of some fruits

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The use of most medicinal plants discovered by traditional societies has not been verified scientifically and bioassays can provide initial screening data about the biological activities of these plants. Two different bioassays (antibacterial and antitumor) were performed to show the biological activities of nine different aqueous and ethanol extracts of fresh or dried fruits [*Viburnum opulus* L. (guelder rose), *Viburnum lantana* L. (wayfaring tree), *Cornus mas* L. (cornelian cherry), *Pyracantha coccinea* Roemer (firethorn), *Rubus caesius* L. (dewberry), *Crataegus tanacetifolia* (Lam.) Pers (tansy-leaved thorn), *Crataegus monogyna* Jacq. (hawthorn), *Rosa canina* L. (dog rose) and *Fragaria vesca* L. (wild strawberry)]. The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibacterial activity. Among the tested fruits, best antibacterial activity was obtained with fresh fruits of wayfaring tree, firethorn and hawthorn. Hot ethanol extracts of these fruits showed strong antibacterial activity against *S. aureus*, *S. epidermidis* and *S. pyogenes*. Antitumor activity was evaluated with potato disc diffusion bioassay. Best antitumor activity was obtained with ethanol extract of fresh or dried fruits of guelder rose (100% inhibition). Furthermore, cold water extract of fresh fruits of dewberry showed 100% tumor inhibition. Strong antitumor activities of cold or hot ethanol extracts of fresh fruits of wayfaring tree and hot ethanol extract of fresh fruits of wild strawberry were also observed.

PJ145 Biological activities of meadowsweet (*Filipendula ulmaria* (L.) Maxim)

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Filipendula ulmaria (L.) Maxim (meadowsweet) is a medicinal plant that has been used to treat several inflammatory diseases including gout and rheumatoid arthritis, and for the treatment of coughs, bronchitis, fevers, ulcers and colds. Three different bioassays (antibacterial, antitumor and toxicity) were performed to show the biological activities of meadowsweet. They were evaluated between field-grown plants and *in vitro*-grown plants using eight different extracts (aqueous, ethanol, ethylacetate and hexane). The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibacterial activity [1]. The microorganisms used were: *Streptococcus pyogenes* (ATCC® 19615), *Staphylococcus aureus* (ATCC® 25923) and *Staphylococcus epidermidis* (ATCC® 12228) which are Gram-positive bacteria and *Escherichia coli* (ATCC® 25922), *Pseudomonas aeruginosa* (ATCC® 27853), *Salmonella typhimurium* (ATCC® 14028), *Serratia marcescens* (ATCC® 8100), *Proteus vulgaris* (ATCC® 13315), *Enterobacter cloacae* (ATCC® 23355) and *Klebsiella pneumoniae* (ATCC® 13883) which are Gram-negative bacteria. Generally, antibacterial activities of field-grown plants were better than *in-vitro* grown plants against all used bacteria. Aqueous extract of field-grown plant (FW) exhibited better antibacterial activity than other extracts. *S.epidermidis*, *S. aureus, S. typhimurium, S. marcescens*, *P. aeruginosa*, *P. vulgaris*, *K. pneumonia* and *E. cloacae* were sensitive to FW. Antitumor activity of all extracts was assessed with the potato disc method as modified by McLaughlin's group [2].The inhibition of *Agrobacterium tumefaciens*-induced tumors (or crown gall) in potato disc tissue is an assay based on antimitotic activity and can detect a broad range of known and novel antitumor effects [3,4]. The validity of this bioassay is predicted on the observation that certain tumorigenic mechanisms are similar in plants and animals. It has been shown that the inhibition of crown gall tumor initiation on potato discs and subsequent growth showed good correlation with compounds and extracts active in the 3PS (P388) (*in vivo* murine leukemia) leukemic mouse assay [4,5]. Field-grown plants showed better activity than *in vitro*-grown plants. But, after viability test for *A. tumefaciens*, it was understood that inhibition of crown gall formation on potato disc is caused by decreasing the viability of the *A. tumefaciens*. It is not possible to evaluate the antitumor activity of *F. ulmaria* with potato disc bioassay. Because meadowsweet extracts have very strong antibacterial activity and affect the viability of *A. tumefaciens*. The brine shrimp bioassay was used to assess the general toxicity of meadowsweet extracts [6]. All extracts were toxic at higher doses ($LC_{50} > 2.000$ mg/l) by comparing with MS-222 (Tricaine methane sulfonate). Aqueous extracts of field-grown and *in vitro*-grown plants were less toxic than other extracts (ethanol, ethylacetate and hexane). **References:** [1] Andrews, J.M. (2004) J. Antimicrob. Chemoth. 53:713 – 728. [2] Ferrigini, N.R. et al. (1982) J. Nat. Prod. 45:679 – 686. [3] McLaughlin, J.L., Rogers, L.L. (1998) Drug Inf. J. 32:513 – 524. [4] Coker, P.S. et al. (2003) Phytomedicine 10:133 – 138. [5] Galsky, A.G. et al. (1980) Plant Physiol. 65:184 – 185. [6] Meyer, B.N. et al. (1982) Planta Med. 45:31 – 34.

PJ146 Whey protein and its major peptide fractions ameliorate hepatorenal dysfunction induced by CCL_4 in rats

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The major whey proteins (WP) are α-lactalbumin (α-La) and β-lactoglobulin (β_Lg) possess interesting nutritional, functional and therapeutic properties [1]. This study was designed to evaluate the effect of WP, α-La and β-Lg, on CCl_4-iduced hepatorenal damage in rats. The albino rats were pre-treated with WP, α-La and β-Lg (100 and 200 mg/kg b.wt.) for 15 days before treatment with single dose of CCl_4 (0.5 ml/kg. s.c. in olive oil). The rats were sacrificed 24 hrs later and blood samples were collected for assign serum biochemical parameters. Serum samples were taken to determine the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), triglycerides and cholesterol levels. The histopathological effect on the liver tissue was also investigated to support the above parameters. The results of the present study indicated that the levels of serum AST, ALT, ALP, GGT, triglycerides and cholesterol were significantly ($P < 0.05$) elevated by CCl_4 administration as compared with the control group and significantly reduced at $P< 0.05$ by the treatment with the WP, α-La and β-Lg (100 and 200 mg/kg/b.w for 15 days) compared with the CCl_4-intoxicated rats. Moreover, kidney function tests showed significant ($p < 0, 05$) reduction in the level of creatinine and uric acid by the treatment with the WP, α-La and β-Lg. Microscopic examination of liver of CCl_4 treated animals revealed that lymphoctes infiltration, cytoplasmic vacuolization, fatty degeneration, focal necrosis, and fibrous tissue. Also renal tissues showed hypercellularity and shrinkage of glomeruli, vacuolization and necrotic of epithelial cells with interstitial inflammatory cell infiltration. The histopathological examination also showed ameliorative effect of WP, α-La and β-Lg reduced the alterations that induced in liver and kidney by CCl_4. The higher dose of α-lactalbumin is more effective than whey proteins and β-lactogloblin . **References:** [1] Marshal, K. (2004) Altern. Med. Rev. 9:136 – 156.

PJ147 Antifungal activity of plant essential oils against wild strains of *Candida* spp. isolated from hospitalized patients

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The antimicrobial activity of Basil oil, Chamomile Blue oil, Origanum oil, Tea Tree oil and Thyme oil was investigated using a broth macrodilution method against 12 strains of Candida spp isolated from clinical speci-

mens (wounds, blood and urine), derived from various patients entering the University Hospital of Ioannina plus one ATCC *Candida* strain. The strains used in this study were *C. albicans* (n = 4), *C. parapsilosis* (n = 5), *C. tropicalis* (n = 2), *C. glabrata* (n = 1) and *C. albicans* ATCC 10231. Susceptibility test to antimicrobials was performed using the Bauer-Kirby method and the Vitek II system (Biomerieux). Basil oil (W211907), Chamomile Blue oil (W227307), Origanum oil (W282812), Tea Tree oil (W390208) and Thyme oil (W306509) were obtained from Sigma-Aldrich (Germany). Carvacrol and thymol (*Origanum oil*), thymol, linalool and p-cymene (*Thyme oil*), terpine-4-ol and p-cymene (*Tea tree oil*), methyl chavicol (*Basil oil*) were the main components of the tested oils. The antifungal activity of the selected essential oils was tested also by the viable counts method and the optical density method in addition to the broth macrodilution method and the Minimal Inhibition Concentration (MIC) was determined at 24 hours, 48 hours and 7 days. All experiments were performed in duplicate. All tested essential oils except the Chamomile Blue oil, displayed similar antifungal activity ranging from 0.06 to 0.37% (v/v). Specifically, the MIC values for Origanum oil ranged from 0.06 to 0.25% (v/v), for Basil oil from 0.06 to 0.37% (v/v), for Tea Tree oil from 0.09 to 0.25% (v/v) and for Thyme oil from 0.12 to 0.25% (v/v), while Chamomile Blue oil exhibited no antifungal properties. After the 7th day of incubation the MIC values for the tested oils except Chamomile Blue oil, ranged from 0.12 to 0.5% (v/v). Relevant studies by other researchers report MIC values ranging from 0.04 to 0.12% (v/v) for origanum oil, 0.12 to 0.32% (v/v) for thyme oil, 0.18 to 0.5% (v/v) for basil oil and 0.03 to1% (v/v) for tea tree oil, while there are no data available for chamomile blue oil. *Aknowledgements: This research was financially supported by the HERAKLEITOS project, funded by the General Secretariat of Research and Technology, Greek Ministry of Development.* **References:** [1] Pozzatti, P. et al. (2008) Can. J. Microbiol. 54:950 – 956. [2] Preuss, H.G. et al. (2005) Mol. Cel. Biotechnology 272:29 – 34. [3] Rosato, A. et al. (2008) Phytomedicine 15:635 – 638. [4] Salgueiro, L.R. et al. (2003) Planta Med. 69:871 – 874.

PJ148 Localization of arabinogalactan-proteins in roots of *Echinacea purpurea by* immunofluorescent labelling

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From the high molecular weight fraction of an aqueous extract from roots of *Echinacea purpurea* (L.) MOENCH, arabinogalactan-proteins (AGPs) were purified and characterized with special regard to the structure of the polysaccharide moiety. It is highly branched and shows a linkage composition comparable to AGPs from the aerial parts of *E. purpurea* [1] with 1,3-, 1,6- and 1,3,6-linked galactopyranosyl residues as basic structural elements and arabinofuranosyl residues predominantly as terminal and 1,5-linked residues. Some terminal units of glucuronic acids could also be detected. A new method of localization of AGPs in plant tissue has been developed. The synthetic $(\beta\text{-D-Glc})_3$ Yariv phenylgycoside (βGlcY) is known to bind specifically to AGPs and was used for their isolation. For immunolocalization, polyclonal βGlcY-antibodies have been generated and were used to label Yariv-treated thin sections of roots from *E. pupurea*. After addition of a FITC-conjugated secondary antibody, the sections were analyzed by confocal laser scanning microscopy. AGPs were mainly detected in the central cylinder in xylematic elements. Cell walls of vessels and tracheids are strongly labeled, especially at the inner area of the wall. Furthermore, there was an intense labelling of pit canals. The proposed involvement of AGPs in xylem differentiation [2] thus could be confirmed, and in addition it is suggested that AGPs are involved in the formation of pit canals during xylem development. **References:** [1] Classen, B. et al. (2000) Carbohydr. Res. 327:497 – 504. [2] Motose, H. et al. (2004) Nature 429:873 – 878.

PJ149 Phytochemical investigation and biological evaluation of the secondary metabolites isolated from *Erythrina poeppigiana* – Fabaceae

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In most developing countries, the traditional medicine based on preparations from medicinal plants still stays one of the main appeals. Many *Erythrina* species for example are used in the traditional system of medicine in Cameroon for the treatment of menopause related illnesses. Additionally, the Leguminosae family is well known for its great content in isoflavonoids which have been shown to exhibit numerous biological activities including estrogenic/anti-estrogenic activity (so called phytoestrogens) [1]. In our endeavour, aiming to discover novel active natural products based on the long-established empirical traditional knowledge, phytochemical investigation of *Erythrina poeppigiana* (Fabaceae), a Leguminosae species growing in humid and sub-humid tropical lowland is undertake. After a qualitative evaluation of DCM and MeOH extracts using TLC and HPLC-PDA, further investigation was performed on both extracts leading to isolation and structure determination of a number of secondary metabolites using several chromatographic (VCC, CC, FCPC, HPLC) and spectroscopic (UV, MS, 1&2D NMR) techniques, respectively. At this stage of the work, 10 isoflavonoids (5 novel compounds), one pterocarpan and 3 simple phenolic ester were isolated from the DCM extract and 4 *Erythrina* alkaloids from the MeOH extract. The ability of the isolated compounds derived from the DCM extract to bind to the estrogen receptor (ERα & ERβ) was estimated. Most of them found to be potent ligands. **References:** [1] Veitch, N.C. (2007) Nat. Prod. Rep. 24:417 – 464.

PJ150 A new flavonoid from *Cassia bicapsularis*

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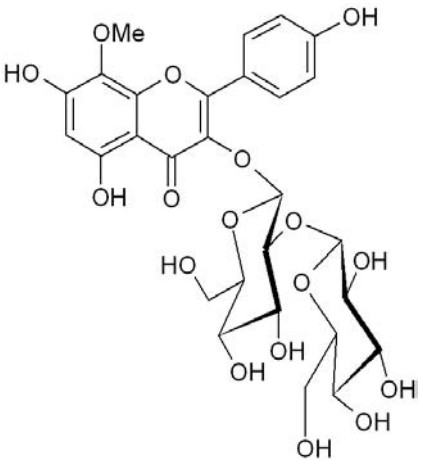
Genus *Cassia* is considered one of the most important genera of family Leguminosae [1]. It was reported to contain flavonoids, anthraquinones, triterpenes, sterols and carbohydrates [2]. *Cassia bicapsularis* L. is a semi-evergreen shrub native to South America [3]. We studied the phenolics of the hydroalcoholic extract of *C. bicapsularis* flowers and we isolated 11 compounds: kaempferol 8-*O*-methyl ether, vanillic acid, rutin 4'-*O* -glucopyranoside, rutin, isorhamnetin 3-*O*-galactopyranoside, isoquercitrin, hyprin, luteolin 7-*O*-glucopyranoside, luteolin 4'-*O*-galactopyranoside, luteolin 3'-*O*-β-galactopyranoside and a new flavonoid 8-methoxy kaempferol 3-*O*-glucopyranosyl(1'''→2'')-glucopyranoside. LD_{50} tests showed that *C. bicapsularis* L. hydroalcoholic flowers' extract was non toxic up to 5 g/kg which is the maximum soluble dose. It possessed a significant anti-inflammatory activity on the carrageenan induced paw edema at a dose of 1000 mg/kg, compared with indomethacin which was given as reference drug at a dose of 25 mg/kg.



References: [1] Polhill, R.M., Raven, P.H. (1981) Advances in Legume systematics. Royal Botanic Gardens, Kew. [2] Anu, S.J. and Rao, J.M. (2002) Phytochemistry 59:425 – 427. [3] Wiggins, I.L., Porter, D.M. (1971) Flora of the Galapàgos, Stanford University Press.

PJ151 Evaluation of some types of fennel (*Foeniculum vulgare* Mill.) newly introduced and adapted in Egypt

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Exports of local fennel (*Foeniculum vulgare* Mill.) from Egypt in last years have been affected due to its high estragole but low anethole contents in the oil. Therefore, fennel seeds were imported from different countries to investigate the adaptability of such strains in different locations in Egypt in comparison with the local one. The obtained results indicated that Holland fennel surpassed other fennel strains under study, as it showed the best growth in terms of number of umbels, seed production, seed oil (%) and oil production per plant and unit area. The seed oil of this strain had the highest content of anethole (75.93% in average) and low estragole percentage (4.22%). The Indian strain showed poor characters. The German strain although contained high oil percentage, but the oil yield/plant or/unit area were poor. Menia location led to the best characters for Holland, Indian, Florence and local fennel however, it did not favor the growth of the German strain. It would be recommended to cultivate the Holland fennel strain at Menia to obtain the best growth and oil yield and characters.

PJ152 Antimicrobial effects of Sumac (*Rhus coriaria L.*) extract in minced meat

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In recent years, there is a great interest in use of natural preservatives in food. One of the most important one is plant extracts [1]. *Rhus coriaria* L., commonly known as Sumac, grows wild in the region extending from the Canary Island to Iran and Afghanistan. It used traditionally as a flavor in food in Iran [2]. So, this study was done to assess antimicrobial effects of Sumac extract on spoilage and pathogenic bacteria in minced meat. Ethanolic extract of Sumac was prepared and minimum inhibitory concentration of it was evaluated. Sterile plastic bags with 50 grams minced meat were prepared. Different amounts of Sumac extract were added into the bags (5, 10 and 15 µl/g). Total bacterial count of the meat was measured and definite number of *Salmonella Typhimurium* was inoculated. Two control group (alcohol and without extract) was mentioned. All samples were stored at 4 °C and evaluated at the days 7, 14, and 21. Result showed that there was a significant difference between tests and control groups in total microbial count in the first week (for example: 6.6 ± 0.1 $\log_{10}$ cfu/g in tests group and 8.7 ± 0.7 in control group), but this was not significant in second and third weeks ($p < 0.05$). Also there was a significant difference between tests and control group in *Salmonella* count in the first week but this was not significant in second and third weeks ($p < 0.05$). The results are similar to those obtained from previous studies of sumac [2,3].Therefore it seems that Sumac extract can have an antimicrobial effect on total microbial and *Salmonella* count in minced meat for one week. **References:** [1] Becerril, R. et al. (2007) Anal. Bioanal. Chem. 388:1003 – 1011. [2] Fazeli, M.R. et al. (2007) Food Control. 18:646 – 649. [3] Nasar-Abbas, S.M., Kadir, H.A. (2004) Int. J. Food Microbiol. 97:63 – 69.

PJ153 Effect of sowing date and nitrogen on yield, components yield and oil characteristic of medicinal flax

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In order to investigate the effect of sowing date and different nitrogen (N) levels on quality and its features of medicinal flax, an experiment performed in split plot in form of Randomized Complete Block Design was carried out with 4 replications and during farming years 2005 – 2006 and 2006 – 2007 in Yasooj Agriculture Research Station. 5 sowing date included 4, 6, 8, 10, 12 on the basis of depth temperature of 5 cm in main plots and 4 fertilizer levels in sub-plots included sample (no fertilizer) 50, 100 and 150 kg/ha pure N from urea source that 50% was used at the time of sowing and 50% in the way of top-dressing. The results of complex 2-year analysis of data indicate that with delayed sowing plant height, number of branch, number of fruits, grain yield, 1000-seed weight, leaf area index, dry mater, crop growth rate and oil percentage were reduced significantly. The use of 100 kg/ha pure N significantly increased plant height, number of branch, number of fruits, grain yield, leaf area index, dry mater, crop growth [1]. First sowing date with 1801.12 kg/ha had the most yields and fifth sowing date with 760.48 had the least product. The most and the least yield rates of seed in 100 kg/ha pure N and sample were 1895.22 and 1351.87. At last, the most of seed yield rate is obtained 2135.26 and 2128.14 kg/ha orderly in the first sowing date and the consumption of 100 and 150 hg pure N/ha and the least seed yield rate is obtained in the fifth planting date without the pure N consumption. The most oil rate was 34.6% that obtained in the first date. The first sowing date with 52.38% produced the most and the fifth sowing date with 50.58% produced the least linolenic acid. The sowing date had no significant effect on oleic acid and linoleic acid. The most linolenic acid 52.64% and linoleic acid 15.36% were obtained by the use of 150 kg/ha pure N and the most oleic acid 20.59% were obtained without use of nitrogen [2].

PJ154 Histochemical screening of flavonoids in secretory trichomes of *Zataria multiflora* leaves

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Zataria multiflora Boiss (Lamiaceae) grows wild in central and southern Iran, and is used in traditional herbal medicine for antiseptic, analgesic and carminative properties. *In vitro* antibacterial and antioxidant properties of essential oil and methanol extracts of *Z. multiflora* have recently been reported [1]. Preliminary phytochemical screening indicated the presence of flavonoids in ethanol extract of *Zataria* with antinociceptive and anti-inflammatory activities in mice [2]. Pharmacological effects of *Z. multiflora* are reported, but the botanical data are missing and the secretory aspect in relation to *Zataria* development is unknown. The present work focused on flavonoids because of their ecological importance as UV radiation filters and antioxidants. *Z. multiflora* branches were collected from Fars province, south western Iran, in spring. Fresh sections of leaves were first investigated for the aspect and density of trichomes. Flavonoids were detected based on their fluorescence and using Neu's reagent; sections were immersed in 1% 2- aminoethyl diphenyl borate in absolute methanol for 2 – 5 minutes and examined with an epifluorescence microscope upon excitation at 450 nm. Four types of glandular trichomes were recognized on the emerging leaves of the shoot tips, differing by number and shape of the secretory head. All trichome types secrete flavonoids characterized by a yellow color in the cytoplasm or in subcuticular space. Results of the present study are in support of the phytochemical reports indicating promising antioxidant and protective activities of this popular Iranian spice. **References:** [1] Sharififar, F. et al. (2007) Food control 18:800 – 805. [2] Hosseinzadeh, H. et al. (2000) J. Ethnopharmacol. 73:379 – 385.

PJ155 Effect of potassium fertilizer on lemon balm (*Melissa officinalis* L.) grown under water stress conditions

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This work was carried out to study the effect of K fertilizer rates and water stress levels on the growth and essential oil content of *Melissa officinalis* L. A pot experiment was carried out under the natural conditions of the greenhouse of the National Research Centre, Dokki, Giza, Egypt. Growth characters (herb fresh and dry weights g plant $^{-1}$ leaves number and leaf area) and essential oil content of *Melissa officinalis* L. were significantly decreased with the rise in water stress levels, but proline synthesis was stimulated in response to water stress levels. Application of K fertilizer rates counteracted the above adverse effects of water stress. Irrigation at 80% available soil moisture and fertilization with 0.8 g K pot $^{-1}$ dose in the 1st cut resulted in the highest mean values of herb fresh and dry weights (51.01 and 12.05 g plant $^{-1}$, respectively) and leaves number (420.80 plant $^{-1}$), while 40% available soil moisture treatment resulted in the lowest values (13.73; 3.22 and 113.60) of these parameters, respectively. The maximum mean value of essential oil content (0.131%) was obtained from plants irrigated with 80% available soil moisture with K fertilizers rate (0.6 g K pot $^{-1}$), while the lowest mean value (0.097%) was determined in the plants irrigated with 40% available soil moisture with no potassium fertilization in the 3rd cut. Increasing the dosage of K fertilization significantly increased the proline content while, increasing water irrigation decreased the proline content. The highest mean value of proline was determined in the plants received 0.8 g K pot $^{-1}$ and irrigated with 40% of available soil water increase an average of 68.69% than that for the plants irrigated with 80% available soil moisture in the 3rd cut. Geranial and neral were identified as the two major compounds in the essential oil extracted from *Melissa officinalis* L. Increasing both of K rates and available soil moisture tended to increase the contents of geranial and neral. The results revealed that, the highest amount of geranial (49.75%) was recorded from the combination of irrigation at 80% available soil moisture and fertilization with 0.8 g K pot $^{-1}$ treatment in the 3rd cut while, the highest amount of neral (35.71%) was recorded at 80% available soil moisture treatment in the 2nd cut.

PJ156 Antibacterial activity of *Curcuma longa* extract against bacteria isolated from infected burn wounds

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Burns are suitable sites for antibiotic resistant infections. Thus search for effective drugs against this problem is necessary. Medicinal herbs with antimicrobial activity have always been important in traditional medicine. The aim of this study was to determine the antibacterial activity of a methanol extract from roots of *Curcuma longa* against bacteria isolated from infected burns and their comparison with selective antibiotics in vitro. First, a sample of methanol extract of dried roots of *Curcuma longa* (1 mg: 5 ml) by maceration method was prepared and then its antibacterial activity against 8 bacterial isolates obtained from 100 samples of infected burns was tested for the determination of MIC (minimum inhibitory concentration) using well diffusion and agar serial dilution (0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10, 20, 40) assays. Also the antibacterial activity of penicillin, oxacillin, vancomycin, ceftazidime, tobramycin, imipenem, amikacin was tested by the disk diffusion method. Statistical methods were used to analyze the data. The results demonstrated that the *Curcuma longa* methanol extract had been effective against more than 75% of *Staphylococcus aureus, S. epidermidis, S. saprophyticus*, against 80% of *Pseudomonas aeruginosa* and 69% of *Acinetobacter spp.* The MIC of the extract against *Staphylococcus* spp. was about 3/7 mg/mL. The MIC against *Pseudomonas aeruginosa* and *Acinetobacter spp.* was 13/95 mg/mL and 14/55 mg/mL, respectively. This study demonstrates that a methanol extract of *Curcuma longa* is effective on most of bacteria isolated from infected burns and its effect is even better than that of selective antibiotics. Further investigations will be necessary.

PJ157 Constituents of ginger rhizome (*Zingiber officinale* Roscoe) inhibit Interleukin-1β maturation and secretion in stimulated human monocytes by a phospholipase A2 dependent manner

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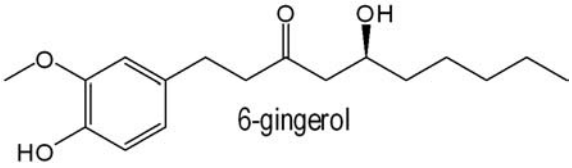
Ginger (*Zingiber officinale* Roscoe) is widely used against emesis and inflammatory diseases [1] but the exact mode of action remains to be elucidated. Functional inhibition of NF-κB and MAP kinases [2], or inhibition of COX-2 [3] were reported. The main constituent of ginger rhizome (6-gingerol) is believed to be the dominant active compound, but results on PGE2 inhibition [4] also indicates that several other chemically related compounds seem to be involved in the immunomodulatory effects.



We tested ginger extracts (50 μg/ml) and pure compounds (10 – 50 μM) in stimulated human whole blood and could show an inhibition of different cytokines in dependence of the used stimuli with the exception of interleukin-1β (IL-1β) being consequently down-regulated (up to 74% inhibition). Using Cytometric Bead Arrays and Western Blots we could demonstrate that in isolated human monocytes the $iPLA_2$ dependent maturation and the $cPLA_2$ dependent secretion are reduced, depending on assay conditions, to 3 – 22% and 61 – 88% respectively (at 10 μg/ml), whereas transcription/translation was unaffected. Using flow cytometry we show that ion fluxes are not affected. The suppression of secreted mature IL-1β as a key player in inflammatory diseases like rheumatism and the possible inhibition of phospholipases are likely to contribute to the anti-inflammatory potential of ginger preparations. **References:** [1] Srivastava, K.C. et al. (1992) Med. Hypotheses 39:342 – 348. [2] Kim, S.O. et al. (2004) Biofactors 21:27 – 31. [3] Tjendraputra, E. et al. (2001) Bioorg. Chem. 29:156 – 163. [4] Jolad, S.D. et al. (2004) Phytochemistry 65:1937 – 1954.

PJ158 Investigations on the antiinflammatory way of action of a *Harpagophytum* extract using microarray technology

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Preparations from the tubers *Harpagophytum procumbens* are monographed and used in the treatment of degenerative rheumatic diseases, but a clear antiinflammatory mode of action is still lacking. Several in vitro investigations revealed inhibitory effects on COX-2, iNOS, NF-kB, MMP-9 and MMP-2 and, in very high concentrations, on TNF-alpha. We tried a new approach to gather new information on the antiinflammatory way of action of *Harpagophytum* by using the microarray ('gene chip') technology. THP-1 cells were incubated with 50 μg/ml *Harpagophytum procumbens* dry extract (DER 1.5 – 3: 1; 60% ethanol; A. Vogel Bioforce AG) for 1 hour, afterwards stimulated with 0.1 μg LPS/ml and then incubated for 18 hours. The isolated mRNA was transcribed to cDNA and labelled and then transferred on a Whole Human Genome Chip (Agilent Technologies). The *Harpagophytum* extract inhibited only few genes statistically significant. From these genes, we investigated with rt-PCR those further which are involved in inflammatory and/or rheumatic diseases and in particular some genes which are regulated by the TLR-4 pathway. The *Harpagophytum* extract showed after an incubation time of 3 hours an antiinflammatory effect by inhibiting the transcription of NF-kB, TNF-α, CCl_2 and MMP9. These experiments confirm

there is no distinct antiinflammatory way of action but rather a moderate inhibition of several inflammatory targets.

PJ159 A multicentre open clinical trial to assess the tolerability and efficacy of Sage tablets in menopausal patients with hot flushes

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Hot flushes figure amongst the most common symptoms in general medical practice and alternatives to HRT are of growing interest. Sage has been traditionally used to treat menopausal symptoms. In this study including 71 women aged 50 – 65, menopausal since at least half a year, with a minimum of 5 flushes daily, a proprietary Sage mono-product (*Salvia off*. *folium* rec. T., *spissum* extract, DER 1:17) applied once daily during 8 weeks proved to be well tolerated and efficacious in reducing intensity and frequency of hot flushes and menopausal symptoms assessed via patient diary and Menopause Rating Scale (MRS), respectively. The decrease over the treatment period of 8 weeks was statistically significant for the global MRS Score and each of the related Subscores: MRS by about 6.4 ± 0.9 score points, and Somato-vegetative, Psychological and Urogenital subscale by about 3.3 ± 0.4 2.7 ± 0.5, and 0.3 ± 0.1 score points, respectively. The total score of the mean number of hot flushes (TSNMH) decreased significantly compared to the previous week from week 1 to week 8. Tolerability was rated as very good or good by 87.3% of the physicians and by 87.3% of the patients. The evaluation of the safety laboratory parameters also demonstrated a high degree of safety and tolerability. In this clinical trial Sage tablets clearly demonstrated good clinical value in terms of efficacy, safety and tolerability in the treatment of menopausal hot flushes and climacteric symptoms. Overall, the results suggest that Sage tablets are a promising herbal treatment alternative for menopausal women with climacteric complaints and a safe and effective herbal approach for the treatment of hot flushes in menopause.

PJ160 Antibacterial activity of *Terminalia catappa* extract against bacteria isolated from infected burn wounds

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Burn wound is suitable site for incidence of resistant infections. Thus, the research for finding of effective drugs against this problem is necessary. Medicinal herbs with antimicrobial activity have been important role in traditional medicine. The purpose of this study was determine of antibacterial activity of methanol extract from fruit of Terminalia catappa against bacteria isolated from burn wound infections and comparison with effects of selective antibiotics In vitro. First, a sample of methanol extract of the plant fruit (1 mg: 5 ml) by maceration method was prepared and then its antibacterial activity against 8 bacterial isolates obtained from 100 samples of infected burns was tested for the determination of MIC (minimum inhibitory concentration) using well diffusion and agar serial dilution (0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10, 20, 40) assays. Also the antibacterial activity of penicillin, oxacillin, vancomycin, ceftazidime, tobramycin, imipenem, amikacin was tested by the disk diffusion method. The frequency distribution tables, diagrams, Kay square, Fisher exact and t- test (by applying spss computer programs) were used to describe and analyze the data. The results demonstrated that the *T. catappa* methanol extract had been effective against more than 80% of *Staphylococcus aureus/epidermidis/saprophyticus*, *Pseudomonas aeruginosa*, *Acinetobacter spp*. and against 50% *E.coli*. The MIC of the extract against all the bacteria was 20 mg/ml. This study demonstrated that methanol extract of *T. catappa* have excellent antibacterial activity against most of bacteria isolated from infected burns and its effect is better than the selective antibiotics. However, we need more investigation *in vitro* and *in vivo*.

PJ161 Antibacterial activity of extract *Curcuma amada* against *Staphylococcus aureus*

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Staphylococcus aureus is an important pathogen and produce widespread infections. Increasing of antibiotic usage for *S. aureus* infections, created antibiotic resistance and subsequently to produce new antibiotics. Medicinal herbs with antimicrobial activity have been important role in traditional medicine. The purpose of this study was to determine the antibacterial activity of hydroalcoholic extract from root of *Curcuma amada* against *S. aureus* (25923 ATTC). The roots of *C. amada* were collected from India and its hydroalcoholic extract (1 mg: 5 ml) by maceration method was prepared and then its antibacterial activity against *S. aureus* was evaluated by disk diffusion and broth serial dilution methods (0.039, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5, 10, 20, 40) for determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration). The results from the antibacterial tests demonstrated that *C. amada* hydroalcoholic extract had been effected against *S. aureus*. The MIC and MBC of the extract against the *S. aureus* were 2.5 and 5 mg/ml, respectively. This study demonstrated that hydroalcoholic extract of *C. amada* have excellent antibacterial activities against *S. aureus* and are beneficial to human health. They have the potential to be used for medical purposes and to be utilized as antibacterial additives in making paper products. However, we need more investigation *in vitro* and *in vivo*.

PJ162 Chromatographic analysis of phenolic acids from *Iris* species. Comparison of different methods of extraction

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Phenolic acids have been reported to possess important biological and pharmacological properties, especially antioxidant activity, which have been widely described in the literature. The aim of this study was the quantification of phenolic acids in extracts obtained with different extraction techniques from several *Iris* species. A pressurized liquid extraction (PLE) was performed in an ASE 100 accelerated solvent extractor (Dionex, USA) at 100 °C using 80% and 100% methanol. To compare the effectiveness of PLE, the ultrasound-assisted extraction (UAE) procedure was also carried out, as well as traditional Soxhlet extraction (SX). To purify the PLE, UAE, SX extracts, the well-known solid phase extraction (SPE) technique was applied. Next, SPE eluates were qualitatively and quantitatively analyzed using an Agilent 1100 liquid chromatograph with UV-visible diode-array detector (DAD). As the result of the study, seven phenolic acids were identified by SPE-RP-HPLC: vanillic, caffeic, chlorogenic, protocatechuic, ferulic, p-coumaric and gallic acids. The calibration curves for all standards were linear ($R^2 > 0.999$, n = 3) in a concentration range of 0.01 – 2.00 mg 10 ml^{-1}. For most acids (vanillic, protocatechuic, p-coumaric, gallic, ferulic) isolated from the investigated *Iris* species, the highest yield was achieved by ASE at 100 °C repeated three times with 80% methanol as a solvent, while the worst results were obtained by ultrasound-assisted extraction (UAE). A relatively high yield of caffeic acid was also obtained with a Soxhlet apparatus (11.10 mg/100 g dry wt. in aerial parts of *Iris bungei* Maxim.). *Acknowledgments: This work was financially supported by grant no N N 405 374835 from The Polish Ministry of Science and Higher Education.*

PJ163 A novel traditional use of *Jovibarba heuffelii* leaves in Romanian ethno botanical veterinary practice in S-W Carpatians

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Romanian medicinal plant *Jovibarba heuffelii* (Schott) A&D Love (= JH) is a characteristic carpato-balcanic perennial monocarpic crassulacean; native to mounts of the N Greek peninsula, the Balkans and throughout

the Romanian S-E Carpatian Mounts; thriving in arid, rocky habitats [1,2,3]. They are an enjoyed food ingredient in some Romanian regions [3,4]. Traditionally planted on tile-roofs they are highly prized ornamental plants also in graveyards. We hereby report novel traditional uses of JH leaves in Romanian ethno-botanical veterinary practice discovered by us in S-W Carpatians, together with some biochemical and ecological considerations with regard to biotic and abiotic factors involved in its use. Data were gathered from locals with a semi-structured questionnaire about the occurrence, traditional knowledge and uses for the *Sempervivum s.l. spp* in Mehedinti Mounts, SW Romania, were complemented by a literature survey. In 1998, 1 informant from Gornenţi, com. Podeni, Mehedinti county, reported a novel and surprising traditional use of JH leaves in households in the region. Positive plant ID was done by indicating live specimens *in cult*. and *in situ*. Data obtained document a novel traditional use by Romanian locals from SW Mehedinţi, of JH leaves for chicken feeding with the aim to increase egg quality/production, and a probable vitaminizing effect. This use is not mentioned in any region or neighbouring countries, nor in other parts of Europe or Turkey where *Sempervivum* is widely used, not even for this closely related later genus. Secondly, it is worth mentioning the profound knowledge by the local peasants of the taxonomy, cultural, organoleptic and pharmaco-biological characteristics of *JH* and *S. marmoreum* -a very similar species co inhabiting the region, but accurately distinguished by them from JH. **References:** [1] Barca, V. and Niculae, M. (2005) Contrib Bot. Cluj. XL.:28 – 39. [2] Barca, V. and Niculae, M. (2006). Contrib Bot. Cluj:XLI: 223 – 233. [3] Ravarut, M. (1953) Flora RPR, Crassulaceae, Edit. Acad RPR, Bucharest.

PJ164 Essential oil constituents of leaves and fruits of *Myrtus communis* L. from Iran

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Abstract: *Myrtus communis* L. is an evergreen aromatic plant growing wild in Iran. Essential oil constituents of leaves were analyzed from two origin and two developmental stages [1]. Major oil components of two origins at flowering stage were α-pinene (3.8 – 23.0%), 1,8-cineole (9.9 – 20.3%), limonene (5.5 – 17.8%), linalool (12.3 – 17.6%) and α-terpinyl acetate (1.8 – 7.0%). Oil composition at fruit ripening stage was highly similar to those of flowering stage. Concentrations of major oil components were 1,8-cineole (24.0%), α-pinene (22.1%), limonene (17.6%), linalool (11.4%), linalyl acetate (4.5%), α-terpinyl acetate (2.2%), and geranyl acetate (1.2%). Major constituents of fruit oil were α-pinene (28.6%), 1,8-cineole (26.7%), limonene (18.0%), α-terpinyl acetate (5.4%), linalyl acetate (3.4%) and linalool (2.3%). **Reference:** [1] Adams, R.P. (1995) Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream: Allured.

PJ165 Role of SOD in the protection of *Rhizophora mangle* on gastric injury induced by ethanol, ischaemia-reperfusion and acetic acid in rats

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Gastric antiulcer property was found for *R. mangle* at very low doses. The butanolic fraction from its bark extract (BuOH-Fr) showed an antiulcer activity at 0.5 mg.Kg^{-1}, linked to an antioxidant property, as demonstrated in this study. In the present work we aimed to assess the activity of superoxide dismutase (SOD) in gastric injury induced by ethanol and ischaemia-reperfusion, and the expression of SOD in gastric ulcer induced by acetic acid. We found a significant increase on SOD levels in gastric mucosa pretreated with BuOH-Fr in both models of gastric injury, ethanol and ischaemia-reperfusion. The expression of SOD on the gastric tissue was demonstrated with the immunohistochemistry technique in the acetic acid method, which confirmed our results (data not shown). Table 1: SOD activity on ethanol and ischaemia-reperfusion induced gastric injury.

Method	Treatment	SOD activity (Units per mg of protein)
Ethanol	Saline 0.9%	2.243 ± 1.315
	Sham	28.82 ± 2.888***
	Lansoprazole 30 mg.Kg^{-1}	4.296 ± 3.006
	BuOH-Fr 0.5 mg.Kg^{-1}	21.87 ± 3.314***
Ischaemia-reperfusion	Saline 0.9%	10.61 ± 0.3043
	Sham	13.42 ± 1.987
	Lansoprazole 30 mg.Kg^{-1}	25.14 ± 2.363***
	BuOH-Fr 0.5 mg.Kg^{-1}	18.48 ± 1.882*

Results expressed by the mean ± standard deviation. ANOVA followed by Dunnett's t test, *P< 0.05 and ***P< 0.001.

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PJ166 Evaluation of anti-inflammatory and radical scavenging activity of an aqueous extract of *Barleria cristata* leaves

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Barleria cristata Linn (family Acanthaceae) has been used traditionally for the treatment of variety of diseases including anemia, toothache and inflammatory disorders [1]. Due to lack of sufficient scientific evidence indicating the utility of this plant in the treatment of inflammation, the present study was aimed at investigating the potential anti inflammatory and free radical scavenging activity of the plant in different experimental screening methods. Anti-inflammatory activity of BCW at dose of 125, 250 and 500 mg/kg was evaluated in acute inflammatory model, against carrageenan induced paw edema in rats and prostaglandins inhibitory activity in mice. Radical scavenging activity of BCW was evaluated by *in vitro* methods by DPPH (1,1-diphenyl-2-picryl-hydrazyl) (IC_{50}= 206.61 μg/ml) and NO (Nitric oxide) (IC_{50}= 289.01 μg/ml) radicals. Experiment was performed in triplicate to minimize the errors. When evaluated *in vivo* in the acute inflammation, BCW significantly inhibited edema induced by carrageenan in rats and showed significant prostaglandin inhibitory activity in mice. Ascorbic acid and Indomethacin (10 mg/kg) was used as a positive control. Results were analyzed by One-way ANOVA followed by Dunnett's test p< 0.05 and considered significant as compared to control. It is concluded that, methanol extract of *Barleria cristata Linn* leaves exhibited significant anti-inflammatory and radical scavenging activity. **Reference:** [1] Khare, C.P. (2009), Indian Medicinal Plants: An Illustrated Dictionary. 1st ed, Springer Verlag.

PJ167 Ameliorative effect of *Psoralea corylifolia* seeds against experimental myocardial oxidative stress-induced injury in rats

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The effect of methanolic extract of *Psoralea corylifolia* seeds (PCM) was assessed using isoproterenol-induced myocardial infarction model in rats. PCM was administered orally to Wistar rats (150 – 200 g) in two different doses, by gastric gavage (250 mg/kg, 500 mg/kg) for 21 days followed by subcutaneous administration of isoproterenol (85 mg/kg). Isoproterenol administration resulted in significant increase in lipid peroxides (MDA) levels in heart tissue as a result of oxidative stress. A significant decrease was observed in the activity of the myocardial marker enzymes viz. alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatine kinase (CK) with a concomitant increase in their activity in serum in isoproterenol treated rats. Isoproterenol administration also had a significant effect on lipid profile as evidenced by increased triglycerides, total cholesterol, LDL-cholesterol and VLDL-cholesterol levels with a significant decrease in HDL-cholesterol levels. These levels were significantly ameliorated by pretreatment with PCM. Activities of heart antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glutathione reductase (GR) and

reduced glutathione (GSH) were significantly lowered owing to myocardial infarction in isoproterenol treated rats. PCM pretreatment was found to ameliorate the effect of isoproterenol on lipid peroxide formation and retained the activity of marker enzymes. It also prevented the isoproterenol-induced decrease in antioxidant enzymes in heart and improved lipid profile. The results indicated that pretreatment with *Psoralea corylifolia* prevents the damage induced by isoproterenol in rat heart.

PJ168 Phytochemical and pharmacological studies on the leaves of *Couroupita guianensis* Aubl

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The present work aimed to give the detailed description of phytochemistry and pharmacological profile of isolated constituents from the leaves of *Couroupita guianensis* Aubl. Fractionation studies on the leaves of *C. guianensis* resulted in the isolation of three compounds. Petroleum-ether extract of the plant was chromatographed over silica gel G and the column was eluted with pet-ether (60 – 80 °C), benzene, chloroform and ethanol in succession. Eluted fractions were subjected to rechromatography. Pet-ether eluted fraction gave compound **1**, mp 79 – 81 °C. Physicochemical and spectral studies on compound **1** revealed it to be aliphatic hydrocarbon. The chromatographic column, on further elution with benzene gave two more compounds. Compound **2**, mp 95 – 97 °C was obtained in small yields and further work was not possible. Compound **3**, mp 273 – 275 °C, was isolated in crystalline form and identified as triterpene alcohol on the basis of physicochemical and spectral data. Lipid soluble compound **3** was evaluated for psychopharmacological activity in animal models. Antidepressant activity studies using tail-suspension test and despair swim test in mice, revealed its antidepressant potential. In conclusion, phytochemical and pharmacological studies on the leaves of *C. guianensis* resulted in the isolation of a novel constituent, ompound **3**, a triterpene alcohol, possessing antidepressant potential.

PJ169 Antibacterial activity of unifloral honeys against clinical isolates of methicillin-resistant *Staphylococcus aureus*

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Wounds infected with methicillin-resistant *Staphylococcus aureus* (MRSA) is getting more difficult and expensive to treat as drug resistance is widespread and the incidence of MRSA in the community increases [1]. Approval of medical grade Manuka and Medihoney impregnated dressings by European countries and Food and Drug administration of USA as therapeutic agents for the treatment of such infections [2] has led to the search for new honeys with high medicinal values which are locally produced and affordable. Therefore, this study had main aim to investigate the antibacterial activity of indigenous unifloral honeys (Black Seed, Beri and Shain honey) as well as Manuka honey (UMF-21). Minimum inhibitory concentration (MIC) of honeys against twenty five clinical isolates of MRSA and three ATCC reference strains, *Staphylococcus aureus* (25923), *Escherichia coli* (25922), *Pseudomonas aeruginosa* (27853) were determined by an agar incorporation assay. Phenol 6% was used as positive control. All MRSA strains were susceptible to Manuka honey at 4 – 5% (v/v) dilution, whereas Black Seed, Beri and Shain honey inhibited these isolates at 5 – 6%, 6 – 7% and 10 – 11% (v/v) dilution, respectively. The MIC range of Manuka honey against ATCC strains were in accordance with previous studies [3]. This study has shown that honey other than those approved can have comparable antibacterial activity and therefore, these newly identified honeys may add value to the existing honey resource. *Acknowledgements; We are extremely grateful to Microbiology Department of AFIP, Pakistan, for donating clinical isolates of MRSA and ATCC reference strains. We also wish to thank Professor Peter C Molan of Waikato University, New Zealand for kind donation of Manuka-21 honey sample. We are grateful to the University of Health Sciences, Lahore, for funding of this research project.* References: 1. James, F. et al. (2008) Int. J. Low. Extrem. Wounds. 7:28 – 31. 2. Visavadia, B.G. et al. (2008) Br. J. Oral Maxillofac. Surg. 46:696 – 697. 3. French, V.M. et al. (2005) J. Antimicrob. Chemother. 56:228 – 31.

PJ170 *In vitro* antimicrobial activity of propolis (bee glue) against pigmented anaerobic periodontal pathogens

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Periodontitis is one of the most common causes of tooth loss worldwide [1]. Recently, special attention has been paid to natural medication for its treatment [2]. For this purpose, propolis activity has also been investigated worldwide. Its antibacterial properties are mainly attributed to flavonones pinocembrin, flavonols galangin and to the caffeic acid phenethyl ester [3]. This study aimed at evaluating the antimicrobial effects of propolis from our region on 35 clinical isolates of pigmented anaerobic periodontal pathogens. Included were *Porphyromonas asaccharolytica* (n = 9), *Porphyromonas gingivalis* (n = 13), *Prevotella intermedia* (n = 9), *Prevotella melaninogenica* (n = 4). Minimum inhibitory concentration (MIC) to three antibiotics was obtained by Etest method. All strains were sensitive to amoxicillin plus clavulanic acid and metronidazole but 100% of *P asaccharolytica* and *P melaninogenica* strains displayed intermediate resistance to tetracycline while 69.2% *P. gingivalis* and 100% *P. intermedia* strains exhibited complete resistance to tetracycline. Screening for antibacterial activity of propolis extract was done by agar well diffusion assay and all strains were found sensitive to ethanolic extract of propolis. MIC was obtained by agar incorporation technique with values ranging from 0.064 to 0.512 mg/ml. It was also noticed that percentage yield of ethanolic extract of propolis prepared from ultrasonic extraction method was significantly higher compared to extract obtained with maceration. These results indicate that propolis from our region has potent antimicrobial activity against pigmented anaerobic periodontal pathogens. Taking into consideration the increasing resistance in anaerobic bacteria, this effective antimicrobial activity of propolis gives hope in the treatment of oral cavity diseases. *Acknowledgements: We are grateful to University of Health Sciences for providing finances for this research project and special thanks to Dr. Waseem Ahmed Gillani of National Agricultural Research Council, Islamabad, Pakistan and Dr. Nasreen Muzzafar of Punjab University Lahore, Pakistan for donating propolis.* References: 1. Loesche, W.J., Grossman, N.S. (2001). Clin. Microbiol. Rev. 14:727 – 752. 2. Gebara, E.C.E. et al. (2002) Braz. J. Microbiol. 33:365 – 369. 3. Koru, O. et al. (2007) Anaerobe 13:140 – 145.

PJ171 Anti-typhoid potential of *Punica granatum* (pomegranate) – An *in vitro* study

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Typhoid fever remains a significant clinical problem all over the world, especially the third world [1]. Emergence of antimicrobial resistance especially to the fluoroquinolones has led to difficulties in the management of typhoid fever [2]. Plants containing alkaloids, flavonoids and polyphenols are reported to exhibit several biological properties [3]. The pomegranate plant possesses an immense therapeutic value. Antimicrobial activity of pomegranate's constituents/extracts of different parts is well established. Antimicrobial activity of pomegranate peel has been tested against only one strain of *S. typhi.* Besides, no comparative study regarding the different parts of pomegranate against *S. typhi* has been done yet. The aim of this study was to evaluate the anti-bacterial potential of pomegranate against *Salmonella typhi*. Ethanolic extracts of pomegranate's different parts; peel, pericarp and fruit were screened for anti-bacterial activity against "Multi-Drug Resistance" (MDR) strain (UHS-14) by agar well diffusion method. 6% phenol was used as positive control. The peel extract had the largest inhibitory zone of 22.73 ± 0.26 mm against *Salmonella typhi* followed by pericarp (22.47 ± 0.36 mm) and fruit (15.55 ± 0.29 mm) extracts at neat concentration. The peel, pericarp and fruit extracts were further evaluated for minimum inhibitory concentration (MIC) against forty five clinical isolates of *Salmonella typhi.* The peel extract showed MIC of 22 mg/ml; followed by pericarp (MIC 24 mg/ml) and fruit extract (64 mg/ml). Etha-

nolic peel extract displayed highest antibacterial activity against *Salmonella typhi* in both agar diffusion and agar dilution assay as compared to pericarp and fruit extracts. This warrants further evaluation of peel extract in suitable typhoid animal model. *Acknowledgements: We are extremely grateful to University of Health Sciences for funding of this research project.* References.1. Wain, J.and Kidgel, l.C. (2004) Trans. R. Soc. Trop. Med. Hyg. 98:423 – 430. 2. Parry, C.M. et al. (2002) N. Engl. J. Med. 347:1770 – 1782. 3. Ajaikumar, K.B. et al. (2005) J. Ethnpharmacol. 96:171 – 176.

PJ172 Evaluation of anti-mycobacterial activity of garlic (*Allium sativum*) against clinical isolates of non-MDR and MDR *Mycobacterium tuberculosis*

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Tuberculosis (TB) continues to be the disease of public health problem [1]. Emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) TB throughout the developing world is very disturbing in the present scenario of TB management [2]. Therefore there is an urgent need to develop alternative anti-TB agents. Garlic (*Allium sativum*) has been shown to possess antibacterial, antifungal and antiviral properties [3]. We evaluated garlic for its antibacterial activity against non-MDR and MDR strains of TB. Ethanolic extract of garlic was prepared by maceration method and minimum inhibitory concentration (MIC) was performed by using 7H9 middle brook broth dilution technique to evaluate its anti-mycobacterial activity against non-MDR (n = 5) and MDR (n = 15) strains of MTB. All the test organisms were inhibited by the garlic at MIC range of 1.0 – 3.0 mg/ml. The results of present study support the use of garlic extract as supplement against TB along with conventional anti-TB drugs. Therefore, complementary and substitute medicines practices with plant extracts including garlic as a means of decreasing the burden of drug resistance and reducing the cost of management of diseases would be of public health importance. *Acknowledgments: We are grateful to University of Health Sciences for providing finances for this research project and special thanks to Dr. Barakat Hussain, Dr. Sidrah Saleem and Mr. Faisal Nadeem, University of Health Sciences, Lahore, Pakistan.* **References:** [1] WHO Report 2008. [cited 06 – 04 – 09]; Available from: URL: http://www.who.int/tb/publications/global_report/2008/en/index.html [2] Granich, R.M. et al. (2005) JAMA 293:2732 – 2739. [3] Iwalokun, B.A. et al. (2004) J. Med. Food. 7:327 – 333.

PJ173 Estrogenic and cytochrome p450 enzyme inhibitory effects of *Salvia officinalis* tincture and its subextracts

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Herbal medicinal products (HMPs) are widely used as an alternative to common hormone replacement therapy for the relief of menopausal symptoms [1]. It has been reported that menopausal women experienced a significant reduction of hot flushes during treatment with a HMP containing *Salvia officinalis* [2], but the mechanism underlying this effect has remained unknown. In order to obtain mechanistic insights into this biological effect, we investigated estrogenicity as a possible mode of action of a 66% ethanolic *S. officinalis* tincture as well three subextracts (*n*-hexane, $CHCl_3$ and aqueous-EtOH) obtained by solvent-solvent partition of this tincture. Estrogenicity was evaluated using a reporter gene construct in the human breast cancer cell line (T47D-Kbluc) with luminescence as the endpoint [3]. While the tincture showed no apparent effect, the aqueous-EtOH subextract exhibited estrogenic activity with a half maximal effective concentration (EC_{50}) value of 64 µg/ml. Additionally, the inhibitory potential of the *S. officinalis* tincture and the subextracts against the cytochrome p450 enzyme (CYP) isoform 3A4 was assessed in a fluorescence based microtiter plate assay [4]. The tincture, the *n*-hexane and the $CHCl_3$ subextracts were found to inhibit CYP3A4 moderately, whereas no CYP inhibition activity was detected with the estrogenic aqueous-EtOH subextract at concentrations under 70 µg/ml. Although estrogenicity could not be identified in the *S. officinalis* tincture, the activity found in the aqueous EtOH subextract may contribute to the overall effects of the tincture for amelioration of menopausal symptoms. *Acknowledgements: Funding from Bioforce AG Switzerland is gratefully acknowledged.* **References:** [1]Geller, S. et al. (2005) J. Womens Health 14:634 – 649. [2] De Leo, V. et al. (1998) Minerva Ginecol. 50:207 – 211. [3] Wilson, V. et al. (2004) Toxicol. Sci. 81:67 – 77. [4] Crespi, C. et al. (1997) Anal. Biochem. 248:188 – 190.

PJ174 Gene expression analysis of hypothalamic and hippocampal tissues of rats treated with St. John's wort extract or fluoxetine in a chronic restraint stress model

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Hypericum perforatum L., known as St. John's wort (SJW) is indicated as phytotherapeutic agent for the treatment of mild to moderate forms of depression. Data from literature support the hypothesis that chronic stress is a major risk factor for psychiatric illnesses. The aim of the present study was to evaluate the effect of SJW extract (STW3-VI; 250 and 500 mg/kg; p.o.) and fluoxetine (10 mg/kg, p.o.) on genes involved in the pathogenesis of depression using a chronic restraint stress (CRS) model in rats (1 h for 21 consecutive days). Hypothalamic and hippocampal tissues were analyzed using the Affymetrix gene chip Rat Genome 230 2.0 Array, which comprises more than 30,000 rat transcripts. Limma analysis and PANTHER database were used to evaluate the microarray data. Our first results show that chronic stress for 21 days differentially regulated 256 genes in the control group, whereas treatment with fluoxetine in stressed animals influenced 43 genes in the hippocampus. However, in stressed animals treated with 250 mg/kg of the SJW extract 140 genes were altered and 223 genes in the 500 mg/kg group. In all groups several pathways were identified which provide a link between the various hypotheses of depression. Gene expression profiles for hypothalamic tissues will provide additional information about brain circuits involved in depression.

PJ175 Anthocyanins and fatty acids from the flowers of *Lathyrus odoratus* L. and their antimicrobial activity

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Flowers of sweet pea, *Lathyrus odoratus* L. (Leguminoseae) have an attractive color and pleasant odor. Anthocyanins of different cultivars of sweet pea were belonging to delphinidin, cyanidin, and pelargonidin series. Anthocyanins were reported to possess antioxidant, antiulcer and anti-inflammatory activities. Total anthocyanins of sweet pea flowers of different color, pale red, dark red and dark pink were determined [1]. The total anthocyanins were 0.026, 0.131 and 0.338 (g/100 g) fresh weight, respectively. Four anthocyanins (cyanidin-3-glucoside, cyaniding-3,5-gluco-galactoside, malvidin-3,5-diglucoside and delphinidin-3,5-diglucoside) were isolated and identified from dark pink flowers. Pale red and dark red types contained similar anthocyanins (pelargonidin-3,5-gluco-xyloside and cyaniding-3,5-gluco-galactoside). GC/MS analysis of the ethanolic concrete extract of the sweet pea flowers revealed the presence of 30 components, 98% of the constituents were identified. The major components were palmitic acid (52.303%), linoleic acid (9.655%), methyl linolenate (6.683%), uncosanoic acid methyl esters (6.644%) and heptadecanoic acid (3.085%). Antimicrobial screening of total, isolated anthocyanins and the ethanolic concrete extract of sweet pea were conducted using disc diffusion assay against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Aspergillus niger,* and the human pathogenic yeast, *Candida albicans* [2]. The total anthocyanins of pale red type was the most potent one as anti-bacterial, anti-yeast, anti-fungal followed by the total anthocyanins of dark red type and dark pink type. The total anthocyanins of the three types were more potent than their isolated compounds as antibacterial. The ethanolic extract of the concrete showed the lowest effect as anti-bacterial and yeast but showed the highest effect as anti-fungal. In conclusion, anthocyanins of sweet pea flowers might be valuable as antimicrobial agent that can

be exploited for development of an alternative remedy for bacterial infection. **References:** [1] Fuleki, T. and Francis, F.J. (1968) J. Food Sci. 33:72 – 77. [2] Gould, J.C. (1952) Edinb. Med. J. 59:178 – 199.

PJ176 Inhibitory effect of panduratin A on the expression of matrix metalloproteinase-2 in *Porphyromonas gingivalis* supernatant-treated human gingival fibroblasts

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Porphyromonas gingivalis, a type of Gram-negative periodontobacteria, causes periodontal disease by activating intracellular signaling pathways that produce excessive inflammatory responses such as matrix metalloproteinases (MMPs). Panduratin A, a chalcone compound isolated from *Kaempferia pandurata* Roxb., has been found to possess anti-biofilm activity and MMP-9 inhibitor for treatment of periodontal disease [1,2]. In this study, we investigated the molecular mechanism by examining signaling pathways that are likely to be involved in the downstream effects of panduratin A on MMP-2 expression in *P. gingivalis* supernatant-stimulated human gingival fibroblast (HGF-1) cells by performing gelatin zymography, Western blotting, and reverse transcription-PCR. Our results demonstrated that exposure of HGF-1 cells to *P. gingivalis* supernatant significantly up-regulated MMP-2 expression. Specific MAPK inhibitors (U0126, SB203580, and SP600125) effectively blocked MMP-2 expression, indicating that MAPK signalings contributed to the induction of MMP-2 expression in HGf-1 cells in response to *P. gingivalis* supernatant. Panduratin A was found to partially attenuate the level of phosphorylated extracellular signal-related kinase (ERK) 1/2, p38, and *c-jun* N-terminal kinase (JNK). Panduratin A also inhibited CREB phosphorylation, a transcription factor element of MMP-2 promoter gene, in *P. gingivalis* supernatant-stimulated HGF-1 cells. These findings strongly suggest that a decrease of MMP-2 expression by panduratin A in HGF-1 cells in response to *P. gingivalis* supernatant can be mediated by the inhibition of MAPKs- and CREB-dependent signaling pathways. **References:** [1] Yanti, et al. (2009) J. Oral Sci. 51:87 – 95. [2] Yanti, et al. (2009) Biol. Pharm. Bull. 32:110 – 115.

PJ177 New polyketides from the endophytic fungus *Pestalotiopsis* sp. isolated from the Chinese mangrove plant *Rhizophora mucronata*

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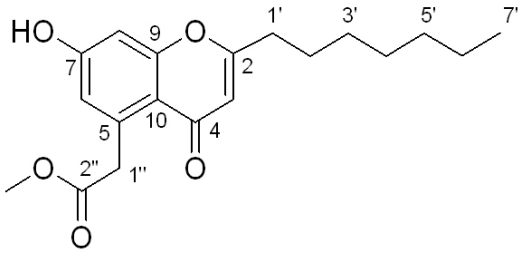
Fungi of the genus *Pestalotiopsis* are known as endophytes of tropical plants and as prolific producers of structurally unusal natural products. In continuation of our ongoing search for new bioactive compounds from fungal endophytes [1,2], we have isolated sixteen new polyketide derivatives as well as four known compounds from an unknown *Pestalotiopsis* strain obtained from leaves of the Mangrove plant *Rhizophora mucronata*. The new compounds include chromones (**1**), coumarins (**2**) and cytosporone derivatives (**3**). All compounds were unambiguously elucidated based on one and two dimensional NMR and mass spectrometry. Some of the isolated constituents displayed antibiotic or cytotoxic activities [3,4]. Our findings suggest that Mangrove derived endophytes are a promising source of new bioactive constituents.



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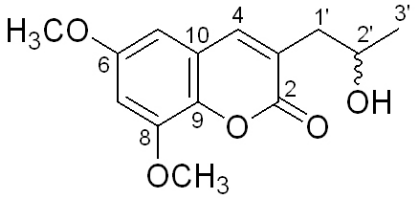

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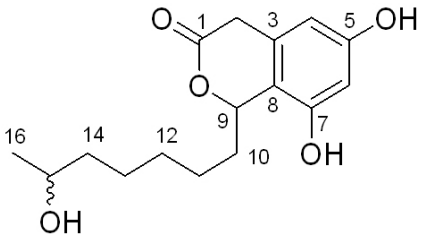

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References: [1] Aly, A.H. et al. (2008) J. Nat. Prod. 71:972 – 980. [2] Aly, A.H. et al. (2008) Phytochemistry 69:1716 – 1725. [3] Xu, J. et al. (2009) J. Nat. Prod. 72:662 – 665. [4] Xu, J. et al. (2009) Bioorg. Med. Chem. Submitted.

PJ178 Evaluation of antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata*

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Oxalis corniculata (family: Oxalidaceae) is used in folk medicine for the treatment of dysentery, fever, cardiopathy, hepatopathy, and various inflammatory aliments [1]. Free radicals have been implicated in a variety of conditions including inflammation, atherosclerosis, diabetes, ageing and hepatic toxicities [2]. Thus the aim of the present study was to investigate antioxidant and anti-inflammatory activity of methanol extract of whole plant *Oxalis corniculata* (MOX) using different techniques. *In vitro* antioxidant activity was studied by DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical [3], nitric oxide radical scavenging activity [4] and TBARS (Thiobarbituric acid Reactive Substances) methods [5]. Experiment was performed in triplicate to minimize the errors. *In vivo* anti-inflammatory activity was studied using carrageenan induced rat paw edema [6] and cotton pellet induced granuloma formation in rats [7] at the dose levels of 125, 250 and 500 mg/kg. Indomethacin (10 mg/kg) was used as a positive control in *in vivo* activity. Results were analyzed by One-way ANOVA followed by Dunnet's test, and considered statistically significant at $P < 0.05$ when compared with negative control. MOX showed significant antioxidant activity by scavenging DPPH (IC_{50}= 296.40 μg/ml) and NO radicals (IC_{50}= 200.45 μg/ml) and TBARS (IC_{50}= 165.43 μg/ml) method. Oral administration of MOX showed significant ($p < 0.01$) biphasic response by decrease in paw inflammation induced by carrageenan. MOX also showed significant percent inhibition viz. 35.12, 56.45, and 68.45 in granuloma formation at the doses of 125, 250 and 500 mg/kg respectively. It is concluded that, methanol extract of whole plant of *Oxalis corniculata* exhibited significant antioxidant and anti-inflammatory activity in acute and chronic phase of inflammation. **References:** [1] Basu, K. (1984) Indian Medicinal Plants 1:437. [2] Soni, K. et al. (2003) Indian J. Pharm. Sci. 65:27 – 30. [3] Govindarajan, R. et al. (2003) Indian J. Expt. Biol. 41:875 – 879. [4] Sumanont, Y. et al (2004) Biol. Pharm. Bull. 27:170 – 173. [6] Okhawa, H. et al (1979) Anal. Bio-

chem. 95:351 – 358. [7] Kale, M. et al. (2007)J. Ethnopharmacol. 112:300 – 304.

PJ179 Effect of summer savory (*Satureja hortensis* L.) density on essential oil yield to Persian clover (*Trifolium resupinatum* L.) intercropping

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In order to evaluate intercropping of summer savory (*Satureja hortensis* L.) and Persian clover (*Trifolium resupinatum* L.), an experiment was conducted in the Agricultural Research Station of Ferdowsi University of Mashhad in 2004 growing season. Treatments were: sole crop of Persian clover (eight rows), double-row intercropping of Persian clover and summer savory with 27 plant.m^{-2}, 40 plant.m^{-2} and 80 plant.m^{-2} (eight rows), sole crop of summer savory with 27 plant.m^{-2}, 40 plant.m^{-2} and 80 plant.m^{-2} (eight rows). For this purpose a complete randomized block design with 7 treatments and 4 replications was used. Effect of different treatments on essential oil percentage was not significant but on essential oil yield was significant. essential oil yield of summer savory in sole crop treatments were significantly higher than in intercrop ($P > 0.05$). Highest of essential oil yield was in sole crop of summer savory with 40 plant.m^{-2} and lowest was in intercropping of summer savory with 27 plant.m^{-2} and Persian clover. In intercropping treatments, this parameter increased by increasing plant density. The result of this increasing, plant dry weight was highest by increasing plant density. In sole crop of summer savory treatments, essential oil yield in 27 plant.m^{-2}, 40 plant.m^{-2} and 80 plant.m^{-2} density were 55, 59/7 and 58/2 kg/h. **References:** [1] Baher, Z.F. et al. (2002) Flavour Frag. J. 17:257 – 277. [2] Evans, P.M. et al. (2005) Aust. J. Exp. Agric. 42:135 – 141.

PJ180 Metabolite profiling of plant extracts by ultra-high pressure liquid chromatography at elevated temperature coupled to time-of-flight mass spectrometry

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Detailed metabolite profiling of crude plant extracts, mandatory for both quality control and metabolomics purposes, requires high-resolution separation and sensitive detection with a reasonable sample throughput. In this respect, the use of ultra-high pressure liquid chromatography working at high temperature and coupled to time-of-flight mass spectrometry (HT-UHPLC-TOF-MS) was evaluated in terms of achievable peak capacity for a given analysis time. In a first step, it was shown that the longest column does not compulsory provide the maximal peak capacity for a given analysis time in UHPLC, using representative natural products. From a theoretical point of view, a 150 mm column should be preferentially selected for gradient lengths up to 60 min at 30 °C, while longer columns are attractive only for higher analysis times. Compared to 30 °C, peak capacities were increased by about 20 – 30% for a constant gradient length at 90 °C and gradient time decreased by 2-fold for an identical peak capacity [1]. In a second step, profiling of natural crude sample, as example of complex mixtures, was evaluated. Extracts from the model plant *Arabidopsis thaliana* and from a *Ginkgo biloba* phytopreparation were analyzed. For metabolites spread over a large polarity range (e.g., methanolic extract of *Arabidopsis thaliana*) the use of high temperature (HT) was found beneficial with similar improvements as those recorded with the standard mixture. On the other hand, for the analysis of extracts containing more polar analytes (e.g., *Ginkgo biloba*), HT was found detrimental and causes a decrease in retention and thus resolving power [2]. Stability under HT conditions was evaluated and no apparent degradation was evidenced for both standard mixtures and crude extract analyses [2]. HT represents thus an additional parameter that can be considered for improving high-resolution profiling of extracts with metabolites spread over a large polarity range. **References:** [1] Guillarme, D. et al. (2009)J. Chromatogr. A 1216:3232 – 3243. [2] Grata, E. et al. (2009)J. Chromatogr. A, submitted.

PJ181 Distribution of Huperzine A in Malaysia Lycopodiaceae

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Alzheimer's disease is a neurodegenerative disorder affecting the elderly population throughout the world. Huperzine A found in the genera of Lycopodiaceae is a potent, reversible and selective acetylcholinesterase inhibitor and is a promising drug for treatment of symptoms of Alzheimer's disease. The content of Huperzine A in the *Lycopodium* species collected from the subtropics in Malaysia was evaluated. The dried *Lycopodium* species were dried, pulverized and macerated in 70% aqueous methanol. After five cycles of extraction, the pooled methanol extract was dried under reduced pressure with a rotary evaporator. The dried methanol extract was dissolved in methanol before subjecting for further analysis. A high performance liquid chromatography system with a diode array detector connected to a reverse phase column was developed to evaluate the content of huperzine A in the *Lycopodium* species. The separation was achieved with a gradient mobile phase system with an increasing amount (20 – 70%) of methanol in 0.01% trifluoroacetic acid for 30 minutes monitored from 200 to 500 nm. The peak area was linear ($r^2 = 0.998$) from 5 to 100 μg/mL and has a repeatability between 0.4 to 1.4% relative standard deviation within five replicate chromatograms. The evaluated *Lycopodium* species collected in Malaysia contained Huperzine A ranging from 0.00 to 0.04% of the crude plant.

PJ182 Benzophenone derivatives from the fruits of *Garcinia multiflora* and their anti-inflammatory activity

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Garcinia multiflora (Guttiferae) Champ. is a small evergreen tree, distributed in South China, Hong Kong, and Taiwan. Xanthones [1-4], biflavonoids [5], benzophenones [6], and their derivatives are widely distributed in plants of the genus *Garcinia*. Many of these compounds exhibit cytotoxic [1,2,6], anti-inflammatory [3], antitubercular [4], anti-HIV [5], and antioxidant [6] activities. In our studies on the anti-inflammatory constituents of Formosan plants, many species have been screened for *in vitro* anti-inflammatory activity, and *G. multiflora* has been found to be one of the active species. Five new benzophenone derivatives, 13,14-didehydoxyisogarcinol (**1**), garcimultiflorone A (**2**), garcimultiflorone B (**3**), 13-hydroxygarcimultiflorone B (**4**), and garcimultiflorone C (**5**), and seven known compounds (**6-12**) have been isolated and identified from the fruits of *G. multiflora*. 13,14-Didehydoxyisogarcinol (**1**), garcimultiflorone A (**2**), garcimultiflorone B (**3**), and 13-hydroxygarcimultiflorone B (**4**) exhibited inhibition with an IC_{50} range of 0.11-5.58 μM on superoxide anion generation and elastase release by human neutrophils in response to fMet-Leu-Phe/cytochalasin B (fMLP/CB).This symposium describes the structural elucidation of **1-5** and the anti-inflammatory activities of the isolates. *Acknowledgements: This work was supported by grant from the National Science Council of the Republic of China.* **References:** [1] Asano, J. et al. (1996) Phytochemistry 41:815 – 820. [2] Chen, J. J. et al. (2004) Planta Med. 70:1195 – 1200. [3] Chen, L.G. et al. (2008) Food Chem. Toxic. 46:688 – 693. [4] Chen, J. J. et al. (2006) Planta Med. 72:473 – 477. [5] Lin, Y. M. et al. (1997)J. Nat. Prod. 60:884 – 888. [6] Baggett, S. et al. (2005)J. Nat. Prod. 68:354 – 360.

PJ183 *seco*-Abietane diterpenoids, a phenylethanoid derivative, and antitubercular constituents from *Callicarpa pilosissima*

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Callicarpa pilosissima (Verbenaceae) is an endemic evergreen shrub that grows in low- to medium-altitude forests throughout Taiwan. Diterpenoids [1], lignanoids [2], flavones [3], and their derivatives are widely distributed in plants of the genus *Callicarpa.* Many of these compounds exhibit cytotoxic [1], and fish-killing [3] activities. In our studies on the antitubercular constituents of Formosan plants, many species have been screened for *in vitro* antitubercular activity, and *C. pilosissima* has been found to be an active species. Investigation of the EtOAc-soluble fraction of the leaves and twigs of *C. pilosissima* has led to the isolation of six new compounds, including five *seco*-abietane diterpenoids, 12-deoxy-*seco*-hinokiol methyl ester (**1**), 12-deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**), callicarpic acid A (**3**), 9α-hydroxycallicarpic acid A (**4**), and callicarpic acid B (**5**), and a phenylethanoid derivative, 4-hydroxyphenethyl tetradecanoate (**6**), along with 14 known compounds (**7**-**20**). 12-Deoxy-11,12-dihydro-*seco*-hinokiol methyl ester (**2**), callicarpic acid B (**5**), and α-tocopherol trimer B (**15**) exhibit antitubercular activities (MICs ≤ 63.6 μM) against *Mycobacterium tuberculosis* H_{37}Rv *in vitro*. This work describes the structural elucidation of **1**-**6** and the antitubercular activities of the isolates. *Acknowledgements: This work was supported by grant from the National Science Council of the Republic of China.* **References:** [1] Jones, W.P. et al. (2007) J. Nat. Prod. 70:372 – 377. [2] Shao, Y. et al. (2006) Chim. Acta 89:64 – 72. [3] Nagai, M. et al. (1973) Yakugaku Zasshi 93:1087 – 1088.

PJ184 LC/PDA/ESI-MS/MS Chemical profiling, radical scavenging and antibacterial activities of two *Abutilon* spp. native to Sudan

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The present communication represents the first attempt to subject *Abutilon pannosum* Forst. f. Schlecht. and *A. figarianum* Webb (Malvaceae) to bioactivity-directed fractionation in order to detect their bioactivities and the profile of the secondary metabolites associated with these activities. Dried flowers were extracted separately by petroleum ether, chloroform and 80% methanol. The extracts were tested against two gram positive and three gram negative human pathogenic bacteria. The chloroform extracts of *A. figarianum* and *A. pannosum* exhibited inhibitory activity against *Bacillus subtillus* of 18 mm and 16 mm, at a concentration of 1 mg/ml, respectively. Meanwhile, higher concentration (5 mg/ml) of the methanolic extract of both *A. figarianum* and *A. pannosum* exhibited significant inhibitory activity against *Bacillus subtillus* (80%, 76%), *Escherichia coli* (52%, 60%), *Pseudomonas aeruginosa* (70%, 80%), *Staphylococcus aureus* (70%, 60%) and *Proteus vulgaris* (80%, 70%), respectively. The aqueous residue of the methanolic extract was further partitioned with ethyl acetate and *n*-butanol. The ethyl acetate fractions of both plants possessed a prominent antioxidant activity (80% inhibition) at a concentration of 1 mg/ml using DPPH (1,1-diphenyl-2-picrylhydrazyle) as a free radical source. The chemical structures of the compounds present in the bioactive extracts and their respective fractions were elucidated by tandem mass spectrometry (HPLC-MS/MS-ESI) leading to the identification of 18 compounds: 13 flavonols, 3 flavones, 1 flavanons and 2 anthocyanins derivatives. A number of reports of the antioxidant and antimicrobial activities of other *Abutilon* species have already been published [1,2]. **References:** [1] Porchezhian, E., and Ansari, S.H. (2005) Phytomedicine 12:62 – 64. [2] Matlawska, I. and Sikorska, M. (2005) Acta Pol. Pharm. Drug Res. 62:135 – 139.

PJ185 Anti-inflammatory study and phytochemical analysis of *Hyphaene thebaica* (Doum plant)

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The anti-inflammatory study (*in vivo*) was carried out on the different extracts (200 g/kg) of Doum plant parts in comparison with Asprin (100 g/kg). High activity was found in the chloroform and ethanol extracts of the seeds (27%, 22%), roots (24%, 20%), and leaves (25%, 20%), respectively. The chloroform extract of the seeds was the most active (27%). The anti-inflammatory study (*in vitro*) of the seed extracts was monitored using atropinized rat fundus strip by induced inflammations with kidney homogenate. The kidney homogenate (0.5 ml) stimulate rat fundus strip contraction up to 20 mm for 3 min. Pre-incubation of the kidney homogenate in indomethacin (as standard, 5 μg/ml) completely (100%) inhibited their stimulant effect. Whereas pre-incubation of the kidney homogenate in Doum chloroform extract of the seeds (5 ng/ml) markedly inhibited the stimulate effect by 60%. Pharmacological studies (*in vitro*) of Doum seeds extracts on the isolated smooth muscles showed that chloroform extract of the seeds inhibit the spontaneously contracting rabbit jejunum by 40% for 0.8 min at a concentration of 5 μg/ml. However the seeds cold aqueous extract increase the contraction of rabbit jejunum by 110% for 1 min. Meanwhile, the hot aqueous extract stimulates the same tissue by 150% for 2 min. In comparison the acetylcholine (50 ng/ml) showed 120% increase. The chloroform extract of the seeds (100 ng/ml) possessed no effect on the aortic strip whereas the aqueous extract (cold & hot) stimulates the same tissue by 3 mm (1 min) in comparison to adrenaline (5 ng/ml) (8 mm stimulant for 3 min). Chromatographic profiling of the chloroform extract of the seeds using preparative thin layer chromatography (TLC) lead to separation of three fractions. Testing the anti-inflammatory activity (*in vivo*) of these fractions lead to the selection and separation of the most active fraction. Spectroscopical (NMR, Mass, UV, IR) data were recorded for the isolated major compounds which were found to be triterpenoids in nature.

PJ186 Amides and Benzenoids from *Zanthoxylum ailanthoides* with Inhibitory Activity on Superoxide Generation and Elastase Release by Neutrophils

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Zanthoxylum ailanthoides is a medium to large-sized tree found at low altitude in forests of China, Korea, Japan, the Philippines, and Taiwan [1]. Its leaves are used as a folk medicine to treat the common cold in Taiwan [2]. Benzo[*c*]-phenanthridines, quinolines, coumarins, flavonoids, lignans, and terpenoids have been identified as constituents of this plant. Antiplatelet aggregation [3] and anti-HIV [4] activities have been reported for some of these compounds. In our studies on constituents of Formosan plants for *in vitro* inhibitory activity on neutrophil proinflammatory responses, *Z. ailanthoides* was found to be an active species. Five new compounds, ailanthamide (**1**), *N*-(4-methoxy-phenethyl)-*N*-methylbenzamide (**2**), (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide (**3**), 4-(4′-hydroxy-3′-methylbut-oxy)benzaldehyde (**4**), and (*E*)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (**5**), and 17 known compounds have been isolated and identified from the stem bark of *Z. ailanthoides*. The structures were determined through spectroscopic and MS analyses. Compounds **1**, **3**, xanthyletin, decarine, (+)-episesamin, (-)-hinokinin, and evofolin-B exhibited inhibition (IC_{50} ≤ 5.34 μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds **1**, xanthyletin, decarine, and (+)-episesamin also inhibited fMLP/CB-induced elastase release with IC_{50} values ≤ 5.53 μg/mL. *Acknowledgements: This work was supported by a grant from the National Science Council of the Republic of China.* **References:** [1] Chang, C.E. and Hartley, T.G. (1993) Rutaceae in Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol. 3. [2] Gan, W. S. (1970) Manual of Medicinal Plants in Taiwan, National Research Institute of Chinese Medicine, Taiwan, Vol. II. [3] Sheen, W. S. et al. (1994) Phy-

tochemistry 36:213 – 215. [4] Cheng, M. J. et al. (2005) Bioorg. Med. Chem 13:5915 – 5920.

PJ187 Analgesic activity of fractions of *Stereospermum kunthianum* stem bark

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Stereospermum kunthianum (Bignoniaceae) is a woody shrub indigenous to Africa and Asia where the plant parts are used in traditional human medicine for its analgesic properties [1]. We have recently reported the analgesic activity of its aqueous stem bark extract [2]. Vacuum liquid chromatography (VLC) technique [3] produced 3 fractions: A, B, and C while further column chromatography (CC) analysis [3] of the VLC fractions yielded fractions L, S and Y, respectively. The fractions were evaluated for possible analgesic activity using the acetic acid [4] and formalin pain [5] tests. Fractions A, B and C (100, 200, and 400 mg/kg) significantly ($p < 0.0001$) inhibited abdominal writhes in mice. While fractions L and Y (100 – 400 mg/kg) inhibited both phases of the formalin-induced pain in mice, with a more pronounced effect on the late-phase than the early-phase, fraction S at the same doses inhibited both phases but with a more marked effect on the early phase. The results indicate that the VLC and CC fractions of *Stereospermum kunthianum* may inhibit pain responses mediated via both central and peripheral mechanisms. The present study has demonstrated and confirmed that *Stereospermum kunthianum* stem bark contains pharmacologically active constituents which possess analgesic activity justifying its popular use in treating painful conditions. **References:** [1] Gill, L. S. (1992) Ethnomedicinal uses of plants in Nigeria. University of Benin press, Benin, Nigeria. [2] Ching, F.P. et al. (2009) Acta Poloniae Pharmaceutica-Drug Research 66:83 – 88. [3] Braithwaite, A., Smith, F.J. (1996) Chromatographic Methods, Blackie academic and Professiponal, Amazon, Glasgow, 5th Ed. 4. Koster, R. et al. (1959) Fed. Proc. 18:412. [5] Dubuisson, D. and Dennis, S.G. (1977) Pain 4:161 – 174.

PJ188 Cytotoxic effects of hydroalcoholic extracts of *Cucurbita pepo* and *Solanum nigrum* compared with hydroalcoholic extract of *Taxus baccata* and cisplatin on normal and cancer cell lines

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In recent years, there has been a global trend toward the use of natural substances present in fruits, vegetables, oilseeds, and herbs as antioxidants and functional foods. Also, isolation and identification of some potent anti-tumor compounds from medicinal plants, has motivated researchers to screen different parts of plant species for determination of anti-tumor effects. *Zataria multiflora* (vernacular name of Avishan Shirazi) has traditionally used as antiseptic, anesthetic and antispasmodic drug. On the other hand, this plant is extensively used as flavor in Iranian food. The main constituents of its essential oil are phenolic compounds, such as carvacrol and thymol. Also, inhibitory effects of essential oil of *Z. multiflora* on *Salmonella typhimurium* and *Staphylococcus aureus* were reported. In this study cytotoxic effects and IC_{50} of specific concentrations of hydroalcoholic extract leaves of *Zataria multiflora* were compared with hydroalcoholic extract of bark of *Taxus baccata* and cisplatin, as well known anticancer compounds on normal cell lines (CHO and mice fibroblast) and cancer cell lines (HepG2 and SKOV3). Hydroalcoholic extracts of the plant were prepared by perculation. The cytotoxic effects and IC_{50} of the extract on the cell lines were studied followed by colonogenic assay after 72 hours incubation. The results showed that IC_{50} of *Zataria multiflora* extract was significantly higher than the extract of *Taxus baccata* and cisplatin on all 4 normal and cancer cell lines ($P < 0.05$). As a result, it is concluded that the extract of *Z. multiflora* has almost similar cytotoxicity with the extract of *Taxus baccata* on cancer cells.

PJ189 Comparison of artemisinin levels in *Artemisia annua* L. cultivated at three distinct geographic regions in Rwanda

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Artemisia annua L. (Asteraceae) is listed in the Chinese pharmacopoeia as a remedy for various fevers including that caused by malaria. The plant contains the well-established antimalarial compound artemisinin [1]. A hybrid (*Artemis*) of *A. annua* was successfully cultivated in Rwanda, one of the malaria-endemic regions. We performed HPTLC-densitometric analysis of aerial parts of *A. annua* grown at distinct geographic regions under different climatic conditions (Mukoni, Rwabuye and Ruhengeri). Of special interest was the influence of soil and altitude on the production of artemisinin. The artemisinin concentration estimated in n-hexane extracts was 0.46 – 1.17% per dry weight of aerial plant material. The plants grown at higher altitude between 1800 m and 2000 m in the southern province, on sandy soil (Mukoni) or marshy soil (Rwabuye) were richer in artemisinin (1.17% or 1.11%, respectively) than plants cultivated at altitude between 1500 m and 1650 m in the western province on volcanic soil (0.46%). These results suggest an influence of the factors altitude and soil on the artemisinin content in *Artemisia annua* hybrids. *Acknowledgement: The Rwandese Government and the Institute of Scientific and Technological Research are gratefully acknowledged for the grant to Marie Jeanne MUKAZAYIRE.* **Reference:** [1] Mueller, M.S. et al. (2000) J. Ethnopharmacol. 73:487 – 493.

PJ190 Chemical constituents of *Angelica lucida* fruits

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The genus *Angelica* is well documented for the presence of coumarins, which are known for a broad spectrum of pharmacological properties. In the framework of our research concerning chemical composition of different Umbelliferae plants, we report in this study the chemical composition of the fruits of *Angelica lucida* L. Tender parts of the plant have been used as food, analgesic and tonic against common colds by Eskimos. The roots as well as the young stems of the plant have been taken as a preventative medicine [1]. In this study, from the petroleum ether and the methanolic extract of the fruits of the plant, five known coumarins have been isolated (imperatorin, isoimperatorin, heraclenol, heraclenin and oxypeucedanin hydrate). Their structure elucidation was performed by modern spectral means (1D- and 2D-NMR) and literature data [2]. These compounds have been also isolated from other species of the genus *Angelica* [2, 3] but for the first time from *A. lucida*. Biological activities (antimicrobial and cytototoxic activities) of all isolated compounds are also under investigation. **References:** [1] Lawrence, B.M. and Morton, J.K. (1974) Phytochemistry 13:528. [2] Harkar, S. et al. (1984) Phytochemistry 23:419 – 426. [3] Bergendorff, O. et al. (1997) Phytochemistry 44:1121 – 1124.

PJ191 Comparative effects of fructose, glucose and sucrose on growth and atropine production in *Datura metel* callus culture

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Datura metel is regarded as an important source of atropine. Evaluating of variable factors in callus growth and atropine production in cultured cells of *Datura metel* is considered a very interesting research subject. It is known that different sugar and concentration can affect metabolite production in plant cell culture. The aim of this research is to examine the influence of sucrose, glucose and fructose on growth and atropine production in *Datura metel* callus culture. Callus culture of *Datura metel* were estabilished by transferring seedlings on solidified Murashig & Skoog medium containing 2,4-dichlorophenoxyacetic acid and kinetin as plant regulators. Calluses were subcultured to medium supplemented with 1.5%, 3% and 6% of sucrose, glucose and fructose separately. After 28 days callus were collected and their fresh and dry weight were recorded. Atropine was extracted from dry calli and analysed using HPLC. Acetonitrile, methanol and three ethylamine (4:18:78) were used as mobile phase. The results showed remarkable differences in atropine production and callus growth using different sugar treatments. The maximum product of atropine was seen in medium culture containing 3% of sucrose or glucose. The maximum of fresh weight was observed in medium culture containing 1.5% and 3% of sucrose. It seems that atropine production in *Datura metel* callus culture is influenced by the type and concentration of the sugars. The results can provide the information on optimal carbohydrate sources for atropine production in *Datura metel* callus culture.

PJ192 LC/PDA/ESI-MS/MS Metabolites profiling, radical scavenging and antimicrobial activities of *Combretum hartmannianum* Schweinf

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Decoctions of the bark and leaves *of Combretum hartmannianum* are commonly used in Sudanese traditional medicine against jaundice, external skin infections, malaria and similar febrile diseases [1,2]. Air dried ground bark and leaves of *C. hartmannianum* were extracted sequentially using petroleum ether, chloroform and finally the marc was extracted using 80% methanol. *In vitro* screening of the antioxidant activity of 12.5 µg/ml ethyl acetate extract of both the bark and leaves using 1,1-diphenyl-2-picrylhydrazyle (DPPH) as free radical source revealed significant inhibitory concentration of DPPH radicals of 96% and 92%, respectively while the 12.5 µg/ml concentration chloroform and acetone extracts exhibited 67% and 59% inhibition, respectively. The aforementioned extracts of *C. hartmannianum* were tested against two Gram positive and three Gram negative bacteria as well as two fungi. Most active among the leaves extracts was the ethyl acetate phase at both concentrations (1 mg, 5 mg) against *Staphylococcus aureus* (70%, 70%) and *Escherichia coli* (70%, 70%). Furthermore, this fraction at a concentration of 5 mg possessed activity against *Proteus vulgaris (64%), Pseudomonas aeruginosa* (70%), *Aspergillus niger* (70%) and *Candida albicans* (70%). The leaves chloroform extract (1 mg, 5 mg) possessed activity against *Bacillus subtilis* (86%, 80%) and *S. aureus* (86%, 80%). Barks ethyl acetate extract was the only extract possessing antimicrobial activity. Most sensitive to it was *S. aureus* at both concentrations tested (1 mg, 5 mg) being inhibited by 80% and 70% respectively. RP-HPLC-DAD coupled with tandem mass spectrometry performed on the ethyl acetate fraction of the leaves of *C. hartmannianum* led to the identification of 16 flavonoids and a phenantherene which were believed to be responsible of the activities mentioned above. **References:** [1] Ali, H. et al. (2002) J. Ethnopharmacol. 83:219 – 228. [2] El Ghazali, et al. (1994) Medicinal plants of the Sudan. Part III, Medicinal plants of the White Nile province. Khartoum University Press, Sudan.

PJ193 Susceptibility of oral pathogenic microorganisms to Brazilian medicinal plants extracts

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The plants have been used in the popular medicine for treatment of diverse diseases. However, only recently, its pharmacology, toxicicity and effectiveness against oral microorganisms have been scientifically studied. The objective of this study was to verify *Candida albicans, Streptococcus mutans, Staphylococcus aureus* and *Agregatibacter actinomycetemcomitans* susceptibility to the *Lithraea molleoides* Marchand (aroeira) (Air), *Stryphnodendron barbatiman* Mart. (barbatimão) (Bb), dejanira (Dj), *Lafoensia pacari* (pacari) (PC), *Croton campestris* A. St. Hil.(velame) (VL), *Anacardium humile* A. St. Hil. (cajuzinho do campo) to Brazilian native plants extracts. Records of antibiogram had been absorbed with 20 µL of each extract and distributed on the agar surfaces previously sown with 1.5×10^8 UFC/mL and cultured at 37ô¤C during 24 – 48 hours, obeying the norms of the CLSI. After that, the inhibition zones had been measured and the averages and shunting lines standards had been priced. Records contend nystatin and vancomycin served as positive inhibition controls for *C. albicans* and bacteria, respectively. The statistical analysis Kruskal-Wallis tests had been evaluated. The results demonstrated that all the tested extracts had inhibited the *in vitro* growth of the microorganisms. The continuity of these studies, through clinical assays in patients, will be important to confirm the antimicrobial effectiveness of extracts *Acknowledgements (italic): FAPEMIG – Fundação de Apoio a Pesquisa do Estade de Minas Gerais; CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico; CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*).

PJ194 Antimicrobial activity of bioactive glass associated to Brazilian red and green propolis

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Bioactive glasses are a group of surface reactive glass-ceramics and are able to induce the formation of mineralized tissue in vivo, maxillofacial and periodontal repair. In this work we are studied the 58S glass type produced in the UFMG Biomaterials Laboratory using the sol-gel process comprising SiO_2 (46.1 mol%), P_2O_5 (4 mol%) and CaO (26.9 mol%) associated with green propolis originated by *Baccharis dracucunlifolia* (GP), red propolis originated by *Dalbergia ecastophylum* (RP) and tetracyclin (TC) and shaped in 6.0 mm diameter discs. The antimicrobial susceptibility test for the different discs was conducted according to CLSI (2007) guidelines. 1.0×10^8 CFU/mL of *Enterococcus faecalis, Streptococcus mutans, Staphylococcus aureus* were plated on Mueller-Hinton agar. The discs were placed on the agar surface and incubated at 37ô¤C during 48 h. TC standardized discs 30 mg, Blanc stelilized discs content 20 µL of GP and RP ethanolic extract, bioglass without propolis and TC were used as controls. After incubation, the inhibition zones were measured and reported as mean ± standard deviation. Kruskal-Wallis test: $p < 0.5$ was considered significant. All tests were made in triplicate. The results show that RP was more efficient and equal against three microorganisms (22.5 ± 0.0 mm) whereas GP showed 15 mm (*S. aureus*), 12 mm (*E. faecalis*), 19 mm (*S. mutans*). The GP and RP extracts had shown similar effectiveness. The bioglass associate with Red Propolis demonstrated to greater antimicrobial activity that observed for tetracyclin and other controls. *Acknowledgments: FAPEMIG – Fundação de Apoio a Pesquisa do Estado de Minas Gerais; CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico; CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Pharm. Dra. Sheila Rago Lemos Abreu (Pharmanéctar- Belo Horizonte- Brazil); Coordenação dos Cursos de Pós-Graduação da Faculdade de Odontologia da UFMG.*

PJ195 Periodontal pockets control with Brazilian green propolis mucoadherent gel

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Propolis has been shown to exibit *in vitro* antimicrobial activity against periodontal pathogens. The aim of this study was to evaluate the efficacy of Brazilian Green Propolis Mucoadherent Gel (BGPMG) for the treatment of patients diagnosed with gingivitis and Chronic Periodontitis (CP). Six patients, 2 males (36/42 years old) and 4 females (42, 46, 49,51 years old) with dental calculus, gingivitis, oedema, bleeding, gingival recession, pocket depths, attachment loss, suppuration, tooth mobility and alveolar bone loss were submitted at BGPG 10% treatment. Dental archs were divided in the following quadrants. Superior Right (SR) – BGPG irrigation; Superior Left (SL) – scraping/smoothing dental root (RAR) and BGP irrigation inside the periodontal pocket; Inferior Right (IR) – RAR; Inferior Left (IL)-control. Dental brushing with BGPG and washing mouth with propolis solution daily was carried through during the treatment. BGPMG was applied in each periodontal pocket once a week, during 4 weeks, having used barren dismissable syringe. The results shown a regression of 95% gingivitis and suppuration in all the teeth irrigated with BGPMG, as well as a pocket depths reduction in all unsubmitted and submitted teeth previously to the RAR. It was not observed alveolar bone reorganization. Increase of gingival contraction and dental mobility reduction was noted. In this clinical study, the patient treated with the BGPMG showed periodontitis/gingivitis regression. The results suggest that 10% BGPG could be used as an adjuvant therapeutic method assigned for the treatment of CP. Other studies need to be conducted with more significant number of patients in order to stablish this treatment as an alternative approach for peridontal diseases conditions. *Acknowledgments: FAPEMIG, CNPq, CAPES, Coordenação dos Cursos de Pós-Graduação da Faculdade de Odontologia da UFMG.*

PJ196 Mechanisms underlying the anti-inflammatory actions of shikonin, a natural naphtoquinone, in experimental murine ulcerative colitis

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Shikonin (5,8-dihidroxy-2-[(1*R*)-1-hidroxy-4-methyl-3-pentenyl]-1,4-naphthoquinone) is a natural naphtoquinone commonly found in *Lithospermum erythrorhyzon* Sieb. et Zucc roots (Boraginaceae). *In vivo*, shikonin significantly reduced the extent and severity of the injury of colon as shown by scores of macroscopic damage and prevented body weight loss observed in dextran sulphate sodium (DSS)-treated mice. *In vitro*, shikonin at 1 μM inhibited the NF-κB activation induced by lipopolysaccharide (LPS) in macrophages RAW 264.7 [1]. In this study, we investigated if the shikonin-mediated inhibition of induced ulcerative colitis is due to the suppression of COX-2 expression and whether shikonin has an inhibitory effect on nuclear factor (NF)-κB activation. Acute colitis was induced in mice by administration of DSS (5% DSS in water, for 7 days). Shikonin (25 and 50 mg/kg) was administred orally just before DSS administration and four days later. The animals were killed on day 8, the colon removed and the distal parts were submitted for evaluation of colonic myeloperoxidase (MPO) activity, and other colonic specimens for extraction of cytosolic and nuclear proteins. MPO activity was determined spectrophotometrically at 620 nm [2]. COX-2 expression and NF-κB nuclear translocation were analyzed by Western blot assay MPO activity, COX-2 levels, NF-κB p65 nuclear translocation were significantly increased in DSS-induced colitis tissues. Shikonin at 50 mg/kg, markedly inhibited these inflammatory parameters. This naphtoquinone could be a new therapeutic and/or preventive agent for gastrointestinal diseases. *Acknowledgements: This study was supported by grants from the Spanish government (SAF2006 – 06726) and from the Generalitat Valenciana (GVPRE/2008/387).* **References:** [1] Andújar, I. et al. (2008) Meth. Find. Exp. Clin. Pharmacol 29:86. [2] Giner, R.M. et al. (2000) Eur. J. Pharmacol. 389:243 – 252.

PJ197 Comparative botanical – pharmaceutical and pharmacognosy research of species of genus *Potentilla* L. (*P. anserina* L., *P. reptans* L. and *P. palustris* L.) from Bosnia and Herzegovina (Western Balkan)

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Many species of order *Potentilla* L. at the area of Dinarides (W. Balkans) have distinguished ethno botanical role [1]. Considering their phylogenetic affinity with currently known medicine and edible plants in this genus, we could expect similar biological – chemical characteristics and similar use. In this research we conducted basic comparative research of microscope – botanical identification, concentration of basic metabolites, dominant secondary metabolite and anti-microbic activity of three species of genus *Potentilla*. Material was gathered on natural biotopes in Sarajevo surrounding, while rare specie *P. palustris* was gathered on Kupres field in 2007. Microscopic analysis in accordance with Ph Eur IV determined value of stomata index (25 for *P. reptans* and *P. anserina* and 15 for *P. palustris*). In acetone dissolution of leafs, using spectrophotometry, determined was different concentration of plant pigments. Chlorophyll a was between 0.58 mg/l (*P. reptans*) and 9.745 mg/l (*P. palustris*). Chlorophyll b is contained in amount of 24.140 mg/l in leafs of *P. palustris*, which indicates distinguished anti-oxidant activity [2]. Methods of thin-layer and planar chromatography in ethanol extract of subsurface and surface parts of all researched species, determined tannin (UV – 254 nm), and in ethanol extract determined was flavonoids (UV – 366 nm). Lowest level of tannin was discovered in above surface parts of P. *palustris* which could indicate different chemical – taxonomic affiliation of this species in relation to other species in this genus, since many species of *Potentilla palustris* are separated in different taxa of *Comarum palustre* [3]. **References:** [1] Redzic, S.S. (2007) Collegium Antropologicum, 31, 869 – 890. [2] Redzic, S. et al. (2007) Planta Med. 73:887. [3] Tomczyk, M., Latté, K.L. (2009) J. Ethnopharmacol. 122:184 – 204.

PJ198 A new facile and efficient synthesis of trans-(+)-sobrerol by biotransformation of α-pinene using extremophiles microorganisms

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Trans-(+)-sobrerol is a hydroxyl containing monocyclic terpenoid resulting from the autoxidation of α-pinene in presence of air and water. It presents pharmacological properties as mucolytic agent and clinical trials have confirmed its efficacy in relieving obstructive symptoms in patients suffering from chronic bronchitics [1]. Synthetic pathways have been established to obtain trans-(+)-sobrerol but in general they consist of several steps and the overall yields are low with poor stereoselectivity [2]. Essential oils are rich mixtures of natural products, mainly terpenes, and are obtained from aromatic plants by steam distillation or hydrodistillation. Some terpenes are very useful starting materials for the synthesis of more complex molecules [3]. α-Pinene, a hydrocarbon monoterpene present in several essential oils, can be easily obtained by rectification. It has been transformed in products of more added value by chemical reactions but also by biotransformation using fungi species, resulting in complex mixtures of oxygenated compounds [4]. In this work, we report for the first time the use of extremophiles microorganisms for the biotransformation of α-pinene. This terpene was transformed by a halophilic bacterial strain belonging to the genus *Jeotgalicoccus* after cultivation in liquid medium under aerobic conditions at 30°C for 48 h. GC analyses of the reaction mixture, after extraction in ethyl acetate, showed the presence of few components. The component present in the highest concentration was isolated and identified as trans-(+)-sobrerol by NMR spectroscopy. *Acknowledgements: We are grateful to SIDA-SAREC (Sweden) for financial support.* **References:** [1] Braga, P.C. et al. (1987) Int. J. Clin. Pharmacol. Res. 7:381 – 400. [2] Wang, Q. et al. (2003) Synth. Comm. 33:2125 – 2134. [3] Monteiro, J.L.F., Veloso,

C.O. (2004) Top.Catal. 27:169 – 180. [4] Lindmark-Henriksson, M. et al. (2003) J. Nat. Prod. 66:337 – 343.

PJ199 Antimicrobial and antiviral activities of some Egyptian medicinal plants

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Eight Egyptian traditional plants belonging to four families were tested for their bioactivities: *Carum carvi* L. and *Foeniculum vulgare* Mill. (Apiaceae), Raphanus sativus L. (Brassicaceae), *Ocimum basilicum* L., *Origanum majoran*a L., *Rosmarinus officinalis* L. and *Salvia palaestina* Benth. (Lamiaceae), *Cassia fistula* L. (Leguminosae). These plants are collected from local herbal market. Methylene chloride/methanol (Me_2Cl_2/MeOH) extracts of each species were separately prepared prior to be examined against eleven micro-organisms include six bacteria; *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*), two fungi (*Candida albicans* and *Aspergillus niger*) and three viruses; Herpes simplex-1, Poliomyelitis-1 and *Vesicular stomatitis*. Antibacterial and anti-fungi activities are determined by using micro-titer dilution method to calculate MIC, MBC and MFC [1] while anti-viral activity is evaluated by means of end point titration technique (EPTT) to calculate the reduction factor of each extract [2]. The results revealed that *Salvia palaestina*, and *Rosmarinus officinalis* possess bactericidal effect against *B. cereus* (MIC: 31.25 μg/ml and MBC: 125 μg/ml) and have inhibitory effect against *S. aureus* at MIC: 31.25 and 62.5 μg/ml, respectively. In addition, *Origanum majorana* are found to possess an inhibitory effect against *B. cereus* at MIC 500 μg/ml. **References:** [1] Vanden Bergha, D.A., Vlietinck, A.J. (1991) Methods in Plant Biochemistry, Academic Press London. [2] Vanden Bergha, D.A. et al. (1986) B I Pasteur 84:101 – 147.

PJ200 Imupret modulates the innate and adaptive immune system parameters *in vitro*

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Imupret is an alcoholic-aqueous extract of seven different herbal drugs. It is used for the treatment of recurrent infections of the respiratory tract, especially tonsillitis. We hypothesized that immunostimulatory actions of Imupret correlate with its clinical effects. The aim of the study was the assessment of the influence of Imupret on immune parameters *in vitro,* i.e. by analysing possible *in vitro* effects of Imupret on immune cells from healthy subjects. The effect of Imupret on the phagocytotic activity of macrophages and polymorphonuclear granulocytes was determined by the quantitative determination of leukocyte phagocytosis in whole blood. Imupret did not affect the phagocyte activity of granulocytes or monocytes. The effect of Imupret on the oxidative burst was determined by measuring the production of reactive oxidants in macrophages and polymorphonuclear granulocytes in whole blood. Imupret stimulated the oxidative burst of fMLP primed phagocytes at a concentration of 5 – 100 μg/ml. Possible cytokine modulating effects of Imupret were analysed in *in vitro* stimulated human peripheral blood mononuclear cells. Low concentrations of Imupret (0.001 – 1 μg/ml) increased the IL-6 and TNF secretion and decreased IL-10 secretion of LPS-stimulated monocytes. Furthermore, IFNγ and GM-CSF secretion of CD3/CD28-stimulated T-cells was increased by Imupret in a similar concentration range (0.001 – 1 μg/ml). At concentrations of 0.2 – 100 μg/ml, Imupret increased IL-6 secretion of CD3/CD28-stimulated T-cells. Finally the effect of Imupret on T-cell surface marker CD4, CD8, CD25 and CD69 were determined in whole blood. High concentrations of Imupret increased the number CD25+ T-cells. Altogether, we detected effects of Imupret on different characteristic parameters of the innate and adaptive immune system. Interestingly, we found different effects for a high and a low concentration range of Imupret. This reflects the different recommendation for the use of Imupret, i.e. higher doses for acute infections and lower doses for improving the immune status. A clinical study is planned to corroborate these findings in human volunteers.

PJ201 A randomised, double-blind, placebo controlled, parallel group study of the standardised extract SHR-5 of the roots of *Rhodiola rosea* in the treatment of subjects with stress-related fatigue

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The aim of the study was to assess the efficacy of the standardised extract SHR-5 of roots of *Rhodiola rosea* L. in the treatment of individuals suffering from stress-related fatigue. The phase III clinical trial took the form of a randomised, double-blind, placebo-controlled study with parallel groups. Participants, males and females aged between 20 and 55, were selected according to the Swedish National Board of Health and Welfare diagnostic criteria for fatigue syndrome. A total of 60 individuals were randomised into two groups, one (n= 30) of which received four tablets daily of SHR-5 extract (576 mg extract/day), while a second (n= 30) received four placebo tablets daily. The effects of the extract with respect to quality of life (SF-36 questionnaire), symptoms of fatigue (Pines' burnout scale), depression (Montgomery Asberg depression rating scale – MADRS), attention (Conners' computerised continuous performance test II – CCPT II) and saliva cortisol response to awakening, were assessed on day 1 and after 28 days of medication. Data were analysed by between-within analyses of variance. Repeated treatment with SHR-5 seems to have a positive effect on fatigue level, attention (as measured by a computerised performance test, $p < 0.05$) and saliva cortisol response to awakening stress ($p < 0.05$). It is suggested that the inhibitory effect of Rhodiola on the increased basal level of cortisol is associated with improvement in cognitive function. This is in line with other studies demonstrating that optimal corticosteroid level is a requirement for efficient cognitive function since significant changes (up or down) in circulating levels of corticosteroids results in cognitive impairment. Modulation of cortisol content is considered to be a key mechanism of action of phytoadaptogens. It may be concluded that Rhodiola, acting as an adaptogen, increases attention and endurance in situations of decreased performance caused by fatigue and sensation of weakness, and reduces stress-induced impairments related to the function of neuro-endocrine and immune systems.

PJ202 Medicinal plants used in traditional pain management in Jos, Nigeria

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Pain management is a concern in many diseases. A survey was conducted on various medicinal plants used in management of pain in Jos, North-central, Nigeria. Five of the commonly used plants; *Amaranthus viridis* Linn (seeds) (Amaranthaceae) [AV], *Paullinia pinnata* Linn (stem bark) (Sapindaceae) [PP], *Solanum incanum* Linn (whole plant) (Solanaceae) [SI], *Ximenia americana* Linn (stem bark) (Olacaceae) [XA] and *Fadogia agrestia* Schweinfurth (leaves) (Rubiaceae) [FA] where evaluated for analgesic activity (acetic acid- induced writhing assay [1] and hot-plate method [2]) in mice and compared with standard drugs (aspirin and morphine). Phytochemical screening [3] of the various plants showed tannins, flavonoids, alkaloids, steroids, anthraquinones and cardiac glycoside. Only tannins and flavonoids were common to all the plants. Ethanolic extracts of the plants at 100 and 200 mg/kg were administered orally and compared with standard drugs. In the acetic acid-induced writhing assay the order of activity was XA>AV>PP>SI>FA. Activity of XA, AV and PP was significant ($P < 0.05$) compared to aspirin. On the other hand, in the hot-plate method, the order of activity was PP>XA>AV>SI>FA. Similarly to the acetic acid assay, the activity of XA, AV and PP was significant ($P < 0.05$) compared to morphine. These findings support the traditional use of these plants in pain management in Jos, Nigeria. **References:** [1] Tjolsen, A., Berge, O. G., et al. (1992) Pain. 51:5. [2] Turner, R.A. (1965) Screening methods in Pharmacology. Academic press, New York, p 158. [3] Sofowora, A. (1993) Standardization of herbal medicine. In: medicinal plants and traditional medicine in Africa. Spectrum Book Limited, Lagos, Nigeria. pp. 56 – 61.

PJ203 Development of clinical trial guideline for Diabetes Mellitus on traditional Korean medicines

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This study was conducted to establish "Clinical trial Guideline for Diabetes Mellitus on Traditional Korean Medicines (TKM)". With the tremendous expansion in the use of traditional medicines worldwide, safety and efficacy as well as quality control have become important issues. Researchers and pharmaceutical manufacturers were increasingly requesting KFDA to provide standards and information on these concerns. This guideline was carefully developed through the various experts' opinions and guidelines of KFDA, WHO, EMEA, FDA, NCI, TGA and ICH related to clinical trial. The methodologies are composed of basic principles, investigational product, inclusion and exclusion criteria, control groups, safety and efficacy assessment, dosage and durations, interaction between drugs, evaluation of quality of life, traditional medicinal diagnosis and other issues related to clinical trials. It would link the TKM with Western medicine for Diabetes Mellitus clinical trial. This is intended to provide necessary recommendations for the development of TKM to researchers in medical and pharmaceutical industry, and to facilitate new drug development through its practical application on clinical trials for Diabetes Mellitus.

PJ204 Australasian *Huperzia* as potential sources of Huperzine A and B

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The alkaloids, Huperzine A (HupA) and Huperzine B (HupB), have been reported to be highly selective, potent and reversible inhibitors of acetylcholine esterase. Clinical trials in both China and USA have demonstrated HupA to be of therapeutic benefit for patients suffering from neurodegenerative disorders such as Alzheimer's disease. Means of synthesising isomerically pure HupA have yet to be optimised and to date all pharmaceutical production of HupA has been from the Chinese club moss *Huperzia serrata* which contains on average only 0.18 mg g^{-1} DW HupA. As a consequence, *H. serrata* has been listed as endangered in China. A potential solution to this problem would be to establish commercial *Huperzia* plantations to supply the ever growing need for HupA. Identifying species or individuals with larger biomass and/or higher HupA concentrations would be critical in establishing such large scale plantations. As little is known about the huperzine concentrations of Australasian *Huperzia* species, this study examined sixteen Australasian *Huperzia*, including three undescribed species, for their HupA and HupB contents. Concentrations of HupA and HupB were observed to vary substantially both inter- and intra-specifically, with the highest yield of HupA (1.01 mg g^{-1} DW) and HupB (0.34 mg g^{-1} DW) observed in one of the yet to be classified *Huperzia* samples originating from the Philippines.

PJ205 Effects of mutation on *Catharanthus roseus* (L.) G.Don. plants and their inheritance

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The plant *Catharanthus roseus* (L.) G.Don. (Apocynaceae), a perennial and self-pollinated plant, is of immense medicinal value due to antineoplastic activities of its leaf alkaloids. A mutation breeding programme was carried out to design a physiologically and chemically efficient plant type with increased production of secondary metabolites. This paper describes the morphology and inheritance of induced mutants, which were produced by physical and chemical mutagens. Induced mutagenesis with gamma rays and EMS in *Catharanthus roseus* produced six distinct mutants that differ in their growth habit and plant morphology. These mutants were dwarf mutants with obovate leaf (dwob), medium tall mutants with small leaf area (mtsl), nontrichomeous mutants (nt), upright oriented elliptical leaf mutants (upel), spoon shape leaf mutants (sp) and variegated leaf mutant (vg). Among them, dwob and mtsl mutants exhibited digenic recessive inheritance. The vg and upel leaf mutants were monogenic recessive and sp leaf mutant supports complementary gene action. Effect of mutation has positive response to the alkaloid content of *Catharanthus* but it varies in different mutants.

PJ206 Studies on leaf epidermal micromorphology, wood element characters and phytochemical screening of three medicinal plants of Convolvulaceae

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The scientific evaluation of ethnomedicinally important plants is now being done thoroughly covering various aspects of study like efficacy of the crude drugs, chemistry of active principles, different pharmacognostic parameters, etc. The use of micromorphology and anatomy is now a recognised tool in the field of plant systematics. Therefore, in this investigation the micromorphology of leaf epidermis, stem xylem element characters and phytochemical screening of three ethnomedicinally important members of the family Convolvulaceae namely *Evolvulus alsinoides, Evolvulus nummularius* and *Ipomoea cairica* have been studied. The epidermal cells are found to be irregular in shape and the outlines of the cells are wavy in every species. Stomata are amphistomatic and mainly of paracytic type except in *Evolvulus nummularius*. Trichomes are glandular and non-glandular, unicellular or multicellular, straight or curved. The range of stomatal index varies from 11.40 to 20.00. Palisade ratio ranges from 6.2 to 9.8. The vessel element length ranges from 60.71 um to 357.10 um and the diameter varies from 21.78 um to 66.06 um. Perforation plate is simple and transverse or obliquely placed. Fibres are typical libriform, very long and diameter ranges from 10.71 um to 16.78 um. In every case, tracheids are long with spiral to condensed spiral type of sidewall thickening and diameter is from 07.14 um to 16.07 um. The active compounds are identified by the chemical colour reaction tests belonging to the phytochemical groups of amino acids, alkaloids, reducing sugars, flavonoids, saponins, steroids and triterpenoids, tannins, etc. The findings will be a useful marker for identification of the crude drugs obtained from the selected taxa. The purpose of the study is to know the leaf epidermal micromorphology, wood element characters and phytochemical screening of three ethnomedicinally important plants of the family Convolvulaceae as it has not been properly worked out. These micromorphological features and phytochemical screening will be very helpful in proper identification of respective crude drugs obtained from these three members of Convolvulaceae and also be used in detection of drug adulterants. Thus, it will be a tool in maintaining the quality of the drug obtained from these three plant species.

PJ207 Expression profiling and network-based analysis for the effect of curcumin on pancreatic cancer cells

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Pancreatic cancer is one of the most aggressive human malignancies with an extremely poor prognosis. The paucity of curative therapies has translated into an overall 5-year survival rate of less than 5%, underscoring a desperate need for improved therapeutic options. Over-expression of cyclooxygenase-2 (COX-2) enzyme in human tumors is associated with poor prognosis. Therefore, COX-2 is considered as one of the crucial targets for cancer therapy. A dietary compound curcumin hardwires to multiple cellular processes, with suppression of cell proliferation, induction of apoptosis, and inhibition of metastasis considered as the major mechanisms underlying its anticancer properties. Here, we undertook a gene expression profiling study to identify novel molecular targets of curcumin in pancreatic cancer cells. Our data demonstrated that curcumin inhibited cell growth and induced apoptosis

in pancreatic cancer cell lines through COX-2 dependent and independent mechanisms of action. Moreover, Using the Ingenuity Pathway Analysis tool, we distributed the differentially expressed genes into biological networks and evaluated their functional significance. In addition, we identified several pathways affected by curcumin including cell cycle and apoptosis. Furthermore, we identified that the effect of curcumin is mediated through modulation of multiple signaling pathways. The present analysis is a starting point for the generation of hypotheses on candidate genes and for a more detailed dissection of the functional role of individual genes for the activity of curcumin in cancer.

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