Planta Medica

Planta Medica

An International Journal of Natural Products and Medicinal Plant Research

September 2006 Volume 72 No. 11 Page 961–1090

11
Volume 72
September 2006
Page 961–1090

Official Organ of the Society for Medicinal Plant Research

www.thieme-connect.com
www.thieme.de/plantamedica

This journal is indexed in Current Contents/Life Sciences, Science Citation Index, MEDLINE, EMBASE/Excerpta Medical Abstract Journals, and CABS (Current Awareness in Biological Sciences)

54th Annual Congress on Medicinal Plant Research

29 August – 2 September 2006,
Helsinki, Finland

Abstracts

Organisers:
The Society for Medicinal Plant Research
Faculty of Pharmacy, University of Helsinki

Issue Editors:
Raimo Hiltunen
Heikki Vuorela

Planta Med ISSN 0032-0943

Georg Thieme Verlag KG, Postfach 30 11 20, 70451 Stuttgart
E 21804 PVSt. DPAG. »Entgelt bezahlt« Planta Med 11/2006

Thieme

Imprint

Editor
Prof. Dr. Matthias Hamburger, Institute of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4053 Basel, Switzerland, E-mail: matthias.hamburger@unibas.ch, Fax: +41 61 267 14 74

Manuscript submission exclusively via:
http:// mc.manuscriptcentral.com/plamed

Publishers
Georg Thieme Verlag KG Stuttgart · New York
Rüdigerstr. 14, D-70469 Stuttgart
P.O. Box 30 11 20, D-70451 Stuttgart
phone +49-711-8931-0
fax +49-711-8931-298
www.thieme.com
www.thieme.de/plantamedica
www.thieme-connect.com

Customer service:
phone ++49-711-8931-900
fax ++49-711-8931-901
email: kunden.service@thieme.de

Production manager
phone ++49-711-8931-241
fax ++49-711-8931-392
email: gudrun.fuchs@thieme.de

Advertising Representative
thieme.media Pharmedia GmbH, Rüdigerstr. 14, D-70469 Stuttgart; P.O. Box 30 08 80, D-70448 Stuttgart; phone +49-711-8931-466; fax +49-711-8931-392; email: Ulrike.Bradler@thieme.de

General information
Planta Medica, ISSN 0032-0943, is published monthly.
All subscription orders are entered for the calender year. The rate of subscription is invoiced in advance at the end of the year for the following year and becomes due for payment for the full calendar year. Subscriptions are automatically extended each year unless notice of cancellation is received from the subscriber prior to September 30 of each year (applies to Germany, Austria, and Switzerland only).

Subscriptions from countries outside the American continents
Order from Thieme Verlag KG, Rüdigerstr. 14, D-70469 Stuttgart; P.O. Box 30 11 20, D-70451 Stuttgart; phone +49-711-8931-421; fax +49-711-8931-410; email: custserv@thieme.de
Subscription rates for 2006: Institutional subscription € 898.00; for Members of the Society for Medical Plant Research € (D) 498.00. Plus postage: Germany € (D) 43.80; Surface (Europe) € 63.80; Rest of World (excl. North, Central and South America) € 76.00. Single issue price € (D) 74.00.
Subscribers are asked to inform the publisher immediately in case of address changes in order to ensure correct delivery of the journal. The Deutsche Post AG is entitled to inform the publisher of address changes. Note according to § 4 of the German "Postdienst-Datenschutzverordnung": Objection to this transmission of data can be raised to the address of the publisher.

Subscriptions from North, Central and South America
Order from Thieme New York, 333 Seventh Avenue, New York, NY 10001, USA. Order toll free ++1-800-782-3488 (U.S. only) or ++1-212-760-0888, fax ++1-212-947-0108;
email customerservice@thieme.com.

Manuscripts
For details regarding manuscript submission please refer to "Instructions for Authors". In principle only papers will be accepted which have not been published previously, domestically or abroad. Furthermore, manuscripts may not be be offered to other publications at the same time as they are under consideration for this journal. With the acceptance of a manuscript the publishers acquire – for the duration of the legal period of protection – the exclusive right for the use of the rights of exploitation according to German copyright law, especially also the rights of translation, of duplication, and of electronic data processing. Authors will receive 25 reprints free.

Online
The scientific text of this journal is available online through Thieme-connect, http://www.thieme-connect.de. Access to Thieme-connect is free of charge for personal subscribers. For information concerning licences and prices for institutional access, please contact Bernd Hess, email: sales@thieme-connect.de. Customers from North, Central and South America and Canada please contact Kristina Kurz, email: kkurz@thieme.com.
Articles may be submitted to this journal on an open-access basis. For further information, please send an e-mail to plantamedica@thieme.de

Important note

Medicine is an ever-changing science undergoing continual development. Research and clinical experience are continually expanding our knowledge, in particular our knowledge of proper treatment and drug therapy. Insofar as this journal mentions any dosage or application, readers may rest assured that the authors, editors and publishers have made every effort to ensure that such references are in accordance with the state of knowledge at the time of production of the journal.
Nevertheless this does not involve, imply, or express any guarantee or responsibility on the part of the publishers in respect of any dosage instructions and forms of applications stated in the journal. Every user is requested to examine carefully the manufacturer's leaflets accompanying each drug and to check, if necessary in consultation with a physician or specialist, whether the dosage schedules mentioned therein or the contraindications stated by the manufacturers differ from the statements made in the present journal. Such examination is particularly important with drugs that are either rarely used or have been newly released on the market. Every dosage schedule or every form of application used is entirely at the user's own risk and responsibility. The authors and publishers request every user to report to the publishers any discrepancies or inaccuracies noticed.

Product names
Product names which are registered trademarks may not have been specifically designated as such in every case. In case that a product has been referred to by its registered trademark it cannot be concluded that the name used is in the public domain. The same applies to patents and registered designs.

Printed in Germany
Typesetting: Sommer, D-91555 Feuchtwangen
Printing: Druck- und Verlagshaus Alois Erdl, Trostberg

Planta Medica

An International Journal of Natural Products and Medicinal Plant Research

Georg Thieme Verlag KG
Stuttgart · New York
Rüdigerstraße 14
D-70469 Stuttgart
Postfach 30 11 20
D-70451 Stuttgart

Thieme New York
333 Seventh Avenue
New York, NY 10001

Planta Medica

An International Journal of Natural Products and Medicinal Plant Research

54th ANNUAL CONGRESS ON MEDICINAL PLANT RESEARCH

29 August – 2 September 2006, Helsinki, Finland

ABSTRACTS

Organisers:

The Society for Medicinal Plant Research
Faculty of Pharmacy, University of Helsinki

Issue Editors:

Raimo Hiltunen
Heikki Vuorela

Technical Assistant:

Kari Kreander

Georg Thieme Verlag KG
Stuttgart · New York
Rüdigerstraße 14
D-70469 Stuttgart
Postfach 30 11 20
D-70451 Stuttgart

Thieme New York
333 Seventh Avenue
New York, NY 10001

Contents

online www.thieme-connect.com

11 72 (2006), 961–1090, No. 11, September

Cover photograph: City of Helsinki (Picture Bank/Matti Tirri)

PL 001

Discovery of natural products by chemical and pharmacological profiling

Hamburger M
Department of Pharmaceutical Sciences, Institute of Pharmaceutical Biology, University of Basel, CH-4056 Basel, Switzerland

Over the past decade, a number of new technologies and tools have become available in the biosciences and in analytical chemistry. They enable new approaches in the discovery of bioactive natural products which can be summarized with a few keywords such as miniaturization, on-line analysis of complex samples, study of molecular modes of action, and systems oriented approaches towards the characterization of drug effects in vitro and in vivo. Some of the technologies which are useful in the context of natural products discovery will be discussed and illustrated with examples from our lab. The efficient tracking of bioactivity in an extract remains a major challenge. We have replaced preparative activity-directed isolation by HPLC-based activity profiling at analytical scale. In the search for natural products leads, we prefer assays with high information content and complex endpoints, such as phenotypical screens, over biochemical assays. The molecular targets for these leads are subsequently studied with the tools of molecular and cell biology. HPLC-based activity and metabolite profiling will be illustrated with the example of the anti-inflammatory plant Isatis tinctoria, while phenotypical screening and subsequent characterization of signaling pathways will be discussed with the example of fungal pyridone alkaloids from Paecilomyces militaris. The postgenomic era offers a range of new tools and approaches for an essentially unbiased and global investigation which does not need to be hypothesis-driven. The application of genome-wide expression profiling in the characterization of extracts will be described with our ongoing studies on Cimicifuga racemosa (L.) Nutt. and Leuzea carthamoides DC. Findings from array experiments are confirmed by quantitative PCR and functional assays, followed by HPLC-based activity profiling, eg. for AhR-agonistic activity, and by structure determination with LC-PDA-MS and microprobe NMR in HPLC fractions. **Reference**: 1. Potterat, O., Hamburger, M. (2006), Curr. Org. Chem., in press.

PL 002

Advances in stationary phase development for the analysis of target compounds in proteomics, phytomics and metabolomics

Bonn GK, Stecher G, Huck CW, Bakry R, Feuerstein I
Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University, Innrain 52a, 6020-Innsbruck, Austria

Extraction, purification, preconcentration and separation are the classical steps for the analysis of plant materials. Although a huge number of different techniques are available, the design of novel materials and stationary phases is still needed. In fact, selective extraction, preconcentration and purification prior to analysis is often necessary owing to the complexity of samples. Additionally, analytes are often present in low concentrations, what makes successful analysis a challenge. Within this talk we present different strategies for the synthesis and the modification of stationary phases to produce tailored solutions for the analytical questions. In fact, different possibilities concerning extraction, purification and separation will be presented, e. g. a multidimensional approach for the simultaneous preconcentration and separation of biomolecules and flavonoids. Further on, focus will be placed on open tubular capillaries with special surface modifications, as these systems allow the selective extraction of target compounds and the elution of the sample within a high concentrated fraction. For the separation of analytes newly synthesized materials on the basis of methylstyrene (MS) and 1,2-bis(p-vinylphenyl)ethane (BVPE) will be introduced. The polymer was build in the confines of fused silica capillaries (200 μL I.D.) and was successfully employed for the fractionation of peptides, β-blocker drugs (Pindolol, Metopolol, Alprenolol, Propranolol) as well as flavonoids and stilbenes (epicatechin, epigallocatechin gallate, epicatechin gallate, resveratrol). Next to this, several approaches concerning the pharmacological investigation of analytes will be shown, e. g. the analysis of stilbenemetabolites in human urine using silica C-18 stationary phases or the analysis of salix ingredients using encapsulated silica-C18 poly-(styrene/divinylbenzene) capillaries. Finally within the talk some new instrumental developments and applications in phytomics will be presented, e. g. the use of a contactless conductivity detector in capillary electrophoresis for the detection of flavonoids. At last also the use of matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) for the detection of small molecules such as sugars, glucuronic acid derivatives and glycerol will be showen, accenting its potential for metabolomic investigations.

PL 003

Plant Metabolomics: Small Molecules Take Center Stage

Trethewey R
metanomics GmbH, Tegeler Weg 33, 10589 Berlin, Germany

The advancement of genomics technologies in the last decade has been extremely rapid and the opportunity for novel experimentation profound. However, whilst there has been much focus on large molecules (DNA, RNA and protein), small molecules have been somewhat neglected in international efforts. This is odd given the essential importance of small molecules in determining functional performance and phenotype and our emerging understanding of their role as signals that interplay with and regulate gene expression and protein activity in biological networks. In this presentation the importance of the analysis of small molecules via metabolite profiling will be introduced and illustrated with examples from the work of metanomics, a company which has pioneered industrial metabolomics. Today laboratories are operated with some 60 mass spectrometers allowing a throughput of > 100,000 samples per year. This capability has been deployed in plant functional genomics: the company has generated large, unique, populations of Arabidopsis and crop plants where genes have been systematically overexpressed or knocked out at a genome scale. Screening the metabolite profiles of these transgenic lines enables genes to be rapidly selected which influence and control commercially important areas of metabolism e. g. oils, amino acids, vitamins or sugars. Further the linking of metabolic data to genetic and phenotypic data has been demonstrated to be of particular importance and the status of such system biology approaches based on metabolite profiling data will be reviewed.

PL 004

Clinical trials and systematic reviews of herbal medicine

Ernst E
Director, Complementary Medicine, Peninsula Medical School, Universities of Exeter & Plymouth, 25 Victoria Park Road, Exeter, EX2 4NT, UK

The popularity of herbal medicines begs the question whether a given herbal remedy is safe and efficacious in treating a given condition. The latter question is best answered on the basis of randomised (preferably placebo-controlled, double-blind) clinical trials. Several hundred clinical trials of variable methodological rigour have been published. The emerging evidence is often contradictory. In this situation the best evidence is provided by a systematic review or meta-analysis, i. e. an evaluation of the totality of all the available studies on a specific topic. This approach is aimed at minimising both random and selection biases. Today well over 100 systematic reviews relating to a wide range of herbal medicines have

been published. Examples of some of these systematic reviews will be discussed. They leave little doubt that some herbal medicines are efficacious in treating some clinical conditions. To date the evidence relating to safety is largely anecdotal, i. e. based on case reports. The least biased and most informative tool for summarising it is again the systematic review. Several systematic reviews of safety data will be provided. Their results vary but, by and large, suggest that adverse effects are rare. In conclusion, more systematic research is required to evaluate the balance between risk and benefit for commonly used herbal medicines.

PL 005

Absorption and metabolism of dietary phenolics

Crozier A
Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

Since the early 1990 s there has been growing interest in the protective effects of dietary phenolics and flavonoids. In order to assess the potential health benefits of these compounds information is required on the sites of absorption, the metabolic forms in which they are absorbed and their concentrations in the circulatory systems and body tissues. After consumption of onions, which contain flavonol glucosides, the main components being quercetin-4'-O-glucoside (143 mmoles) and quercetin-3,4'-O-diglucoside (107 mmoles), the flavonols undergo rapid metabolism in the small intestine followed by absorption of glucuronidated, sulfated and methylated quercetin metabolites. These metabolites are detected in the bloodstream reaching a Cmax after 1.0 – 1.5 h. Excretion of metabolites in urine over a 24 h period indicates that absorption is ~4 % of intake. In subjects with an ileostomy, the major components in ileal fluid after ingestion of onions are quercetin-3-glucuronide, quercetin-3'-sulfate and quercetin in quantities corresponding to ca. 20 % of intake, suggesting that absorption is substantially higher than 4 %. A comparative study on the absorption an metabolism of 164 mmoles of quercetin-3-rhamnosylglucoside (rutin) in tomato juice showed trace levels of quercetin and methylquercetin glucuronides, but no sulfated metabolites, in plasma with a Tmax of ca. 5 h. There was an 85+% recovery of the ingested ruin in ileal fluid and no quercetin metabolites were detected in plasma collected from ileal volunteers These observations indicate that absorption of rutin is more limited than that of quercetin glucosides and that in healthy subjects it takes place on the large intestine. Other studies have demonstrated that colonic bacteria hydrolyse and breakdown rutin to phenolic acids which are excreted in urine in amounts corresponding to 25 % of intake.

PL 006

Bioassay development in natural product drug discovery

Vuorela P
Department of Biochemistry and Pharmacy, Faculty of Mathematics and Natural Sciences, Åbo Akademi University, FI-20520 Turku, Finland

Bioactivity screening is an integral part of the natural product drug discovery process [1]. The bioactive compounds in the natural product extracts are screened utilizing e. g. whole cells, cell fractions, recombinant enzymes or biochemicals as targets. The screening of natural products provides a complementary structural diversity to synthetic chemistry and offers new low molecular weight lead compounds. Our work involves generating and screening of extract/compound libraries of biogenic origin for pharmaceutical purposes. We have used microfractionation of plant and microbial extracts on HPLC combined with design and development of new bioactivity screening assays as an approach to find bioactive principals. Using HPLC microfractionation, components of crude extracts can be divided into fractions collected into microwell plates and subsequently subjected to diverse bioassays. Miniaturized screening assays have been developed with special emphasis on quality of the bioassays for e. g. susceptibility testing of *Chlamydia pneumoniae* utilizing time-resolved fluorometric immunoassay [2, 3]. The coupling of automated bioassay to analytical HPLC microfractionation greatly facilitated the classical process leading from a plant to pharmacologically active compound [4]. **References**: 1. Vuorela, P., Leinonen, M., Saikku, P., Tammela, P., Rauha, J.-P., Wennberg, T., Vuorela, H. (2004), Curr. Med. Chem. 11: 1375 – 1389. 2. Tammela, P. *et al.* (2004), Anal. Biochem. 333: 39 – 48. 3. Alvesalo, J. *et al.* (2006), J. Med. Chem. 49: 2353 – 2356. 4. Tammela, P. *et al.* (2004), Anal. Bioanal. Chem. 380: 614 – 618.

Keynote Lectures

K 001

Merits and limits of computational methods for the discovery of natural acetylcholinesterase inhibitors

Rollinger JM[1], Schuster D[2], Langer T[2], Stuppner H[1]
[1]Institute of Pharmacy / Pharmacognosy; [2]Institute of Pharmacy / CAMD-Group;
Center for Molecular Biosciences Innsbruck, Leopold-Franzens University of Innsbruck, 6020 Innsbruck, Austria

Bioactive natural products and drug substances in general exhibit their pharmacological activity by binding as ligands to biomolecular targets. Functions and 3D-structures of an increasing number of target macromolecules are becoming available. On the other hand, a wealth of potent ligands from both synthetic and natural origin provides a rich pool of structural and biological information. In this light, computational methods contribute to (i) a rapid identification of novel lead compounds and (ii) an improved molecular insight of ligand-target interactions. This study deals with the application of diverse integrated *in silico* tools to increase the efficiency in the search for natural acetylcholinesterase (AChE) inhibitors. In contrast to previous screening results, where we have been able to correctly predict novel bioactive natural products from in house molecular 3D libraries [1, 2], we report here on the limitations of pharmacophore based virtual screening. A highly potent anticholinesterase alkaloid, taspine (IC_{50} 333 ± 70 nM), was isolated by bio-guided fractionation from *Magnolia x soulangiana* Soul.-Bod. However, none of the 3D conformers was able to fit into the elaborated pharmacophore model [1]. Extensive docking studies on human- and *Torpedo californica*-AChE strongly suggest a binding mode of taspine, which is different to that of known ligands in the active binding site (e. g. galanthamine; [3]) and in the peripheral anionic binding site [4]. It may be assumed that taspine does not occupy the catalytic center itself but prevents acetylcholine from accurately being positioned in the binding pocket for cleavage. Concluding, molecular docking studies helped to explore the possible binding mode of taspine as "hydrophobic plug" in the aromatic gorge of AChE. *Acknowledgements:* This work was granted by the FWF Austria (P18379) **References**: 1. Rollinger, J.M. *et al.* (2004), J. Med. Chem. 47: 6248 – 6254. 2. Rollinger, J.M. *et al.* (2005), Curr. Drug Disc. Techn. 2: 185 – 193. 3. Greenblatt, H.M. *et al.* (2004), J. Am. Chem. Soc. 126: 15405 – 15411. 4. Kryger, G. *et al.* (2000), Acta Crystallogr. D 56: 1385 – 1394.

K 002

Flavonoids Target Multiple Enzymes from the Type II Fatty Acid Pathway of Plasmodium falciparum and Do Not Invoke Delayed Death Phenomenon

Tasdemir D[1], Lack G[2], Brun R[3], Kaiser M[3], Rüedi P[4], Perozzo R[2]
[1]Centre for Pharmacognosy and Phytotherapy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, UK; [2]School of Pharmaceutical Sciences, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; [3]Department of Medical Parasitology, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland; [4]University of Zurich, Institute of Organic Chemistry, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

The deciphering of the complete genome of *Plasmodium falciparum* [1] has uncovered a number of biochemical pathways, including the type II fatty acid biosynthesis (FAS-II) that occurs in a recently discovered plastid-like organelle (apicoplast) in *Plasmodium* [2]. The organizational and structural differences between the fatty acid biosynthesis in the malaria parasite and in humans make FAS-II and its enzymes attractive targets for the design of antimalarial agents. The unique enzyme enoyl-ACP reductase (*Pf*FabI) of *P. falciparum* commits the final reduction step during the fatty acid elongation. We recently identified luteolin-7-*O*-glucoside as the first natural product targeting *Pf*FabI [3]. This prompted us to evaluate the inhibitory activity of a large flavonoid library against FabI, as well as two other crucial enzymes (FabG and FabZ) of the FAS-II system of *P. falciparum*. Several compounds, *e.g.* (-)-catechin gallate, luteolin, fisetin inhibited all three enzymes in the low ng/mL to subμg/mL range and were further investigated kinetically to shed light on the inhibitory mechanism. The ability of a single compound to inhibit three enzymes from the same pathway is a very important aspect, as it is unlikely for the parasite to develop resistance to the drug by introducing mutations at all three enzymes at the same time. Previous studies indicate that the inhibition of some other apicoplast functions, *e.g.* replication, translation may not result in immediate parasite death. Rather than inhibiting growth in the first generation (48 h), some antimalarial agents kill the parasites later in the second generation (96 h) [4]. Therefore, we investigated whether the FAS inhibitory flavonoids invoke this so-called delayed death phenomenon. Fortunately, none of the tested flavonoids elicited the delayed death response and demonstrated rapid antiparasitic effects. **References**: 1. Gardner, M.J. *et al.* (2002), Nature 419: 498 – 511. 2. Fadden, G.I. *et al.* (1996), Nature 381: 482. 3. Kirmizibekmez, H. *et al.* (2004), Planta Med. 70: 711 – 717. 4. Surolia, A. *et al.* (2004), Biochem. J. 383: 401 – 412.

K 003

Plant secondary metabolism in the post-genomic era

Oksman-Caldentey KM[1], Häkkinen ST[1], Rischer H[1], Ritala A[1], Ma R[1], Seppänen-Laakso T[1], Goossens A[2], Orešič M[1], Inzé D[2]
[1]VTT Technical Research Centre of Finland, Tietotie 2, Espoo, FIN-02044 VTT, Finland; [2]Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, B-9052 Gent, Belgium

The biotechnological production of high-value plant secondary metabolites in cultivated cells is an attractive alternative to isolation processes from the intact plants or to the total chemical synthesis. However, plant metabolic engineering has met only limited success, in sharp contrast to microorganisms, since our knowledge on biosynthesis of secondary metabolites is still very limited. Despite of the rapid development of not only plant genomics but also of analytical tools genetic maps of biosynthetic pathways are far from complete. Furthermore, regulation of the individual steps leading to the desired end-product is poorly understood. We have developed a SoluCel® technology platform based on genome-wide identification and functional analysis of genes involved in the production of plant-derived small molecules. It allows the exploitation of these genes in order to produce already existing secondary metabolites at higher levels in cell and tissue cultures through metabolic engineering. Moreover our combinatorial biochemistry approach allows to increase the chemical diversity of plant-based molecules thus offering novel molecules for the industry. A proof-of-concept has first been gained using tobacco cells as a model system. The technology was further applied to several medicinal plants. Using cDNA-AFLP based transcript profiling linked to our UPLC-MS or GC-MS metabololite profiling platform, an inventory of hundreds of genes, potentially involved in secondary metabolism, has been built. The functional analysis of these genes alone or in combination has shown clearly enhanced or altered metabolite accumulation patterns both in tobacco and in other plants. With this technology we are able to offer new opportunities to exploit the entire metabolic repertoire of a plant cell, and to create higher quatities of known metabolites or novel compounds that may find applications not only in pharmaceutical but also in chemical or biotechnological industries.

K 004

Capability of Prenylflavanones present in Hops to Induce Apoptosis in a Human Burkitt Lymphoma Cell Line

Riepl HM[1], Diller RA[1], Rose O[2], Frias C[2], Henze G[2], Prokop A[2]
[1]Institute of Technology for Biogenic Resources, Technical University of Munich, Petersgasse 18, 94315 Straubing, Germany; [2]Department of Pediatric Oncology/Hematology, University Medical Center Charité, Campus Virchow, Augustenburger Platz 1, 13353 Berlin, Germany

8-Prenylnaringenin (8-PN), a flavanone present in the female flower of hops (*Humulus lupulus* L.) and in some other plants (*e.g.* in *Anaxagorea luzonensis* A.Gray.*)* [1, 2] is known as being a very potent phytoestrogen [3]. As such it may accelerate proliferation analogous to estradiol in sensitive cell lines. The question was to be clarified whether it may contribute to growth of hormone dependent neoplasms when present in herbal preparations. We found instead antiproliferative and apoptosis inducing effects of 8 –PN. We compared some side chain variants of 8-prenylnaringenin *e.g.* 8 –geranylnaringenin, isolated also from hops and the synthetic variations 8 –furanylmethylnaringenin, 8 –cinnamylinaringenin. These were synthesized by a Mitsunobu reaction and Claisen rearrangement [4]. When applied to BJAB cells, grown in RPMI 1640 medium, these flavanones showed improved cytotoxic and apoptotic activities – only 8-furanylmethylnaringenin is not active. 8-Geranylnaringenin displayed noticeably improved apoptotic effects when compared to 8-PN. 8-Cinnamylnaringenin significantly induced apoptosis in BJAB cells at a concentration of 50 μM. (Fig. 4). The apoptotic effect of 8-cinnamylnaringenin exceeded those of all other naringenins tested in this study. The induction of apoptosis is concentration dependent (11 % apoptotic cells at 50 μM and 38 % at 100 μM). Apoptosis was induced in a mitochondrial dependent manner. Despite low capacity to induce apoptosis, 8-PN induced a decrease of the mitochondrial membrane potential, too. However, 8-geranylnaringenin caused a change in the membrane potential at much lower concentration. At 100 μM we noticed a saturation effect in decrease of mitochondrial membrane potential. But the greatest effect was demonstrated with 8-cinnamylnaringenin. Even at a concentration of 50 μM, it is observed a transition in 77 % of the BJAB cells. The potential of 8 –PN is shown in an *ex vivo* experiment of a multi-drug resistant leukaemia blast. **References**: 1. Zierau, O., Hauswald, S., Schwab, P., Metz, P., Vollmer, G. (2004), J. Steroid Biochem. Mol. Biol. 92: 107 – 110. 2. Kitaoka, M., Kadokawa, H., Sugano, M., Ichikawa, K., Taki, M., Takahashi, S., Iijima, Y., Tsutsumi, S., Boriboon, M., Akiyama, T., (1998), Planta Med. 64: 511 – 515. 3. Milligan, S.R., Kalita, J.C., Pocock, V., Van de Kauter, V., Stevens, J.F., Deinzer, M.L., Rong, H., De Keukeleire, D. (2000), J. Clin. Endocrin. Metab. 85: 4912 – 4915, 4. Gester, S., Metz, P., Zierau, O., Vollmer, G. (2001), Tetrahedron 57: 1015.

K 005

Biobehavioural effects of herbal extracts

Scholey AB, Kennedy DO
Human Cognitive Neuroscience Unit, Northumbria University, Newcastle upon Tyne, NE1 8ST UK

Mainstream pharmaceuticals have largely been developed from the isolation and/or synthesis of active agents with specific targets. On the other hand plant medicines may contain dozens of actives which exert multiple and often subtle effects upon target systems. Individually these components may act either positively or negatively, and together may affect multiple neuronal, metabolic and hormonal systems. Since mental processes are themselves modulated by such systems, the behavioural effects of plant extracts involve complex interactions both within and between physiological systems. Additionally such interactions may be synergistic, resulting in complex dose- and time dependent effects [1]. Over the last few years work in our laboratory has aimed to systematically assess the effects of plant extracts on human functions which are relevant to ageing and dementia. Extracts include those used in traditional medicine systems. Thus we have built up a portfolio of research documenting the biobehavioural effects of *Ginkgo biloba* L., *Panax ginseng* C.A. Meyer, species of *Salvia* L., *Melissa officinalis* L., *Valeriana officinalis* L. and *Paullinia cupana* Kunth ex H.B.K. amongst others. This talk examines methodology for capturing such effects and presents data suggesting that standardised extracts are capable of differentially affecting aspects of memory and mood. The potential for such agents to act as cognition enhancing, anti-stress and anxiogenic treatments is considered. **Reference**: 1. Scholey, A. *et al.* (2005), Psychopharmacology 179: 705 – 707.

K 006

Salicylate: a phytochemist's headache

Verpoorte R, Verberne M, Budi Muljono RA, Mustafa NK
Department of Pharmacognosy, Section Metabolomics, IBL, Leiden University

Acetylsalicylate is one of the most successful drugs ever made, with still novel indications being discovered. It was developed on the basis of the use of Salix bark, which contains salicin which one may consider as a pro-drug for salicylate (SA). Interestingly it was found that SA acts as signal compound in plants, particularly in systemic acquired resistance (SAR) observed after infection with for example a virus. Despite extensive studies in the past 20 years the biosynthesis still poses many questions [1]. Most work has been on the phenylalanine pathway leading to SA. Several enzymes have been proposed to be involved, but the step(s) between the putative intermediate benzoic acid and phenylalanine remain uncertain. Microorganisms produce SA in two steps via the isochorismate pathway. Verberne et al. (2000) proposed that this pathway might also function in plants, and showed that by introduction of microbial genes this pathway can be introduced in tobacco, making the plant more resistant against viral and fungal infections. The effect of the constitutive expression of salicylate and TMV infection in tobacco was studied by means of NMR-metabolomics. This metabolomics approach showed clear differences for the production of phenylpropanoids. In case of TMV infection clear differences between infected leaves and SAR leaves could be detected. In Arabidopsis it was shown that a gene encoding isochorismate synthase is correlated with the formation of SA and SAR. But still the direct chemical evidence is missing that SA is derived from isochorismate and not from phenylalanine. *Catharanthus roseus* (L.) G. Don. cell cultures produce both SA and the closely related 2,3-dihydroxybenzoic acid (DHBA) upon elicitation. Feeding the cultures with 1-^{13}C-glucose we found by means of ^{13}C-NMR-spectrometry that DHBA has a labeling pattern as expected for the isochorismate pathway [3]. However, in case of SA the labeling in the aromatic ring was such that it might be a mixture of both pathways. **References**: 1. Verberne, M. Verpoorte, R. *et al.* (2000), Nature Biotechnology 18: 779 – 783. 2. Verberne, M.C. *et al.* (1999), Salicylic acid biosynthesis. In: Biochemistry and Molecular Biology of Plant Hormones. New Comprehensive Biochemistry. Vol. 33. P.J.J. Hooikaas, M.A. Hall, and K.R. Libbenga, (Eds.) Elsevier, Amsterdam, 1999, pp. 295 – 312. 3. Budi Muljono, R.A. *et al.* (2002), Plant Physiol. Biochem. 40: 231 – 234.

Short Lectures

1. Drug Discovery from Natural Products

S 001

Evaluation of reversible antiandrogenic and antispermatogenic activities of Annona squamosa (Linn) stem bark methanol extract in male albino rats

Gupta RS, Sharma A, Rehwani H
Center for Advanced Studies, Reproduction Physiology Section, Department of Zoology, University of Rajasthan, Jaipur-302004, India

The present study was undertaken to evaluate antiandrogenic activities of (methanol stem bark extract) Annona squamosa L. (Annonaceae) with their respective reversibility in male albino rats. Adult male albino rats were gavaged with 100% methanol extract of Annona squamosa stem bark at the dose level of 50, 100 and 200 mg/rat/day for 60 days. Fertility test was performed before and after 55th day of treatment. Sperm dynamics in cauda epididymides and testis were assessed. Biochemical and histological analysis were also done in blood, serum and in reproductive organs. Recovery of fertility was followed to evaluate the reversibility of drug nature. Annona squamosa stem bark extract brought about a significant decrease in the weights of testes and accessory reproductive organs. Sperm motility and density was also reduced significantly. Significant reduction was seen in protein, sialic acid and glycogen content of testis as well as fructose content of seminal vesicle. An increased level of cholesterol was seen in testis. The blood and serum parameters were found to be within the normal range whereas the serum testosterone levels decreased significantly. The stem bark extract feeding caused a marked reduction in the number of spermatocytes and spermatids in the testis. The diameter of the seminiferous tubules and the numbers of mature Leydig cells were also decreased whereas, number of degenerating cells increased proportionately. In conclusion Annona squamosa stem bark extract have an antiandrogenic and antispermatogenic activity, which were reversible after withdrawal of drug. Acknowledgment: Authors are thankful to the Head, Department of Zoology, Prof. N.K. Lohiya Coordinator CAS, Department of Zoology for providing the neccessory facilities and UGC, Regional Office, Bhopal, INDIA for financial support.

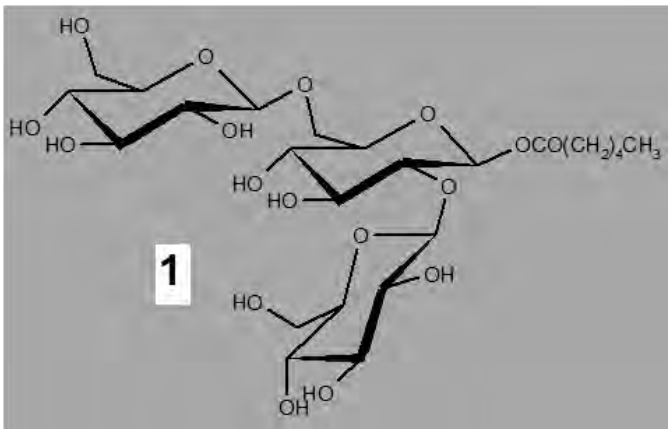
S 002

Phytochemical investigation of Noni fruit (Morinda citrifolia) and Noni-derived commercial products

Potterat O[1], Dalsgaard P[1], Dieterle F[1], Paululat T[2], Kühn T[3], Hamburger M[1]
[1]Institut für Pharmazeutische Biologie, Universität Basel, CH-4056 Basel, Switzerland; [2]University of Siegen D-57068 Siegen, Germany; [3]Bruker Biospin AG, NMR Division, Industriestrasse 26, CH-8117 Fällanden, Switzerland

In recent years, the fruit of the noni tree (*Morinda citrifolia* L., Rubiaceae), a plant used in the Polynesian traditional medicine, has become increasingly popular as a food supplement. Since its approval in 2003 by the European Commission as a novel food, numerous noni products have become available in Europe and are mostly distributed via the internet market. Products are promoted with numerous health claims. However, information about the constituents of the fruit remains scarce [1]. In a phytochemical re-investigation, we identified several new di- and trisaccharide fatty acid esters such as **1** from a fruit extract. Isolation was achieved by a combination of Sephadex LH20, HSCCC and HPLC-ELSD. The composition of various commercial noni capsules and juices was analyzed by LC-

MS and HPTLC. Fatty acid esters, linoleic acid, scopoletin, ursolic acid and asperulosidic acid could be identified in all the investigated products. There were, however, distinct differences in the chromatographic profiles of dry extracts and juices.



Reference: 1. Su, B.-N. *et al.* (2005), J. Nat. Prod. 68: 592 – 595.

S 003

Antigenotoxic effects of Satureja hortensis L on rat lymphocytes exposed to oxidative stress

Behravan J[1,2], Mosaffaa F[2], Karimi G[3], Iranshahi M[2]
[1]Biotechnology Research Centre, Bu-Ali Research Institute, Mashhad University of Medical Sciences (MUMS), Mashhad, IRAN; [2]Department of Pharmacognosy and Biotechnology, School of Pharmacy, MUMS, P O Box 91775 – 1365, Mashhad, IRAN; [3]Department of Pharmacodinamy and Toxicology, School of Pharmacy, MUMS, P O Box 91775 – 1365, Mashhad, IRAN

DNA damage and oxidative stress are widely recognized as major factors in many degenerative diseases and aging [1, 2]. Numerous reports have demonstrated that plant products possess a variety of in vitro antioxidant properties and, that the consumption of food or beverages rich in antioxidant phytochemicals resulted in positive effects on human health and aging process [3]. The protective properties of *Satureja hortensis* L. on the rat lymphocytes DNA lesions were tested. Lymphocytes were isolated from blood samples taken from healthy rats. DNA breaks and resistance to H_2O_2-induced damage were measured with the comet assay [4]. Rat lymphocytes were incubated in *S. hortensis* ethanolic extract (SHE) (0.05, 0.1, 0.5, 1 and 2.5 mg/mL), essential oil (SHEO)(0.05, 0.1, 0.5, 1 and 2.5 µL/mL), H_2O_2 (50, 100 and 200 µM), a combination of H_2O_2 (200 M) with either SHE (1, 2.5 mg/mL) or SHEO (1, 2.5 µL/mL) at 4 °C for 30 min, and the extent of DNA migration was measured using a single-cell microgel electrophoresis technique under alkaline conditions. Treatment of rat lymphocytes with SHE or SHEO resulted in significant reduction of H_2O_2-induced DNA damage compared to controls. SHE exhibited a significant ($P < 0.01$) inhibitory effect on oxidative DNA damage at 2.5 mg/mL. SHEO (1 and 2.5 µL/mL) also showed significant inhibitory effects ($P < 0.01$) on H_2O_2 induced chromosomal damage. In conclusion both the ethanolic extract and the essential oil of the plant were able to reverse the oxidative damage on rat lymphocytes induced by hydrogen peroxide. *Acknowledgements:* Financial support from Mashhad University of Medical Sciences (MUMS) is greatly acknowledged. **References**: 1. Ceruti, P. (1985), Science 227: 375 – 378. 2. Ames, B.N., Gold, L.S. (1990), Med. Onc. 7: 69 – 85. 3. Hertog, M.G., Feskens, E.J., et al. (1993), Lancet 342: 1007 – 1011. 4. Singh, N.P. (1988), Exp. Cell. Res. 175: 184 – 91.

S 004

Synthesis and preliminary colon cancer chemoprevention evaluation of a novel prodrug of 4'-geranyloxy-ferulic acid, active principle of Acronychia baueri Schott

Epifano F[1], Curini M[2], Genovese S[2], Menghini L[1], Tanaka T[3]
[1]Dipartimento di Scienze del Farmaco, Via dei Vestini 31, 66013 Chieti Scalo, Italy; [2]Dipartimento di Chimica e Tecnologia del Farmaco, Sezione di Chimica Organica, Via del Liceo, 06123 Perugia, Italy; [3]Department of Oncologic Pathology, Kanazawa Medical University, 1 – 1 Daigaku, Uchinada, Ishikawa 920 – 0293, Japan

4'-Geranyloxy-ferulic acid is a prenyloxy-cinnamic acid isolated in 1966 from the bark of *Acronychia baueri* Schott, an Australian small tree belonging to the family of Rutaceae [1]. Although known for four decades, only in the last five years some of the pharmacological properties of this secondary metabolite and its synthetic derivatives began to be characterized and some ester derivatives of 4'-geranyloxy-ferulic acid showed interesting pharmacological properties as dietary cancer chemopreventive agents in rodents [2, 3]. In order to achieve a novel approach in the treatment of colon cancer by dietary administered drugs, we carried out the synthesis of a novel prodrug of 4'-geranyloxy-ferulic acid based on the incorporation of the latter into a peptide sequence of general formula X-Ala-Pro-COOH (4'-geranyloxy-feruloyl-L-alanyl-L-proline) structurally built to be hydrolized by intestinal angiotensin-converting enzyme (ACE) so reaching in high concentration the large bowel. The synthesis was accomplished in 7 steps employing commercially available ferulic acid as starting material and led to obtain the desired prodrug in 56.6 % overall yield. Colon cancer was induced in rats by treatment with azoxymethane (AOM) and dextrane sodium sulphate (DSS) in the basal diet for 1 week. The prodrug was then administered in the diet for 19 weeks at two concentration levels, 0.01 % and 0.05 %. Preliminary biological evaluation on colonic tumors developed revealed that at both concentration 4'-geranyloxy-feruloyl-L-alanyl-L-proline was a powerful colon cancer chemopreventive agent with a reduction in cancer incidence of 51.9 % ($p < 0.05$) and 76.6 % ($p < 0.01$) respectively. **References**: 1. Prager, R.H., Thregold, H.M. (1966), Aust. J. Chem. 19: 451. 2. Han, B.S. *et al.* (2001), Jpn. J. Cancer Res. 92: 404. 3. Tanaka, T. *et al.* (2003), Oncology 64: 166 and references cited herein.

S 005

Antitumor activity of Ailanthus excelsa (Roxb.)

Said A[1], Tokuda H[2], Farag A[3], Huefner A[4], Rashed K[1]
[1]Department of Pharmacognosy, National Research Centre, Dokki, Cairo, Egypt; [2]Department of Biochemistry, Kyoto Prefectural University of Medicine, kyoto 602 – 0841, Japan; [3]Department of Organic Chemistry, Faculty of Science, Cairo University, Egypt; [4]Institute for Pharmaceutical Chemistry and Pharmaceutical Technology, University of Graz, Schubertstr.1, A8010 Graz, Austria

In our search for antitumor agents from natural sources, we have assayed the antitumor activity of canthin-6-one isolated from chloroform fraction of methanol (70 %) of *Ailanthus excelsa* (Roxb) stem bark of the Egyptian origin as will as successive extracts. The chloroform extract showed strong inhibitory effect on short term *in vivo* assay for antitumor promoters, Epstein-Barr virus early antigen (EBV-EA) induction assay [1], compared with other fractions petroleum ether, diethyl ether and methanol (70 %). Canthin-6-one also showed the strong activity on this assay against TPA induction. Moreover, these useful materials were investigated for the inhibitory effects in two–stage mouse skin carcinogenesis test. Chloroform extract and its active compound canthin-6-one decrease actually the average number of papillomas per mouse and percent papillomas in the promoting stage. These materials were found to exhibit the excellent anti-tumor promoting activity in the *in vivo* carcinogenesis test [2]. This study provides relevant information regarding the development of the biological effects of alkaloids.

References: 1. Kubota *et al.* (1997), Cancer Lett. 113: 165 – 168. 2. Henle, G., Henle, W. (1966), J. Bacteriol. 91: 1248 – 1256.

S 006

Active anti-head lice component from custard apple seed

Gritsanapan W[1], Intaranongpai J[1], Chavasiri W[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy Mahidol University, 447 Sri-Ayudthaya Rd, Ratchatevi, Bangkok 10400, Thailand; [2]Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

Seeds of custard apple (*Annona squamosa* Linn., Annonaceae) have been used for anti-head lice for a long time. In Thailand, the petroleum ether seed extract prepared as a cream preparation was reported to kill 93 % of head lice within 3 hours [1]. Twenty grams of the 20 % w/w freshly prepared cream can kill 94.5 ± 9.1 % of head lice within 3 hours when applied to school girls and the cream is biologically stable for at least 12 months [2, 3]. There have been no reports on chemically active anti-head lice component of this plant. The present study is focused on the separation and identification of the active compound from the hexane extract of the seeds of custard apple. Chromatographic and spectroscopic techniques revealed that a major component of the hexane seed extract was a triglyceride with one oleate ester (with 2 unknown acyl moieties). The separated pure triglyceride and the crude hexane extract which were separately diluted with coconut oil (1:1), contained 22.25 and 11.49 mg of the triglyceride, respectively were tested *in vitro* for anti-head lice activity and found that they could kill all tested head lice within 11 and 30 minutes, respectively. The triglyceride with one oleate ester was the active compound against human head lice. It could be used as a marker for quality control and standardization of custard apple seeds, the extracts and anti-head lice preparations from the seeds of this plant. **References**: 1. Areekul, M., Chaikledkaew, U. (1944), Antiparasitic cream from *Annona squamosa* Linn. A special project submitted in partial fulfillment of the requirement for the degree of Bachelor of Science in Pharmacy. Mahidol University. Bangkok. 2. Gritsanapan, W. *et al.* (1998), Studies of stability and effectiveness of intensive hair masks from *Annona squamosa* seed extract. 50th IPC and 17th FAPA Congress, Mumbai, India. 3. Tiangda, CH. *et al.* (2000), Southeast Asian J. Trop. Med. Public Health 31 (Suppl 1): 174 – 7.

S 007

Antibacterial proanthocyanidins isolated from the Australian medicinal plant, Planchonia careya (F. Muell.) R. Knuth (Lecythidaceae)

McRae J[1], Yang Q[2], Crawford R[1], Palombo E[1]
[1]Environment and Biotechnology Centre, Faculty of Life and Social Science, Swinburne University, P.O. Box 218, Hawthorn, 3122 Australia; [2]CSIRO Molecular and Health Technologies Division, Bag 10, Clayton South, 3169, Australia

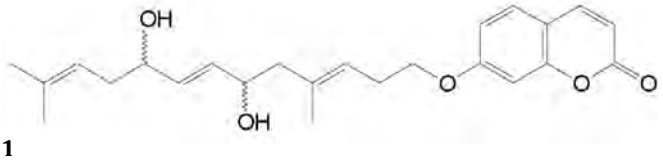
One of the many plants traditionally used for wound healing by the indigenous peoples of northeastern Australia is *Planchonia careya* (F. Muell) R. Knuth. Based on this knowledge, investigation was carried out into the antibacterial activity of the leaf extracts of this species. The chemical constituents responsible for the observed activity were then isolated. The plate-hole diffusion method was used to evaluate the antibacterial activity of the crude aqueous and methanol extracts against a range of Gram positive and Gram negative bacteria. Based on these assays, HPLC-piloted activity-guided fractionation was carried out to isolate the active compounds from the crude aqueous extract. Separation was performed using XAD-16 media, followed by a 20 µm grade Chromatorex® C18 column with a 10 % methanol/ water mobile phase. The active fractions from these columns were separated further with Sephadex LH-20 gel in methanol, and final isolation was attained using an Alltima Preparative C18 (5 µm) column in a 5 % methanol/ water mobile phase. Elucidation of the isolated active compounds was achieved by UV, 1-D NMR (^{1}H, ^{13}C), and 2-D NMR (COSY, HSQC, HMBC) techniques. This analysis yielded (+)-gallocatechin and the prodelphinidin, gallocatechin-(4α-8)-gallocatechin. The structures were confirmed by reference to previously reported NMR spectra of these compounds (1, 2). Further examination of the UV profiles of other active fractions and of the crude methanol extract suggests that the minor active constituents of *P. careya* are also of the flavonoid class. The isolation of these known antibacterial compounds confirms the traditional use of *P. careya* in wound healing. **References**: 1. Sun, D., *et al.* (1987), Phytochem. 26: 1825 – 1829. 2. Cai, Y., *et al.* (1991), Phytochem. 30: 2033 – 2040.

S 008

Antidermatophytic prenylated coumarins from asafetida

Houghton PJ[1], Ismail KM[1], Maxia L[2], Appendino G[2]
[1]Pharmacognosy Research, Pharmaceutical Sciences Research Division, KCL, 150 Stamford St, SE1 9NH London, UK; [2]DISCAFF, 28100 Novara, Italy

Asafoetida is a resinous substances with a smell similar to garlic, which is obtained by drying the exudates from various species of *Ferula* growing in northern Iraq and Iran and surrounding countries. It is widely used in cooking in India and is used medicinally for gastro-intestinal complaints and for treating skin diseases. The botanical source of commercial samples of asafoetida is not easy to determine since several species of *Ferula* exist. Samples of asafetida with proven source were obtained from the pharmacognosy museum of King's College London and compared with some commercial samples obtained from Asian shops in the UK, India and Syria. Samples were examined by TLC and for antifungal activity using serial dilution assay in microtitre plates with two dermatophytes, *Microsporeum gypseum* and *Trichophyton interdigitale* [1]. The most active sample was obtained from India and on TLC conformed most closely with a museum sample from *F. foetida* Regel. From this sample nine prenylated coumarins were isolated and were tested against the two fungal species. Four of the compounds exhibited strong antifungal activity against the dermatophytes with 5,8 dihydroxyumbelliprenin **1** being most active with MIC of 10mM, the positive control miconazole having MIC of 0.5mM. No compounds of this type have previously shown antifungal activity.



1

Reference: 1. Mensah, A.Y. *et al.* (2000), J. Nat. Prod. 63:1210 – 1213.

S 009

Antiviral compounds from Icelandic lichens

Omarsdottir S[1], Óladóttir AK[1], Árnadóttir T[2], Ingólfsdóttir K[1]
[1]Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland; [2]Department of Virology, Landspitali-University Hospital, IS-101 Reykjavik, Iceland

Although one third of prescription drugs are derived from natural sources [1], lichens have only been investigated to a limited extent from a pharmacological perspective. The aim of the study was to investigate whether antiviral compounds could be found in Icelandic lichens, to isolate active compounds in a purified form and elucidate their chemical structure and to confirm antiviral activity of isolated compounds and compare it with that of marketed antiviral drugs. Extracts were made from ten lichen species and screened for antiviral activity in vitro against three different viruses by using the plaque reduction assay (PRA). Two compounds exhibiting potent antiviral activity against respiratory syncytial (RS) virus were isolated and purified and their activity confirmed using both the PRA method and ELISA. The compounds were the depsidone salazinic

acid from *Parmelia saxatilis* (L.) Ach. and the benzyl depside alectorialic acid from *Alectoria nigricans* (Ach.) Nyl. The activity of both lichen compounds was more potent than that of the marketed drug ribavirin, which is used to treat serious respiratory conditions resulting from RS infection. The IC_{50} value for salazinic acid as determined by ELISA was 11.9 μg/mL, for alectorialic acid 17.0 μg/mL and for ribavirin 22.9 μg/mL. The lichen compounds were no cytotoxic at antiviral concentrations. Activity against herpes viruses I and II was less potent than activity against RS virus. *Acknowledgements*: Icelandic Council of Science, University of Iceland Research Fund, The Icelandic Research Fund for Graduate Students. **Reference**: 1. Kinghorn, A.D. (2001), J. Pharm. Pharmacol. 53: 135 – 148.

S 010

Inhibitory effects of cucurbitacin R on lymphocyte proliferation and cytokine production

Escandell JM[1], Recio MC[1], Gil R[2], Merfort I[3], Ríos JL[1]
[1]Departament de Farmacologia, Facultat de Farmacia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain; [2]Departament de Patologia, Facultat de Medicina, Universitat de València, Av. Blasco Ibáñez 15, 46010 Valencia, Spain; [3]Department of Pharmaceutical Biology and Biotechnology, University Freiburg, Freiburg, Germany

Cucurbitacin R (CCR) isolated from tayuya roots reduced both the acute and subchronic inflammation in different experimental models [1]. In addition, its acetyl-derivative showed inhibitory effects in a model of adjuvant-induced arthritis [2]. In order to gain insight into the mechanism of action of CCR, we studied its effect not only on lymphocyte proliferation induced by phytohemagglutinin (PHA), but also on the lymphocyte cell cycle. In addition, we examined its influence on the production of cytokines, and the effects on cyclins A_1, B_1, D_2 and E_2 and the transcription factors involved in inflammation. CCR strongly inhibited lymphoproliferation with an IC_{50} value of 16 μM, arresting the cell cycle in the G_0 phase. Inhibition of lymphoproliferation and on cell cycle disappeared with time. Western blot analysis was used to show CCR's effects on assayed cyclins. The production of mediators such as IL-2, IL-4, IL-10, TNF-α and IFN-γ by human lymphocytes was also significantly inhibited by CCR, with IC_{50} values of 18 μM for interleukins, 12 μM for IFN-γ, and 15 μM for TNF-α. The PCR analysis showed a clear inhibition of all these cytokines. In Jurkat cells, a total inhibition of the nuclear factor of T activated cells (NF-AT) was observed at a 50 μM concentration of CCR. AP-1 remained unaffected. These results indicate that lymphocyte proliferation is inhibited by CCR through NF-AT blocking, which reduces cytokine production. *Acknowledgements*: J.M.E. is recipient of a grant from the Generalitat Valenciana. This work was supported by the Spanish Government (SAF2002 – 00723) **References**: 1. Recio, M.C. *et al.* (2004), Planta Med. 70: 414 – 420. 2. Escandell, J. *et al.* (2006), Eur. J. Pharmacol. 532:145 – 154.

S 011

Four New Natural Products from Mongolian Medicinal Plants Scorzonera divaricata and Scorzonera pseudodivaricata (Asteraceae)

Edrada RA[1], Tsevegsuren N[1,2], Lin W[3], Ebel R[1], Torre C[1], Ortlepp S[1], Wray V[4], Proksch P[1]
[1]Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb. 26.23, 40225 Düsseldorf, Germany; [2]Department of Organic and Food Chemistry, Faculty of Chemistry, National University of Mongolia, Ulaanbaatar, Mongolia; [3]State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing, P. R. China; [4]Gesellschaft für Biotechnologische Forschung, Braunschweig

Eleven *Scorzonera* species are found in Mongolia, one species is endemic, four of which are sub-endemic [1]. Two of these, *Scorzonera pseudodivaricata* Lipsch., a sub-endemic perennial species, and *S. divaricata* Turcz. are used in the Mongolian traditional medicine [2]. Since only a few papers have been published on this genus and no previous chemical work has been recorded on *S. divaricata* and *S. pseudodivaricata*, this arose our interest to do further phytochemical work on these plants. Investigation of the (diphenylpicrylhydrazyl) DPPH-active EtOAc extract of aerial parts of *S. divaricata*, which showed radical scavenging activity, yielded two new 1-*O*-caffeoylquinic acid derivatives. From the cytotoxic EtOAc extract of aerial parts of *S. pseudodivaricata*, a novel phenolic glucoside and an unsual terpene lactone were isolated. The structures of the new compounds were unambiguously established based on NMR spectroscopic (^1H, ^{13}C, COSY, HMBC) and mass spectrometric (ESIMS) data. **References**: 1. Grubov, V. I. (1982), Key to the Vascular Plants of Mongolia, Leningrad, Nauka, pp. 263 – 264. 2. Ligaa, U. (1996), Medicinal Plants from Mongolia Used in Mongolian Traditional Medicine, KSA Press, p. 337.

S 012

Secondary metabolites of Globularia species from the Flora of Turkey

Kirmizibekmez H[2,1], Calis I[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100, Ankara, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, TR-34755, Erenkoy, Istanbul, Turkey

The genus *Globularia* (formerly Globulariaceae, now "new" Plantaginaceae) is represented in the flora of Turkey by nine species, three of which are endemic [1, 2]. Some of these species are used as diuretic, laxative, stomachic, tonic and for the treatment of haemorrhoids in Anatolian folk medicine [3, 4]. Among these species, *G. alypum* is widely used in indigenous systems of medicine in some Mediterrean countries, especially in Morocco as a hypoglycaemic agent, laxative, cholagogue, stomachic and purgative [5]. As a part of our interest on Turkish medicinal plants we have investigated the secondary metabolites of seven *Globularia* species, *G. trichosantha* Fosch. Et Mey., *G. orientalis* L., *G. cordifolia* L., *G. dumulosa* O. Schwarz, *G. davisiana* O. Schwarz, *G. sintenisii* Hausskn. et Wettst. and *G. alypum* L.. Various chromatographic studies (VLC, MPLC, OCC) on the MeOH (or EtOH) extracts of the aerial or underground parts of the species resulted in the isolation of 58 different compounds, which can be categorized under eight chief groups; 27 iridoids, 14 phenylethanoid glycosides, 6 flavone glycosides, 4 lignan glycosides, 3 sugar esters, 2 sterols, an acetophenone glycoside, and a phenylpropanoid glycoside. The structures of the isolates were elucidated by 1D and 2D NMR and MS experiments as well as chemical methods. 15 of the isolated compounds were new for nature, while many of them were new to the genus *Globularia*. The occurrence of such diverse compounds in *Globularia* might be of great chemotaxonomical importance at both the genus and family level. Recent study (6) based on the DNA sequence of the genus *Globularia* placed this genus under the "new" Plantaginaceae family, which was in good accordance with our results. *Acknowledgement:* Hacettepe University, Scientific Research Unit (Project number: 02 G 076) **References**: 1. Edmondson, J.R. (1982), *Globularia* L., in Flora of Turkey and the East Aegean Islands. Vol. 7 (Ed. Davis P.H.), University Press, Edinburgh. 2. Duman, H. (2001), Bot. J. Linn. Soc. 137: 425 – 428. 3. Baytop, T. (1999), Therapy with Medicinal Plants in Turkey (Past and Present), Nobel Tip Kitapevleri, Istanbul, p. 371. 4. Sezik, E. *et al.* (1991), J. Ethnopharmacol. 35: 191 – 196. 5. Bellakhdar, J. *et al.* (1991), J. Ethnopharmacol, 35: 123 – 143. 6. Albach, D.C. *et al.* (2005), Am. J. Bot. 92: 297 – 315.

S 013

Adaptogens modify stress response by suppressing the increase of p-SAPK, nitric oxide and cortisone in the blood of rabbits

Panossian A[1], Hambartsumyan M[2], Hovhanissyan A[2], Wikman G[1]
[1]Swedish Herbal Institute Research and Development, Prinsgatan 12, SE-413 05 Göteborg, Sweden; [2]ExLab" Expert Analytical Laboratory of Armenia Drug Agency, Komitas Ave. 49/4, 375051 Yerevan, Armenia

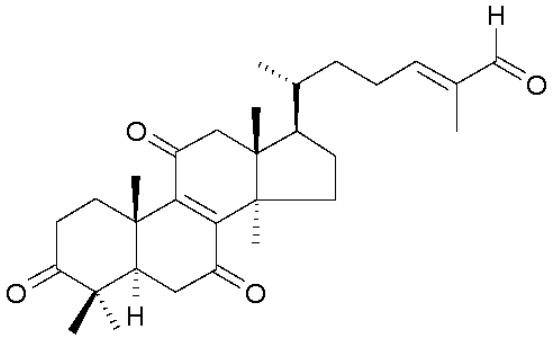
Adaptogens possess anti-fatigue and anti-stress activities that can increase mental and physical working performance against a background of fatigue or stress. The aim of the present study was to ascertain which mediators of stress response are significantly involved in the mechanisms of action of adaptogens, and to determine their relevance as biochemical markers for evaluating anti-stress effects in laboratory animals subjected to immobilisation stress. Basal blood levels of cortisone, testosterone, nitric oxide, prostaglandin E_2, thromboxane B_2, leukotriene B_4, stress-activated protein kinase (SAPK), and phosphorylated-SAPK (p-SAPK/p-JNK) were determined in three groups of rabbits. Group A and B animals were treated orally for 7 days with extracts of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., *Schizandra chinensis* (Turcz.) Baill., *Panax ginseng* C.A. Meyer, *Bryonia alba* L., *Rhodiola rosea* L. and active component rhodioloside; group C received only placebo. Ten minutes after the final treatment, group A and C animals were immobilized for 2 hours, and blood levels of markers in rabbits of all groups re-determined. Only p-SAPK, cortisone and nitric oxide increased significantly (200 – 300 % > basal levels) following immobilization stress (group C). However, following repeated administration of adaptogens, basal levels of these markers remained practically unchanged during acute stress (group A). *S. chinensis, R. rosea* and rhodioloside were the most active inhibitors of p-SAPK formation (group B). It is speculated that the positive effects of adaptogens on mental performance in stress may be associated with the inhibition of p-SARK formation, and that such activity might be beneficial in neurodegenerative disorders associated with loss of neurons in brain regions involved in learning and memory.

S 014

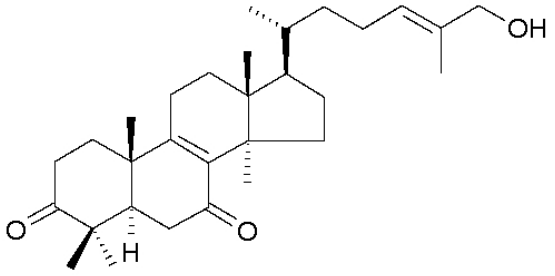
Antiviral Terpenoid Constituents of Ganoderma pfeifferi Bres

Lalk M, Niedermeyer THJ, Mentel R, Lindequist U
Institute of Pharmacy, Ernst-Moritz-Arndt-University, Friedrich-Ludwig-Jahn-Str. 17, 17487 Greifswald, Germany

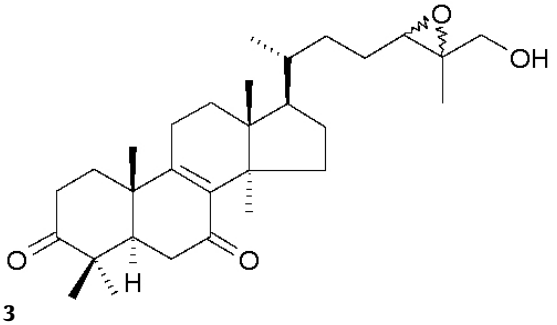
Ganoderma pfeifferi Bres., a weak parasitic and later saprophytic basidiomycete, is a fungus only found in Europe. It is living preferentially on *Fagus* L. and some other deciduous trees such as *Aesculus* L., *Acer* L., *Fraxinus* L. and *Quercus* L.. In contrast to *G. lucidum* (Fr.) Karst. and *G. applanatum* (Persoon) Patouillard, from which a number of biologically and pharmacologically interesting triterpenes, steroids and polysaccharides have been isolated [1], *G. pfeifferi* is one of the phytochemically poorer examined species of the family Ganodermataceae [2]. As part of our continuing interest in compounds from *G. pfeifferi,* four sterols and ten triterpenes were isolated from a DCM-extract of the fruiting bodies of this mushroom. In addition to compounds common in mushrooms and other Ganodermataceae, we isolated the previously unknown triterpenoid constituents 3,7,11-trioxo-5α-lanosta-8,24-diene-26-al **1** (Lucialdehyde D), 5α-lanosta-8,24-diene-26-hydroxy-3,7-dione **2** (Ganoderone A), and 5α-lanosta-8-ene-24,25-epoxy-26-hydroxy-3,7-dione **3** (Ganoderone C). The evaluation of the antiviral properties of the isolated compounds showed strong inhibition of the growth of *Herpes simplex* and influenza viruses with IC_{50} values between 0.01 and 3.0 μg/mL [3].



1



2



3

References: 1. Gao, Y.; Zhou, S.; Huang, M.; Xu, A. (2003), Int. J. Med. Mushrooms 5: 235. 2. (a) Mothana, R.A.A., Jansen, R., Jülich, W.-D., Lindequist, U. (200), J. Nat. Prod. 63: 416, (b) Mothana, R.A.A., Ali, N.A.A., Jansen, R., Wegner, U., Mentel, R., Lindequist, U. (2003), Fitoterapia 74: 177. 3. Niedermeyer, T.H.J; Lindequist, U.; Mentel, R.; Gördes, D.; Schmidt, E.; Thurow, K.; Lalk, M. (2005), J. Nat. Prod. 68: 1728.

S 015

In search of promising antimalarial drugs: Detection of heme-based adducts induced in complex matrixes from Brazilian plants using HPLC-DAD

Castro-Gamboa I[1], Pauletti PM[1], Cavalheiro AJ[1], Siqueira DHS[1], da S. Bolzani V[1]
[1]Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais – NuBBE, Universidade Estadual Paulista – UNESP – Instituto de Química, Departamento de Química Orgânica, Rua Prof. Francisco Degni s/n – 14.800 – 900 – Araraquara – São Paulo – Brasil

The development of fast and efficient detection and HPLC separation methods on a bioprospection program is crucial for speeding-up the selection of natural matrixes. Based on this goal, the induction of an *in situ* heme adduct in crude plant extracts from Brazilian Cerrado and Atlantic Forest turned out to be a powerful tool, foretelling if a crude extract contains promising molecules that may have antima-

larial or antileishmanial properties. Studies of heme adducts were reported using known drugs such as quinine and artemisinin. We initially selected 75 plants from our bank of extracts based on chemosystematics, reported antimalarial activities as well as ethnopharmacological data. Species such as *Arrabidaea samydoides* (Cham.) Sandwith (Bignoniaceae), *Strychnos pseudoquina* St. Hil. (Loganiaceae), *Garcinia gardineriana* Miers ex Planch. Et Triana (Clusiaceae), *Zanthoxyllum rhoifolium* Lam. (Rutaceae), *Sorocea bonplandii* Baill. Burg. (Moraceae) and *Bidens segetum* Mart. ex Colla (Asteraceae) were some of the matrixes that showed adduct formation when incubated with hemine. Through the observation of retention time shifts and comparison of UV spectra after adduct induction, we were able to pin-point the responsible molecules and thus select plant extracts for further specific studies. *C*-glucosylxanthones isolated from *A. samydoides* were the metabolites responsible for adduct formation. *Acknowledgements:* To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), CAPES and CNPq for research funding.

S 016

Inhibition potential of natural based products against Chlamydia pneumoniae infection

Alvesalo J[1,2], Vuorela HJ[2], Tammela P[1,2], Leinonen M[3], Saikku P[4], Vuorela PM[5]

[1]Drug Discovery and Development Technology Center, Faculty of Pharmacy, P.O. Box 56, FI-00014 University of Helsinki, Finland; [2]Division of Pharmaceutical Biology, Faculty of Pharmacy, P.O. Box 56, FI-00014 University of Helsinki, Finland; [3]National Public Health Institute, PO Box 310, FI-90101, Oulu, Finland; [4]Department of Microbiology, PO Box 5000, FI-90014 Oulu University, Finland; [5]Department of Biochemistry and Pharmacy, Åbo Akademi University, Tykistökatu 6A, FI-20520 Turku, Finland

A large number of antimicrobial substances, phytoalexins, are found in nature and they form a variable group of compounds playing important role in the natural defence of living organism. This study was carried out to evaluate whether several groups of natural, natural derived synthetic compounds or natural extracts have an impact on *C. pneumoniae* infection, *in vitro*. 37 % (21/57) of the tested compounds were highly active; 28 % (16/57) active; 11 % (6/57) moderately active; 24 % (14/57) inactive. Highly active compounds were found in many compound groups, but the most active group was that of gallates. Inactive compounds could also be found in many compound classes, but among synthetic coumarins, many compounds had 0 % inhibition. *Chlamydia pneumoniae* is a common cause of acute upper and lower respiratory tract infections, including pharyngitis, sinusitis and pneumonia, but it also has a tendency to cause chronic infections. There is augmenting evidence on the involvement of chronic *C. pneumoniae* infection in the atherosclerotic diseases like coronary heart disease. Even though the acute infections can be successfully treated with several antibiotics, the eradication of chronic *C. pneumoniae* infection seems to be exceedingly difficult. High doses and prolonged treatment is often needed to achieve clinical cure and there is still a risk of the persistence of *C. pneumoniae* in the tissues after treatment. Thus, it is extremely important to find new compounds that can be used in the treatment or prophylaxis of *C. pneumoniae* infections.

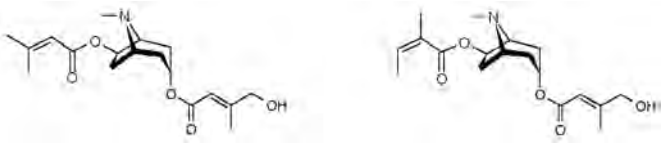
S 017

Two new isomeric tropane alkaloids from Schizanthus tricolor identified by capillary NMR

Humam M[1], Kehrli T[1], Jeannerat D[2], Muñoz O[3], Christen P[1], Hostettmann K[1]

[1]Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; [2]Department of Organic Chemistry, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; [3]Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

The genus *Schizanthus* (Solanaceae) belongs to the tribe Salpiglossidae and is endemic to Chile. It comprises 12 species growing in the northern part, and down to the Andes. Beside pyrrolidine alkaloids, this genus is characterized by the presence of ester derivatives with isomeric C_5 acids, namely angelic, senecioic, tiglic, itaconic and mesaconic acids as well as by dimers and trimers [1]. This esterification leads to the formation of numerous structural and configurational isomers. Two new isomeric tropane alkaloids of 337 Da were isolated from the aerial parts of *Schizanthus tricolor* Grau and Gronbach, namely 3α-*trans*-hydroxysenecioyloxy-7ß-senecioyloxytropane and 3α- *trans*-hydroxysenecioyloxy -7ß-angeloyloxytropane. These isomers were characterized by IR, HRMS and the structures were established by NMR, CapNMR, GC-MS and LC/UV-APCI-MS[3].



3α-*trans*-hydroxysenecioyloxy-7ß-senecioyloxytropane
3α-*trans*-hydroxysenecioyloxy-7ß-angeloyloxytropane

These two isomers are acetylcholinesterase inhibitors, and atypical to the genus *Schizanthus* as the alcohol group is attached to the senecioyl moiety and not to the tropane nucleus. In this work, the structure elucidation of the two isomers, as well as their separation and isolation are discussed. **Reference**: 1. Lounasmaa, M., Tamminen T. (1993), The Alkaloids, Cordell G. A. Ed, Academic Press, San Diego 44: 1 – 113.

S 018

Protoberberine alkoloids from the hairy root cultures of Tinospora cordifolia transformed with Agrobacterium rhizogens

Verma R[1], Juvekar AR[1], Gopalkrishnan R[2], Eapen S[2]

[1]Department of Pharmaceutical Sciences and Technology, Mumbai University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019, India; [2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center,Trombay,Mumbai-400085,India

Recently the production of secondary metabolites using plant cells has become the subject of extended research. These secondary metabolites are a high value and low yield compounds. Evaluation of its medicinal value demands considerable attention. "Hairy" root culture technology represents an advantageous method for the downstream processing of such important bioactive components. Thus, in the present study hairy roots of *Tinospora cordifolia* were induced from the shoot cultures by transformation with *Agrobacterium rhizogenes* strain 2402 on a solid YMB medium. Roots were subcultured on liquid MS medium containing B_5 vitamins and 3 % sucrose, devoid of any plant growth hormone. Optimization of various growth parameters like light, precursor, and elicitor treatment was studied for a period of 28 days. In addition, a time course study was also carried out to understand a basic growth pattern and occurrence of alkaloids in the transformed roots. Time course analysis revealed that, berberine production was maximum on the 21st day. However, it was observed that a higher amount of berberine

(0.034 %) was produced in the cultures treated with 500 mg/L of L-Tyrosine as precursor, than the control. Jasmonate elicitation was found best at the concentration of 200 μmoles/mL (0.047 %). Moreover the berberine content in hairy roots was comparable to that produced by the roots of parent plant. HPLC and HPTLC results show the presence of jatrorrhizhine, in trace amounts. Thus it can be concluded that, the hairy root cultures form a promising source for the production of berberine and related compounds. **References**: 1. Hyeon-J., Jack, M.,(2002), Plant Cell Tissue Organ Culture 69:259 – 269. 2. Kamada, H. *et al*, (1996), Plant Cell Rep. 5: 239 – 242. 3. Ravishankar, G.A., Venkatraman, L.V. (1997), Biotechnological Application of Plant Tissue and Cell Culture, Oxford and IBH publishings Co, New Delhi, pp. 74 – 90.

S 019

Anti-stress anxiolytic and nootropic activity of Nyctanthes arbour tritis leaves

Deshmukh VS, Juvekar AR
Department of Pharmaceutical Sciences and Technology, Mumbai University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019, India

Reports suggest that stress is the most common etiological factor in CNS disorders like anxiety, Schizophrenia, Parkinson's disease and Alzheimer disease for which effective treatment strategies are inadequate due to complexities of the ailment and the limitations of allopathic medications. There are scanty reports[1] on the putative neuro pharmacological effects of the leaves of *Nyctanthes arbor tristis* Linn. (Family: Oleaceae) [NAT]; hence the present work investigated gamut of its neuro-pharmacological effects. The methanolic extract was evaluated for anxiolytic activity using plus maze model, open field test and light dark model. Further, the nootropic potential[2] of extract was evaluated using Morris water maze test and plus maze model. Antistress potential[3] was evaluated in Wistar rats by subjecting the animals to chronic cold restraint stress followed by biochemical estimation of plasma corticosterone, glucose, triglycerides; dopamine, 5-Hydroxy Tryptamine and nor epinephrine from brain. Diazepam 1 mg/kg was used as a positive control. One-way ANOVA followed by Dunnett's test was applied for statistical significance. Pretreatment with NAT extract resulted in preference to open arm in plus maze test, increased exploratory behavior in open field test and increased number of crossings in light dark model. Further it improved cognitive function with respect to spatial and working memory processes. The treatment with NAT extract ameliorated the stress-induced variations in the biochemical levels of corticosterone, glucose, triglycerides; dopamine, 5-HT and nor epinephrine. In conclusion, the NAT extract exhibited anxiolytic, antistress and nootropic activity with utility in oxidative cognitive impairment due to its antioxidant potential. **References**: 1. Saxena R.S., Gupta B. (2002), J. Ethnopharmacol. 81: 321-/325. 2. Vogel, G.H., Vogel, W.H. (Eds) (2005), Drug Discovery and Evaluation- Pharmacological assays, pp. 435. 3. Nachankar, R.S., Juvekar, A.R.A. (2005), Acta Hort. (ISHS) 680:101 – 107.

S 020

New Norterpene Cyclic Peroxides from the Sponge Diacarnus megaspinorhabdosa

Edrada RA[1], Ibrahim S[1], Ebel R[1], Wray V[2], Müller WEG[3], Proksch P[1]
[1]Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, Geb. 26.23, 40225 Düsseldorf, Germany; [2]Gesellschaft für Biotechnologische Forschung, Braunschweig; [3]I nstitut für Physiologische Chemie und Pathobiochemie, Johannes-Gutenberg-Universität, Mainz

Chemical investigation of the n-hexane extract of the sponge *Diacarnus megaspinorhabdosa* has provided a series of norterpenes, including three new norditerpene cyclic peroxides and five new norsesterterpene peroxides together with four known norterpene peroxides: nuapapuin A methyl ester, epimuqubilin B, methyl-2-epinuapapuinoate and methyl diacarnoate A. The structures of the new compounds were established on the basis of one and two dimensional NMR spectroscopic studies (^{1}H, ^{13}C, COSY, HMQC, HMBC and ROESY) as well as on mass spectral analysis. The isolated compounds exhibited moderate (2 – 5 μg) to strong toxicity (0.01 – 0.10 μg) toward L5178Y (mouse lymphoma) and HeLa (human cervix carcinoma) while the same congeners showed weaker activity on the PC-12 (rat pheochromocytoma) cell line. Capon's empirical rules1 were extensively used in this study to derive the relative stereochemistry at C-2, C-3 and C-6. Following Horeau's procedure, the peroxide ring was cleaved to yield its diol congener onto which the advanced Mosher method was utilized to confirm the stereochemistry obtained from Capon's empirical rules. **References**: 1.Capon, R.J., MacLeod, J.K. (1985), Tetrahedron 41: 3391 – 3404. 2. Horeau, A. (1977), Determination of the configuration of secondary alcohol by partial resolution, in Stereochemistry, Fundamentals and Methods, Kagan, H.B ed., Vol. 3., Georg Thieme, Stuttgart, p. 51.

S 021

New materials for extraction, separation and mass spectrometric investigations in phytochemistry

Stecher G, Hashir MA, Bonn GK
Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020, Innsbruck, Austria

The design of novel materials and stationary phases for the selective extraction and fast separation of analytes from plant materials is an important part in phytomics [1]. In fact, preconcentration and purification prior to analysis is necessary owing to the complexity of samples. Add to this, analytes are often present in low concentrations, what means that sample extraction, purification and preconcentration are the starting points to successful analyses. Within this presentation we present different strategies for the synthesis and the modification of stationary phases to produce tailored solutions for the analytical questions. In fact, sample preparation procedures should be shortened as much as possible to save time and consumables and to prevent degradation of target compounds. As an example the combination of extraction and preconcentration or/and separation within one step using selective materials will be presented. In this coherence not only multidimensional chromatography plays a central role, but also the use of new stationary phases within different formats such as columns, capillaries and discs. Further on focus will be placed on the separation of analytes and on the detection. Especially the use of matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-ToF-MS) for the analysis of small molecules using a newly synthesized material as matrix free system will be presented [2]. In this coherence examples of different classes of plant ingredients will be shown. Performance of the introduced material will be compared with different systems described in literature accenting its effectiveness and power for screening plant systems and metabolites. **References:** 1. Stecher, G., Huck, C.W., Stöggl, W.M., Bonn, G.K. (2003), TrAC, 22: 1 – 14. 2. Bonn, G.; Hashir, M.A.; Stecher, G., Bakry, R., (2006), Patent pending.

S 022

Exploration of natural alkylamides and synthetic analogs as source for new ligands for the cannabinoid type-2 receptor

Gertsch J[1], Raduner S[1], Feyen F[1], Altmann KH[1]
[1]Institute of Pharmaceutical Sciences, ETH Zurich, 8093-Zürich, Switzerland

The cannabinoid type-2 (CB_2) receptor is an attractive target for the development of drugs against inflammatory disease, atherosclerosis, and osteoporosis. Based on the discovery that alkylamides (alkamides) from *Echinacea* constitute a new class of CB_2-specific cannabinomimetics[1], we have screened a series of synthetic analogs of *Echinacea* alkylamides as well as other plant-derived natural *N*-alkyl amides for competitive binding to the CB_2-receptor. Because dode-

ca-2*E*,4*E*-dienoic acid isobutylamide from *Echinacea* has a high affinity to the human CB_2-receptor we synthesized analogs with modified head groups, as reported for the endogenous cannabinoid anandamide[2]. The resulting preliminary structure-activity relationship clearly shows that the CB_2 receptor binding mode of *Echinacea* alkylamides is different from anandamide. To further explore the potential of natural *N*-alkyl amides as a new general class of CB_2 ligands we have initiated a screening of plant extracts. The hexane extracts of the medicinal plants *Spilanthes oleracea* L. and *Lepidium meyenii* Walper. which are known to contain distinct types of alkylamides, were tested in receptor binding assays. The alkylamide-fraction of the *L. meyenii* extract showed significant receptor binding and five isolated benzylated alkylamides (macamides) were assessed for both their CB_2-receptor affinity as well as CB_2-mediated functional effects. Our data show that natural alkylamides from *Echinacea* and *Lepidium* are promising candidates for the development of novel CB_2-receptor ligands. *Acknowldgments*: Prof. Ikhlas A. Khan, School of Pharmacy, University of Mississippi for providing the macamide references **References**: 1. Raduner, S. *et al.* (2006), J. Biol. Chem. 281: 14192 – 14206. 2. Khanolkar, A.D. *et al.* (1996), J. Med. Chem. 39: 4515 – 4519.

S 023

Selected phototoxicological assays used for plant metabolites screening

Chobot V[1,2], Vytlačilová J[1], Kubicová L[1]
[1]Faculty of Pharmacy, Charles University, Heyrovskeho 1203,500 05 Hradec Králove, Czech Republic; [2]Present address: Faculty of Life Sciences, University ofVienna, Althanstral!,e 14, A.1090 Vienna, Austria

In the last decade, phototoxins especially attracted the attention of pharmacists, toxicologists, cosmetologists, and food specialists [1]. Progress in phototoxicologal research relies on efforts to develop reliable screening methods. This issue will be discussed with the aid of the thiophene polyacetylene (*E*)-1-[5-(hept-5-en-1,3-diynyl)-2-thienyl]ethan-1,2-diol. Together with the furocoumarin xanthotoxin as positive control phototoxicity in conjunction with UV A radiation was assessed by histidine photo-oxidation assay and *Arlemia* and *Tubifex* bioassays [2]. The determined activities were statistically explored by probit-log calculations of EC_{50} and LC_{50} values and evaluated by comparison of effective and lethal concentrations of dark controls to the irradiated sets. The thiophene polyacetylene showed strong phototoxicity in the histidine photo-oxidation assay and in both organismic bioassays. Xanthotoxin demonstrated higher effects independent of UV radiation in the *Artemia* assay. The differences in the phototoxicity of both photosensitizers may be caused by their variable absorption of the test compound and different mechanisms of activity. The results demonstrate the different sensitivity of the applied assays and suggest combining various phototoxicological assessment methods. *Acknowledgements:* This work was supported by Project MSM 0021620822 of the Czech Ministry of Education. **References**: 1. Chobot, V. *et al.* (2004), Cent. Eur. J. Publ. Health 12: S31.S33. 2. Chobot, V. *et al.* (2006), Fitoterapia 77: 194 – 198.

S 024

Antifouling and Anti-Aggregatory Effects of Bastadins from the Marine Sponge Ianthella basta

Ortlepp S[1], Edrada-Ebel RA[1], Ebel R[1], Hohlfeld T[2], Bohlin L[3], Proksch P[1]
[1]Pharmaceutical Biology and Biotechnology, Heinrich-Heine University, Universitätstrasse 1, Geb. 26.23, 40225 Düsseldorf, Germany;
[2]Pharmacology and Clinical Pharmacology, Heinrich-Heine University, Universitätstrasse 1, Geb. 22.21, 40225 Düsseldorf, Germany;
[3]Pharmacognosy, Medicinal Chemistry, Box 574, 751 23 Uppsala, Sweden

Marine sponges are sessile, soft bodied invertebrates that rely mainly on the accumulation of toxic and/or deterrent natural products as a chemical defence against predators (fishes) and other biotic stress factors such as fouling by epibionts. Overgrowth by fouling organisms can be detrimental to filter feeders like sponges as it will block pores that are needed for inhaling seawater followed by phagocytosis of suspended particles. As marine sponges are usually free of overgrowth a suppression of epibionts by sponge derived natural products is usually assumed. In this study we employed a settling bioassay using barnacle cyprids (*Balanus improvisus* Darwin) in order to investigate sponge compounds for possible anti-fouling activity. The compounds studied are complex brominated tyrosine derived substances named bastadins. The substances were isolated from the marine sponge *Ianthella basta* collected in Indonesia and included a new bastadin congener along with the known compounds bastadin 3, 4, 9 and 16. All bastadins showed pronounced inhibition of cyprid settlement and are suggested be involved in the chemical defence of the sponge against fouling organisms. Additionally, the bastadins were also tested for human platelet aggregation inhibition and gave likewise positive results. Preliminary results suggest that the presence of the oxime group accounts for the antifouling and anti-aggreagtory effects of the bastadin derivatives. *Acknowledgements*: Dr. Mia Dahlström, Dr. Martin Sjögren, Dr. Victor Wray.

S 025

Biological activity of a putative 50-kDa protein purified from Tinospora rumphii Boerl

Charmain J, Bonifacio O[1], Matias RR[2], Natividad FF[2]
[1]Philippine Institute of Traditional and Alternative Health Care, Atlanta Centre, 31 Annapolis St, San Juan, Metro Manila, Philippines

[b] Research and Biotechnology Division-St. Luke's Medical Center, E. Rodriguez Blvd., Quezon City, Philippines *Tinospora rumphii* Boerl. locally known in the Philippines as *makabuhay* is one of the most common plants being used to treat various ailments. Aqueous plant extracts are prescribed in the treatment of indigestion, diarrhea, scabies and topical ulcers. A 50-kDa protein purified from the stem was assayed *in vitro* for its cytotoxic activity in five human cell lines (HeLa, LIM 1215, HT-29, Jurkat and a normal cell line – fetal skin fibroblast). The apoptosis-inducing activity was likewise investigated by flow cytometry, DNA staining and DNA fragmentation using the same set of cell lines as the target cells. Genes that are upregulated in the cells treated with the purified 50-kDa protein were identified by differential display reverse transcription polymerase chain reaction (DDRT-PCR). Five clones from each sample were sequenced and analyzed. In all cell lines studied, the 50-kDa protein demonstrated strong cytotoxicity (IC_{50} from 4 to 6.5 ng/µl) and induced cell death in a dose-dependent manner. Typical morphological and biochemical features of apoptosis including cell shrinkage, chromatin condensation, DNA ladder formation, phosphatidylserine expression using Annexin V were observed in all cell lines used in the study. From DDRT-PCR, a total of 176 genes were differentially expressed, 65 of which were upregulated and 111 were down regulated. Four cDNAs were successfully cloned and sequenced. The sequences showed homology to transcription factors, a chemokine receptor and a voltage-dependent anion channel. The identification of these genes may lead to the elucidation of the molecular mechanism of action of the cytotoxic activity of the protein. *Acknowledgments:* Philippine Council for Health Research and Development – Department of Science and Technology Research and Biotechnology Division – St. Luke's Medical Center Institute of Biology – University of the Philippines Diliman **References**: 1. Mosmann, T. (1983), J. Immunol. Methods 65: 55 – 63. 2. Liang, P., Pardee, A. (1992), Science 257: 967 – 971.

S 026

Chemography and phylogeny – navigating chemical and evolutionary space

Backlund A[1], Gottfries J[2], Bohlin L[1], Larsson J[1]
[1]Division of Pharmacognosy, Department of Medicinal Chemistry, BMC, Uppsala University, Box 574, S-751 23 Uppsala, Sweden; [2]Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, S-431 83 Mölndal Sweden

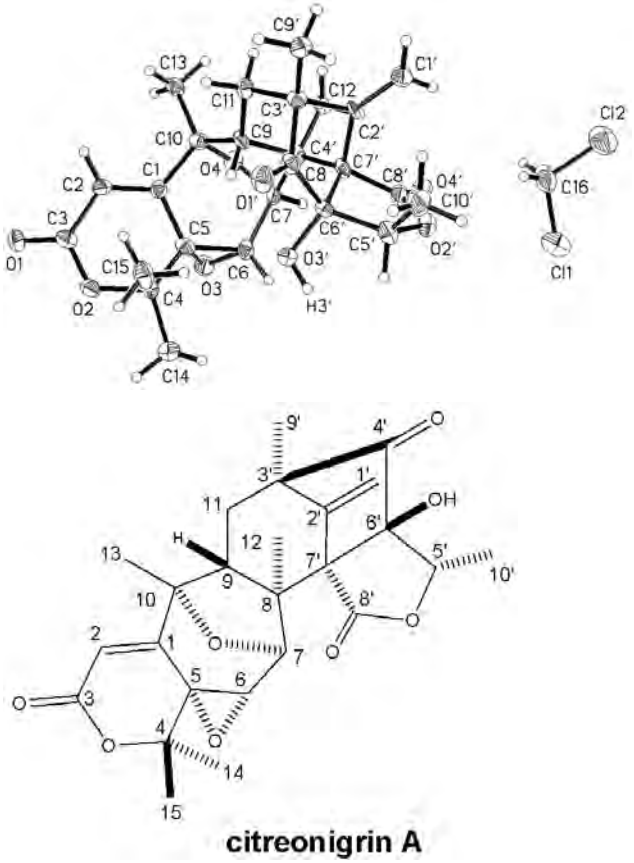
Natural products are, in one respect, forming a mirror image of evolutionary processes, as pointed out already by Abbot [1]. In recent years approaches have been made resulting in *e.g.* consistent mapping devices for the drug-related *chemical space*, such as ChemGPS [2, 3]. Observations made employing this device [3] triggered the development of a device tuned for the biologically relevant sectors of chemical space, ChemGPS-NP [4]. In a similar way the result of evolutionary processes can be regarded as forming an *evolutionary space*, populated by extant and extinct organisms. This space has for the last 50 years been the subject of extensive mapping, lately as phylogenetic studies, aimed at elucidating evolutionary relationships providing robust results for various groups of organisms. In this study we apply ChemGPS-NP to predict chemical traits of natural products with a limited distribution in the phylogeny of living organisms, aiming to cross-validating maps of chemical and evolutionary space. The key findings to be presented include a clearer understanding of the evolutionary driven changes in physical-chemical properties of sets of iridoids, sesquiterpene lactones, and *Strychnos*-alkaloids. Comparisons will be made to previously presented hypotheses of chemosystematic relatedness, and how this tool will aid in a systematic exploration of natural products chemical space. **References**: 1. Abbot, H. (1887), The chemical basis of plant forms, Franklin Institute lecture. 2. Oprea, T.I., Gottfries, J. (2001), J. Comb. Chem. 3: 157 – 166. 3. Larsson, J. *et al.* (2005), J. Nat. Prod. 68: 985 – 991. 4. Larsson, J. *et al.* (2006), submitted manuscript.

S 027

Novel oxygenated meroterpenoids and drimane sesquiterpenoids from the sponge-derived fungus Penicillium citreonigrum

Ebel R[1], Rusman Y[1], Brauers G[1], Proksch P[1], Frank W[2], Wray V[3]
[1]Heinrich – Heine University, Institute of Pharmaceutical Biology and Biotechnology, Universitätsstrasse 1, 40225 Düsseldorf, Germany; [2]Heinrich – Heine University, Institute of Inorganic Chemistry, Düsseldorf, Germany, Universitätsstrasse 1, 40225 Düsseldorf, Germany; [3]Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, 38124 Braunschweig, Germany

In the course of our continuing search for bioactive metabolites from marine-derived fungi, we have investigated an isolate of *Penicillium citreonigrum* obtained from the Indonesian sponge *Pseudoceratina purpurea* (Carter). Besides novel drimane sesquiterpenes bearing close structural similarity to compounds previously described from a marine alga-derived fungus,[1] we obtained a series of highly complex oxygenated meroterpenes exemplified by citreonigrin A. These novel compounds can be divided into different structural types, and are probably biogenetically derived from the "fungal meroterpenoid pathway" leading to known fungal metabolites such as austin or paraherquonine.[2] Details on the structures, their proposed biogenetic relation to previously described natural products, and their biological activity will be reported.



citreonigrin A

References: 1. Belofsky, G.N. *et al.* (1998), Tetrahedron 54: 1715 – 1724. 2. Simpson, T.J. *et al.* (1997), Tetrahedron 53: 4013 – 4034.

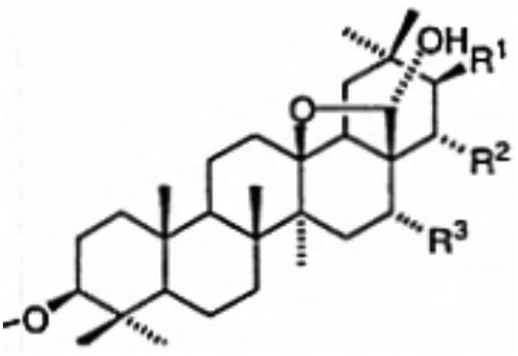
S 028

Structure-activity-relationship (SAR) for in vitro antileishmanial activity of maesabalide (PX-6518) analogue natural products

Maes L[1], Kuypers K[1], Vermeersch M[1], Cos P[1], Pieters L[2], Van Puyvelde L[3]
[1]Laboratory of Microbiology, Parasitology and Hygiene, University of Antwerp, Groenenborgerlaan 171, Antwerp, Belgium; [2]Laboratory of Pharmacognosy, University of Antwerp, Universiteitsplein 1, Antwerp, Belgium; [3]Nat. Centre for natural Science and Technology (NCST), Cau Giay, Hanoi, Vietnam

Maesabalides (PX-6518) were isolated from the leaves of *Maesa balansae* Mez. (Myrsinaceae) and shown to have a strong and selective *in vitro* and *in vivo* action against the intracellular protozoan *Leishmania* [1, 2]. Efforts to establish a SAR by selective chemical derivatisation have been hampered by the complexity of the maesabalide moiety [3]. As an alternative approach exploiting available natural diversity, a literature search for structural analogues was performed based on the sapogenin core, i. e. the triterpenoid skeleton with the presence of the hemi-acetal moiety between C_{-13} and C_{-17}. The search produced >200 molecules belonging to a several plant genera: *Aegicerus*, *Atroxima*, *Anagallis, Anamirta, Androsace, Ardisia, Burseria, Cyclamen, Eleutherococcus, Eucommia, Euptelea, Grindelia, Leucas, Lysimachia, Myrsine, Platycodon, Primula, Polemonium* and *Thevetia*. None of these were ever evaluated for antileishmanial activity. The activity against intracellular amastigotes of *L.donovani* could be determined for some *Ardisia* (IC_{50}= 10 µg/mL), *Maesa* (IC_{50}=< 0.25 µg/mL), *Lysimachia* (IC_{50}= 11 µg/mL), *Anagallis* (IC_{50}=< 0.25 µg/mL) and *Primula* (IC_{50}= 14 µg/mL) species. The IC_{50}-values for the measabalide PX-6518 was 0.06 µg/mL and for the reference drug Miltefosin 8 µM. The fact that several active 'hits'

were identified confirms that specific derivatives do indeed retain antileishmania activity. However, the occurrence of several negatives also shows that the active maesabalide moiety cannot be subject for major structural changes, endorsing its unique potential. Structural factors that may affect the pharmacological activity will be discussed, but more of the above listed plant genera should be investigated to establish a more complete SAR.



Maesabalide sapogenin

Acknowledgements: WHO-TDR (Geneva, Switserland), DGOS (Brussels, Belgium), Tibotec (Mechelen, Belgium) **References**: 1. Maes, L., *et al.* (2004), Antimicrob. Agents & Chemotherap. 48: 130 – 136. 2. Maes, L., *et al.* (2004), Antimicrob. Agents & Chemotherap. 48: 2056 – 2060. 3. Germonprez, N., *et al.* (2005), J. Med. Chem, 48: 32 – 37.

S 029

A screening platform for identification of anti-diabetic compounds in plants used in traditional complementary medicine

Christensen LP[1], Grevsen K[2], Jensen M[2], Kristiansen K[3]
[1]Department of Food Science, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark; [2]Department of Horticulture, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark; [3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

Diabetes is a major health problem due to a massive growth in the number of type 2 diabetes patients. Type 2 diabetes is characterized by insulin resistance, and hence, treatment with thiazolidinedione (TZD) insulin sensitizing drugs is often prescribed. TZDs bind to and activate the nuclear receptor Peroxisome Proliferator-Activated Receptor (PPAR)γ, a master regulator of fat cell formation. TZDs significantly improve insulin sensitivity and restore glycemic control, but unfortunately, the use of TZDs is accompanied by a number of side effects such as weight gain due to fat accumulation. Some of the side effects result from the use of full PPARγ agonists that recruit a "non-desirable" complement of so-called co-activators to PPARγ, and it appears that certain partial PPARγ agonists may recruit a more "beneficial" complement of co-activators. Many types of Traditional Complementary Medicine (TCM) have been used against conditions resembling type 2 diabetes and the aims of the present work were (**i**) to establish a platform for systematic screening of selected classes of TCM for compounds that function as partial PPARγ agonists without promoting fat cell differentiation and (**ii**) to investigate the possibilities for improving the content of potential anti-diabetic compounds in plants by cultivation and breeding. American ginseng is known for its anti-diabetic effects. By using the platform we have performed a systematic screening of several types of ginseng extracts and purified ginsenosides and demonstrated that they have characteristics that warrant further investigations of their effects on glucose (and lipid) homeostasis. We have furthermore shown that the content of ginsenosides can be improved by selection and breeding. Also herbs such as thyme and oregano, not normally considered as "anti-diabetic" plants contain bioactive compounds that activate PPARγ *in vitro*, and hence may affect glucose homeostasis.

S 030

Bioprospecting Program-BIOTA: A Rational Search for Drug Discovery from Brazilian Biodiversity

da S. Bolzani V[1], Siqueira DHS[1], Cavalheiro AJ[1], Castro-Gamboa I[1], Pauletti PM[1], Viegas CJ[1], Araújo AR[1], Lopes MN[1], Furlan M[1], Young MCM[2]
[1]Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais – NuBBE, Universidade Estadual Paulista – UNESP – Instituto de Química, Departamento de Química Orgânica, Rua Prof. Francisco Degni s/n – 14.800 – 900 – Araraquara – São Paulo – Brazil; [2]Secção de Fisiologia e Bioquímica de Plantas-Instituto de Botânica, CP 4009, São Paulo – Brazil

Certainly, the use of natural products has been the single most successful strategy in the discovery of novel medicines, and their importance is evidenced by the new chemical entities (NCE) approved by regulatory authorities around the world in the past decade. The biodiversity found in Brazil makes it a very feasible source of biological active compounds and its preservation is an important goal both for the intrinsic value of this enormous biological resource, and for its huge potential as a source of new drugs. Our collaborative project at Biota-FAPESP has identified antifungal, anticancer, antimalarial and acetylcholinesterase inhibitor compounds from plant species of Cerrado and Atlantic Forest. Among the isolates, the xanthones mangiferin (IC_{50}= 32,55 μM), muraxanthone (IC_{50} 56.72 μM), 2-(2'-*O*-*trans*-caffeoyl)-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (IC_{50} 61.88 μM), 2-(2'-*O*-*trans*-cinnamoyl)-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (IC_{50}= 46.31 μM), 2-(2'-*O*-*trans*-coumaroyl)-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (IC_{50} 55.94 μM), 2-(2'-*O*-benzoyl)-*C*-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone (IC_{50} 55.94 μM) and muraxanthone (IC_{50} 56.72 μM) showed antimalarial activity, using chroroquine as positive control. As acetylcholinesterase inhibitors, piperidine alkaloid acetyl-spectaline (IC_{50}= 24.80 μM) and their derivatives (*2R,3R,6S*)-2-methyl-6-(13-oxotetradecyl)piperidin-3-yl acetate hydrochloride (IC_{50}= 7.32 μM) and tert-butyl *(2R,3R,6S)*-20methyl-6-(13-oxotetradecyl)-piperidin-3-yl carbamate hydrochlride (IC_{50}= 15.10 μM) have been considered lead molecules for Alzheimer disease when compared with standard control: galanthamine (IC_{50}= 3.10 μM).

S 031

Brominated cyclodipeptides from the marine sponge Geodia barretti as selective 5-HT ligands

Hedner E[1], Sjögren M[1], Frändberg PA[2], Johansson T[2], Göransson U[1], Dahlström M[3], Jonsson P[3], Nyberg F[2], Bohlin L[1]
[1]Division of Pharmacognosy, Department of Medicinal Chemistry, Biomedical Centre, Uppsala University, PO Box 574, SE-751 23 Uppsala, Sweden; [2]Division of Biological Research on Drug Dependence, Department of Pharmaceutical Biosciences, Biomedical Centre, Uppsala University, PO Box 574, SE-751 23 Uppsala, Sweden; [3]Department of Marine Ecology, Tjärnö Marine Biological Laboratory, Göteborg University, SE-452 96 Strömstad, Sweden

The production of bioactive compounds by plants, animals and microorganisms has long been exploited in the search for drug candidates to serve as leads in drug development. Traditionally, such bioprospecting for drug candidates has focused on terrestrial microorganisms and plants; the equivalent research in marine systems is in its infancy, but the much larger diversity of major lineages in the sea promises a wealth of new molecular structures with as yet unknown functions. In the ocean, sessile sponges have proved a rich source of bioactive compounds many of which are believed to constitute a chemical defense against predators or foulers aimed at protecting the body surface. We have previously reported on the production of the brominated cyclodipeptides barettin (cyclo[(6-

bromo-8-entryptophan)arginine]) and 8,9-dihydrobarettin (cyclo[(6-bromotryptophan)arginine in the marine sponge *Geodia barretti* Bowerbank and their ability to inhibit settlement of barnacle larvae in a dose-dependent manner [1]. In order to further establish the molecular target and mode of action of these compounds, we investigated their affinity to human serotonin receptors. The tryptophan residue in the barettins resembles that of endogenous serotonin [5-hydroxytryptamine]. A selection of human serotonin receptors, including representatives from all subfamilies (1 – 7), were transfected into HEK-293 cells. Barettin selectively interacted with the serotonin receptors 5-HT_{2A}, 5-HT_{2C} and 5-HT_4 at concentrations close to that of endogenous serotonin, with the corresponding K_i values being 1.93 μM, 0.34 μM and 1.91 μM respectively. 8,9-dihydrobarettin interacted exclusively with the 5-HT_{2C} receptor with a K_i value of 4.63 μM; it failed to show affinity to 5-HT_{2A} and 5-HT_4, indicating that the double bond between the tryptophan and arginine residue plays an important role in the interaction with the receptor proteins. **Reference**: 1. Sjögren, M. *et al.* (2004), J. Nat. Prod. 67: 368 – 372.

S 032

Nanostructured Elasto-Optical Biosensor for Screening on Bioactive Compounds

Keusgen M[2], Botkin N[1], Dähne L[3], Fassbender B[1], Giersig M[1], Hilgendorff M[1], Hoffmann D[1], Knieps H[2], Moske M[1], Pascal R[1], Treitz G[1]
[1]Center of Advanced European Studies and Research, Ludwig Erhard Allee 2, D-53175 Bonn, Germany; [2]Philipps-Universität Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany; [3]Capsulution NanoScience AG, Volmerstraße 7b, D-12489 Berlin, Germany

Biosensors are becoming increasingly important for screening purposes, especially regarding bioactive compounds from nature [1]. In order to parallelize such screening processes, miniaturized sensors are more and more required. In the approach presented here, a so called "elasto-optical" biosensor, based on nanostructures, is developed to fulfil these demands. The detection principle is based on fluorescence energy transfer (FRET), which takes place between two fluorophors F and F' (Figure). These fluorophores are connected by an elastic spacer consisting of polyethylene glycol units. One end of this spacer is immobilized to an oscillating solid support; the other end is attached to a receptor R, which specifically recognizes bioactive compounds (L), *e.g.*, lectins for sugar recognition [2]. If a ligand binds to a receptor, the oscillation properties of the polyethylene glycol spacer will change and as a consequence, the average distance (D) between F and F' will also change. This will then result in an altered FRET. The elasto-optical biosensor will be capable for screening on bioactive compounds from nature with a molecular weight starting at approx. 500 amu.

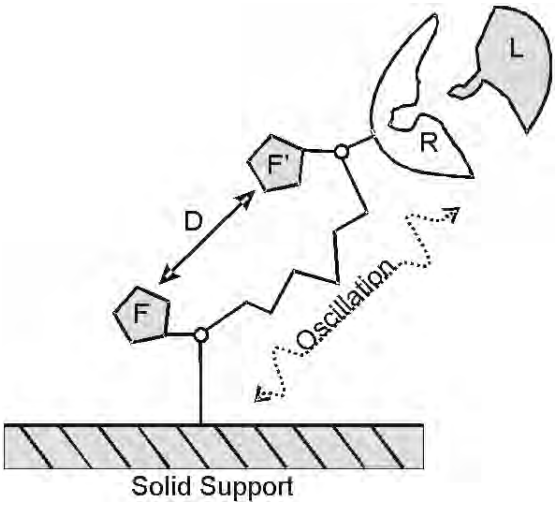


Fig. Principle of the elasto-optical biosensor

Acknowledgements: Research was supported by the German BMBF, as part of the program "NanoBiotechnologie", grant no. 0312022A. **References**: 1. Keusgen, M. (2002), NaturWissenschaften 89: 433 – 444. 2. Hartmann, M., Nikitin, P., Keusgen, M. (2006), Biosens. Bioelectron. (in press).

2. Recent Advances in Analysis of Secondary Metabolites

S 033

Thiolysis-HPLC characterization of the phenolic composition of nut shells of Pinus sibirica (Du Tour) Rupr

Shikov AN[1], Pozharitskaya ON[1], Laakso I[3], Dorman HJD[3], Makarov VG[1], Tikhonov VP[2], Hiltunen R[3]
[1]Interregional Center "Adaptogen", 47/5, Piskarevsky pr, 195067, St-Petersburg, Russia; [2]Open joint-stock company "Diod", 11-A, ul. Derbenevskaya, 115114, Moscow, Russia; [3]Faculty of Pharmacy, Division of Pharmaceutical Biology, University of Helsinki; P.O. Box 56 (Viikinkaari 5E), FIN-00014 Helsinki, Finland

The chemical composition and biological effects of pine nut kernels and the oils obtained from them have been thoroughly investigated; however, the composition of secondary metabolites from their shells, which contain 55 – 60 % of the nut mass, has not been studied. The purpose of the present work was to (i) study the phenolic composition of nut shells of *Pinus sibirica* (Du Tour) (Pinaceae) by RP-HPLC, (ii) determine their average degree of polymerization (DPn) and (iii) evaluate their monomeric units by thiolysis using benzyl-α-thiol. Pine nuts were finely ground and defatted twice with *n*-hexane. After vacuum filtration and air-drying, they were extracted with acetone/water (95:5, v/v). The confirmation of the presence of protocatechuic acid, catechin, epicatechin, vanillic acid, syringic acid, taxifolin, eriodyctiol, *trans*-cinnamic acid and naringenin, was assessed by the addition of authentic compounds to the extract. HPLC-PDA analyses were consistent with the major presence of flavan-3-ol related compounds (proanthocyanidins). After thiolysis, taxifolin, eriodyctiol and flavan-3-ol were identified. Since only the terminal units of tannins become free after thiolysis (catechin or epicatechin as such), it could be seen from the chromatogram that catechin monomers, as terminal units, were more abundant than those corresponding to epicatechin. The DPn of the pine nut extract was 9, which correspond to highly polymerized procyanidins. When the acid-hydrolyzed pine nut extract was analyzed by HPLC, a large amount of polar material was eluted during the first 10 min, followed by one major peak corresponding to eriodyctiol and several minor peaks. Isolariciresinol, lariciresinol and secoisolariciresinol were tentatively identified in the extract. On the basis of comparison of the UV-spectra and retention time of authentic samples, gallic and ellagic acids (structural units of hydrolyzed tannins) were identified. *P. sibirica* pine nut contains considerable quantities of phenolic compounds. This is of great importance to industry, since extracts of these byproducts are finding increasing applications as active substances in pharmaceutical and cosmetic compositions.

S 034

Prevalence of three tetraene alkamide isomers in Echinacea angustifolia and Echinacea purpurea roots

Lehmann RP[1,2], Matthias A[1], Matovic N[2], Penman KG[1], Bone KM[1,3], de Voss JJ[2]
[1]MediHerb Research Laboratories, 3/85 Brandl Street, Eight Mile Plains, Brisbane, 4113 Australia; [2]School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, 4072 Australia; [3]School of Health, University of New England, Armidale, 2351 Australia

Three tetraene alkamide isomers were identified in *Echinacea angustifolia* DC. and *Echinacea purpurea* (L.) Moench. roots by compari-

son with their synthetic cis-trans 8,10 counterparts which were synthesised using novel pathways. The three tetraenes were: (2*E*, 4*E*, 8*Z*, 10*Z*)-isobutyldodeca-2, 4, 8, 10-tetraenamide, the *ZZ* isomer, (2*E*, 4*E*, 8*Z*, 10*E*)-isobutyldodeca-2, 4, 8, 10-tetraenamide, the *ZE* isomer and (2*E*, 4*E*, 8*E*, 10Z)-isobutyldodeca-2, 4, 8, 10-tetraenamide, the *EZ* isomer. The relative concentration of each tetraene was examined in several commercially available samples by GCMS. The amount of each tetraene as a percentage of the total differed between the two species, with 10 % and 29 % of the *ZZ* isomer, 80 % and 63 %of the ZE isomer and 10 % and 8 % of the *EZ* isomer in *E. angustifolia* and *E. purpurea* respectively. These species differences between *E. angustifolia* and *E. purpurea* roots may help to explain experimental differences in the activity of preparations from either species as well as the variations in their efficacy noted in clinical trials.

3. Genomics, Proteomics and Metabolomics in Medicinal Plant Research

S 035

Molecular cloning and characterization of a novel S-adenosyl-L-methionine: coniferyl alcohol O-methyltransferase from suspension cultures of Linum nodiflorum L

Berim A, Petersen M
Institut für Pharmazeutische Biologie, Philipps Universität Marburg, Deutschhausstr. 17A, D-35037 Marburg, Germany

Several methylation reactions occur in the course of aryl tetralin lignan biosynthesis in *L. nodiflorum* L., explaining our interest in this enzyme class. Using a homology-based RT-PCR strategy [1], we have cloned and functionally expressed in *E. coli* a novel 41 kDa methyltransferase displaying high regiospecificity towards the allylic OH-group of coniferyl alcohol (CA). The apparent K_m for CA was determined to be 6.77 µM with V_{max} of 621.19 µkat/kg protein at 30 °C, whereas the K_m for the co-substrate *S*-adenosyl-L-methionine is 18.93 µM Structure-activity relationship studies proved the double bond of the side-chain to be important, as the enzyme activity with dihydroconiferyl alcohol amounted to about 22.95 % as compared to the best substrate (CA). The substitution pattern of the phenol ring is also essential, for both sinapyl and cinnamic alcohols were poorly accepted (7.86 and 15.69 % activity of that with CA, resp.), whereas crotonyl and allyl alcohols are no substrates (< 0.7 % activity), confirming the aromatic ring itself is indispensable. The WU-BLAST2 (EMBL, Heidelberg) search revealed only low homology (< 45 %) to enzymes listed hitherto. The transcription levels, determined by semi-quantitative RT-PCR, were highest between day 2 and 6 of the suspension culture period, whilst the corresponding enzyme activity declined for the first 4 days and rose from day 5 onwards, reaching its maximum of 612.31 nkat/kg on day 7. By identifying a so far undescribed substrate preference of an enzyme with little homology to the already known, function attribution to newly discovered and/or yet unassigned genes might now be facilitated. The physiological role of this side-chain methylation of coniferyl alcohol, a precursor of both lignin and lignan biosyntheses, remains to be assessed yet. **Reference**: 1. Ibrahim, R.K. *et al.* (1998), Plant Mol. Biol. 36: 1 – 10.

S 036

Different strategies for discriminator identification in a NMR based metabonomics matrix of the genus Leontopodium using LC-SPE-NMR, ^{1}H-NMR-guided isolation and classical phytochemistry

Schwaiger S[1], Seger C[1], Godejohann M[2], Humpfer E[2], Hehenberger S[1], Tseng LH[2], Ellmerer EP[3], Spraul M[2], Stuppner H[1]
[1]Institut für Pharmazie/Pharmakognosie, Leopold-Franzens-Universität Innsbruck, Innrain 52c, 6020 Innsbruck, Austria; [2]Bruker-Biospin GmbH, Silberstreifen 4, 76287 Rheinstetten, Germany; [3]Institut für Organische Chemie, Leopold-Franzens-Universität Innsbruck, Innrain 52a, 6020 Innsbruck, Austria

The secondary metabolite profile of Edelweiss (*Leontopodium alpinum* Cass.; Asteraceae), is highly complex – more than 50 analytes have been characterized until now [1 – 4]. Thus, the chemotaxonomical assessment of the genus *Leontopodium* comprising more than thirty mostly Asian species seems a major undertaking not subsumable with a single analytical technique. NMR based metabolic profiling can be envisioned as alternative analytical approach. This technique, combining ^{1}H-NMR spectroscopy of extracts with multivariate statistical data interpretation, allows an unbiased selection of spectral regions responsible for sample discrimination. Profiling of $CDCl_3$-extracts of the roots of twelve *Leontopodium* species resulted in clear species discriminations. The three species with the highest variation compared to *L. alpinum*, the European Edelweiss, were identified as *L. souliei* Beauverd, *L. franchetii* Beauverd and *L. subulatum* Beauverd. Due to varying amounts of available plant material and differences in the obtained extract matrices, identification of the discriminating metabolites was carried out by three different strategies: LC-SPE-NMR experiments for *L. souliei*, ^{1}H-NMR guided isolation for *L. franchetii* and classic phytochemical techniques (*e.g.* silica gel CC, Sephadex LH 20 CC and preparative TLC) for *L. subulatum*. These three distinctly different strategies allowed in each case the identification and structure elucidation of the discriminating constituents. Differences of the applied methods in time consume, amount of the required plant material, yield of the discriminating compounds and costs will be discussed. **References**: 1. Schwaiger, S. *et al.* (2006), Phytochem. Anal., accepted. 2. Schwaiger, S. *et al.* (2005), Tetrahedron 61: 4621 – 4630. 3. Schwaiger, S. *et al.* (2004), Planta Med. 70: 978 – 785. 4. Dobner, M.J. *et al.* (2003), Helv. Chim. Acta; 86: 733 – 738.

4. Health Beneficial Effects of Plant Phenolics

S 037

Inhibition of interleukin-8 secretion by a green tea special extract in the intestinal cell line Caco-2

Netsch M[1], Gutmann H[2], Schmidt A[1], Drewe J[2]
[1]Frutarom Switzerland Ltd, Ruetiwisstrasse 7, 8820 Waedenswil, Switzerland; [2]Dept. of Research and Clinical Pharmacology, University Hospital (Kantonsspital), 4031 Basel, Switzerland

The intestinal mucosa represents a site of active immunologic activity. Accordingly, intestinal epithelial cells, *e.g.* Caco-2, secrete a wide array of inflammatory mediators including chemokines, *e.g.* IL-8, that are able to induce an inflammatory state in intestinal cells or to attract inflammatory immune cells [1, 2]. The chemokine interleukin (IL)-8 is involved in neutrophil attraction and activation and elevated levels have been observed in intestinal inflammation. Natural compounds including green tea have been shown to modulate inflammation *in vitro* and *in vivo* [3, 4]. We investigated the influence of the green tea extract EFLA®942 (GTE) on the secretion of IL-8 protein and on the mRNA expression levels of IL-8 in the human gastrointestinal cell line Caco-2 in an inflammatory state. Therefore, extracellular IL-8 concentrations were determined by ELISA and mRNA expression levels of IL-8 were analyzed by quantitative RT-PCR. Characteristic components in 0.01 mg/mL GTE are 4.02 µM

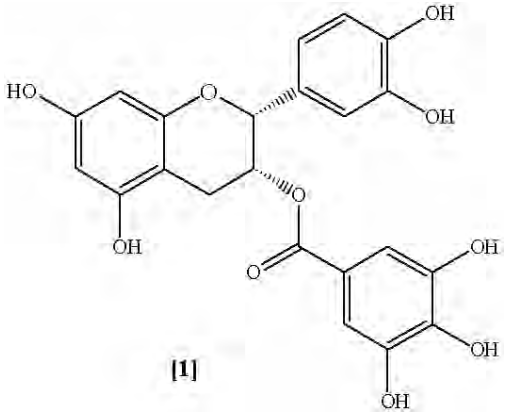
EGCG, 2.27 μM EGC, 4.36 μM caffeine, and 0.99 μM theanine. GTE did significantly inhibit the IL-1β-induced IL-8 secretion in a dose-dependent manner. At highest concentration the GTE-mediated inhibition was comparable to brefeldin A, a fungal inhibitor of vesicular transport. This was not related to a significant down-regulation of IL-1β-induced IL-8 mRNA expression by GTE. These results suggest that GTE may exert an anti-inflammatory activity in enterocytes, which may be useful for the treatment of intestinal inflammation. **References**: 1. Yang, S.K. *et al.* (1997), Gastroenterology 113: 1214 – 1223. 2. Eckmann, L. *et al.* (1993), Gastroenterology 105: 1689 – 1697. 3. Porath, D. *et al.* (2005), J. Pharmacol. Exp. Ther. 315: 1172 – 1180. 4. Varilek, G.W. *et al.* (2001), J. Nutr. 131: 2034 – 2039.

S 038

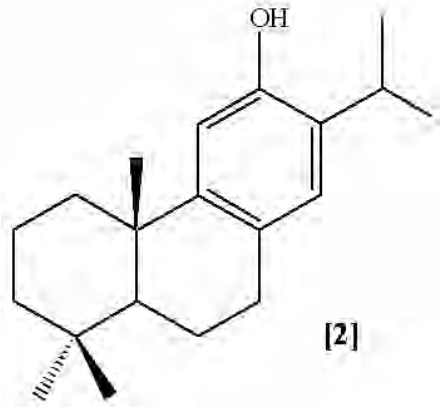
Plant Phenolics as Antibiotic Resistance Modifying Agents

Smith E[1], Williamson E[2], Gibbons S[1]
[1]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, UK; [2]Department of Pharmacy, University of Reading, Whiteknights, Reading, Berks RG6 6AJ, UK

There has recently been renewed interest in plant phenolics, long considered to be toxic and non-specific in their activity. Many activities have been reported for phenolic plant compounds, and of particular interest is their activity as antibiotic resistance modifying agents against Gram-positive bacteria and mycobacteria. Multidrug-resistance (MDR) exhibited by many bacterial species is a major problem in treating both hospital and community acquired infections. A modifying agent is a compound which reduces the minimum inhibitory concentration (MIC) for an antibiotic. This could be of great benefit in combinatory therapy, perhaps facilitating the reintroduction of antibiotics that are no longer effective due to resistance. An example in clinical use is Unasyn™ comprising the β-lactamase inhibitor sulbactam in combination with ampicillin. Polyphenols from green tea, epicatechin gallate **[1]** and epigallocatechin gallate have been shown to potentiate oxacillin activity against methicillin-resistant *Staphylococcus aureus* (MRSA) [1] and to have modest activity in reducing the MIC of some standard antibiotics against effluxing strains of *S. aureus* [2]. Resistance modifying activity has also been reported for the phenolic abietanes, totarol and ferruginol **[2]** isolated from conifer species [3; 4], for carnosic acid from *Rosmarinus officinalis* L. [5], and anacardic acid from the cashew *Anacardium occidentale* L. [6]. Small differences in structure such as the position of a hydroxyl group can have a considerable effect on modulatory activity [3; 4] which suggests a more subtle mode of action for these phenolics than general membrane perturbation. Recent reports have indicated a potentially useful separation between the concentrations required for biological activity and cytotoxicity for both ferruginol and totarol [7].



[1]



[2]

Acknowledgements: We thank Stiefel International R & D Ltd for funding this study. **References**: 1. Shiota, S. *et al.* (1999), Biol. Pharm. Bull. 22: 1388 – 1390. 2. Gibbons, S. *et al.* (2004), Planta Med. 70: 1240 – 1242. 3. Nicolson, K. *et al.* (1999), FEMS Microbiol. Lett. 179: 233 – 239. 4. Smith, E. *et al.* (2006), in preparation. 5. Oluwatuyi, M. *et al.* (2004), Phytochemistry 65: 3249 – 3254. 6. Kubo, I. *et al.* (1992), J. Nat. Prod. 55: 1436 – 1440. 7. Clarkson C. *et al.* (2003), Planta Med. 69: 720 – 724.

S 039

Flavonoids from Vigna angularis – composition and antioxidative effects

Weber N[1], Wätjen W[2], Edrada RA[1], Wray V[3], Lou Y[4], Wang ZQ[4], Proksch P[1]
[1]Heinrich – Heine University, Institute of Pharmaceutical Biology and Biotechnology (a), Universitätsstrasse 1, 40225 Düsseldorf, Germany; [2]Heinrich – Heine University, Institute of Toxicology, Düsseldorf, Germany; [3]Gesellschaft für Biotechnologische Forschung, Braunschweig, Germany; [4]Zhejiang University, Department of Pharmacology and Toxicology, Hangzhou,China

In the last decades interest in the group of flavonoids has markedly increased due to their presumed beneficial effects in humans. Many flavonoids act as radical scavengers and are suggested to prevent chronic diseases such as atherosclerosis or even cancer [1]. For a critical evaluation of the effects of flavonoids with regard to human health, quantitative (daily intake as part of diet or as food supplements) as well as qualitative (structural) aspects have to be taken into consideration [2]. As part of a broader Sino-German project on flavonoids from plants used for human consumption, we analyzed four different cultivars of *Vigna angularis* (Willd.) Ohwi & H. Ohashi, two of them growing in China (Hangzhou) and the other two cultivated in Germany (Düsseldorf). All analyzed plants yielded catechin, myricetin– 3-*O*–rutinoside, and rutin as main compounds but differed with regard to their total flavonoid concentrations which were lower in the Chinese cultivars. We analyzed pharmacological effects of these compounds focussing mainly on their antioxidative properties. All flavonoids exhibited a good radical scavenger activity in a cell free system (DPPH assay). In different cellular systems (rat H4IIE hepatoma and C6 glioma cells) we further analyzed protective effects of these substances against oxidative stress using the fluorescent probe DCF. Beside these antioxidative effects, cytotoxic and pro-apoptotic activities of these compounds were also determined. In consideration of these data it can be emphasized that a known composition of flavonoids is necessary in nutrient uptake. *Acknowledgements*: We thank DFG for financial support and Dr. Ulrike Lohwasser (IPK Gatersleben) for taxonomic identification. **References**: 1. Gordon, M.H. (1996), Nat. Prod. Rep. 13: 265 – 273. 2. Rice – Evans, C. *et al.* (1996), Free Radical Biology & Medicine 20: 933 – 956.

S 040

Composition and antioxidant activities of Salvia halophila and S. virgata from Turkey

Kosar M, Goger F, Husnu K, Baser C
Anadolu University, Faculty of Pharmacy, Department of Pharmocognosy 26470 Eskisehir, TURKEY

Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules [1]. Free radicals are also very important in food products, because oxidative degradation of lipids is one of the main factors limiting their shelf–life [2]. In recent years, natural antioxidants have been focused on because of the harmful effects of synthetic antioxidants in living systems [3] *Salvia* is one of the wide spread members of the family Labiatae (Lamiaceae). *Salvia* species, especially *S. officinalis* L., are an important source of antioxidants used in food and have wider implications for the dietary intake of natural antioxidants [3]. Turkey is an important country for *Salvia* species in the world. The flora of Turkey includes 88 species of the genus *Salvia*. The aerial parts of *S. halophila* Hedge and *S. virgata* L. were used in Soxhlet extraction with different solvents such as *n*-hexane, ethylacetate, methanol and aqueous methanol (50 %). Plants were also extracted with water under reflux. All the extracts were analyzed by HPLC for their phenolic composition and in *in vitro* antioxidant assays for their effects on oxidation. The free radical scavenging activity of the extracts were investigated using 1,1-diphenylpicrylhydrazin ($DPPH^{\cdot}$) radical. Linoleic acid was also used to determine the effects of lipid peroxidation of the extracts. Total phenols, flavonoids and flavonols, and reductive activity of the extracts were also analyzed. Phenolics rich extracts of ethylacetate, methanol and aqueous methanol (50 %) showed scavenging activity on $DPPH^{\cdot}$ whereas non-polar extracts (*n*-hexane and ethylacetate) inhibited the peroxidation of linoleic acid. The aqueous methanol and ethylacetate extracts reduced the ferric(III) to ferro(II) in a certain proportion. Rosmarinic acid was found as the main component and caffeic acid, ferulic acid and luteolin-7-*O*-glycoside were identified in the extracts. **References**: 1. Kumaran, A., Joel karunakaran, R. (2006), Food Chemistry 97: 109 – 114. 2. Pizzalle, L. *et al.* (2002), J.Sci. Food Agric. 82: 1645 – 1651. 3. Kintzios, S.E. (2000), Sage The Genus *Salvia*. Harwood academic publishers,pp. 27 – 53 and 185 – 192.

S 041

Effects of acute and repeated administration of Hypericum perforatum extract (WS 5572®) and its main constituents on extracellular levels of serotonin, noradrenaline and dopamine in the rat brain: A microdialysis study

Kehr J[1,2], Nöldner M[3], Yoshitake T[1,4]
[1]Dept. of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz väg 2, 171 77 Stockholm, Sweden; [2]Pronexus Analytical AB, Karolinska Science Park, Fogdevreten 2a, 171 77 Stockholm, Sweden; [3]Preclinical Research, Dr. Willmar Schwabe GmbH & Co. KG, Willmar-Schwabe-Str. 4, 76227 Karlsruhe, Germany; [4]Dept. of Pharmaceutical Sciences, International University of Health and Welfare, 2600 – 1, Kitakanemaru, Ohtawara-shi, Tochigi 324 – 8501, Japan

The effects of acute and repeated administration of WS 5572®, a hydro-alcoholic extract of *Hypericum perforatum* L. (St. John's wort) on extracellular levels of dopamine (DA), serotonin (5-HT), noradrenaline (NA), and the metabolites 5-HIAA, DOPAC and HVA were examined by use of *in vivo* microdialysis in the prefrontal cortex (PFC), ventral hippocampus or striatum of awake rats. A single oral (*p.o.*) dose of WS 5572® (300 mg/kg) caused a dramatic reduction of metabolites DOPAC and HVA to about 15 % and 53 % of controls, respectively at 180 min after the drug. Extracellular DA levels increased only modestly to about 141 % and there was no effect on 5-HT and NA levels [1]. Hypericum given after sub-chronic pretreatment (300 mg/kg, *p. o.* daily for 14 days), increased most markedly the DA levels to about 260 % and decreased DOPAC, HVA and 5-HIAA, while the extracellular 5-HT and NA increased only moderately to 138 % and 125 %, respectively. In addition, a single dose of the constituents rutin (18 mg/kg, p. o.) and hyperforin (8 mg/kg, p. o.) increased 5-HT levels in the PFC of awake rats to 118 % and 125 % of control values, respectively, where as following sub-chronic 14-days administration rutin, hyperforin and isorhamnetin (0. 3 mg/kg, *p.o.*) increased extracellular 5-HT levels to 142 %, 131 % and 120 % of control values, respectively, and the concentrations of 5-HIAA decreased only to about 90 % of control values for all three treatments. These data suggest that rutin and hyperforin, given at doses corresponding to their content in WS 5572®, were about equally potent to increase 5-HT levels in the PFC of awake rats. In conclusion, the data suggest that WS 5572® inhibits preferentially the metabolism and reuptake of DA, and to a lesser extent affects the extracellular levels of 5-HT and NA, emphasizing the role of dopaminergic system in the mechanisms underlying potential antidepressive effects of WS 5572®. **Reference**: 1. Yoshitake, T. *et al.* (2004), Br. J. Pharmacol. 142: 414 – 418.

5. Clinical Studies with Herbal Medicinal Products

S 042

Effects of hops on clinical efficacy of a valerian-hops-extract combination (Ze 91019) in patients suffering from non-organic sleep disorder

Koetter U[1], Schrader E[2], Brattström A[1]
[1] Max Zeller Söhne AG, CH.8590 Romanshorn, Switzerland; [2]Clinical Drug Research, D-35415 Pohlheim,Germany

The fixed valerian-hops extract combination Ze 91019 is used as a sleep aid. For this combination pharmacodynamic actions [1, 2] as well as efficacy has been demonstrated in patients suffering from sleep problems [3]. While the main focus of research has been on valerian or the extract combination as a single active entity less is known about the contributions of hops to the sleep inducing effect of the combination. In this report, sleep onset latency (SL2) of the combination Ze 91019 was tested in comparison to valerian extract using a mobile device to allow for recording of sleep parameters at home while participating in the trial. Patients suffering from sleep disorders (ICD10: F 51.0, F 51.2) were enrolled when SL2 was prolonged at baseline (≥ 30 min, inclusion criteria). The hypnograms to calculate the objective sleep parameters were recorded with a mobile device (QUISI) twice before including the patient into the study. Treatment period lasted for 4 weeks with placebo, valerian extract (500 mg) or the valerian-hops-extract combination (500 mg plus 120 mg). Both of the extracts were prepared with 45 % methanol m/m with a DER for valerian: 5 – 8:1 and for hops 7 – 10:1. The results of this trial clearly indicate that hops added to the clinical efficacy as Ze 91019 was superior to placebo in reducing the originally prolonged sleep onset latency. **References**: 1. Vonderheid-Guth, B. *et al.* (2000), Eur. J. Med. Res. 5: 139 – 144. 2. Schellenberg, R. *et al.* (2004), Planta Med. 70: 595 – 597. 3. Füssel, A. *et al.* (2000), Eur. J. Med. Res. 5: 385 – 389.

S 043

Efficacy Of A Plant Based Formulation In The Treatment Of Recurrent Airway Obstruction In Horses

Larkins NJ[1], Deaton CM[2], Jones K[1]
[1]Nutritional Laboratories,Monmouth, Monmouthshire, NP15 2DJ, United Kingdom; [2]Animal Health Trust, Newmarket, Suffolk, CB8 7UU, United Kingdom

The purpose of this double blinded placebo controlled cross-over clinical study was to assess the ability of a plant based formulation (*Ginkgo biloba* L., *Zingiber officinale* Roxburgh, *Chlorella pyrenosa*) to prevent or delay the onset, decrease the magnitude of response and/or speed the recovery of lung dysfunction, clinical signs of disease, airway inflammation, and pulmonary oxidative stress in horses with

recurrent airway obstruction (RAO) on exposure to organic dust. The performance of the active supplement was judged on the basis of responses in lung function, clinical examination, airway inflammation and pulmonary oxidative stress following organic dust challenge compared to responses on the placebo diet. Lung dysfunction was assessed by measuring airway reactance and airway responsiveness to histamine by forced oscillation mechanics. Clinical signs were assessed by assigning scores for respiratory rate, nasal discharge, abdominal lift/expiratory effort, nasal flaring, lung sounds and cough. Airway inflammation was determined by cytological analyses of tracheal wash and bronchoalveolar lavage fluid (BALF) samples, and by measuring the concentration of hydrogen peroxide in exhaled breath condensate (EBC). Oxidative stress was assessed by measuring the concentrations of reduced ascorbic acid, dehydroascorbate (DHA, oxidised ascorbic acid), reduced glutathione and oxidised glutathione in tracheal wash and BALF. Results of statistical analyses demonstrated that BALF ascorbic acid concentrations were higher after challenge in horses when fed the active formulation compared to the placebo and that BALF, DHA and ARR (ratio of DHA to total ascorbic acid), were lower after challenge in the active supplement horses irrespective of the order of treatment allocation. **References**: 1. Deaton, C.M., Marlin, D.J. (2004), Am. J. Vet. Res. 65: 80 – 87. 2. Larkins, N.J. (1999), J. Equine Vet. Sci. 19: 84 – 89.

S 044

Strix Forte®, an antioxidant mixture with bilberry anthocyanosides, reduces oxidative stress and immune activation in exfoliation syndrome and exfoliative glaucoma

Blomster H[1], Greilberger J[2], Öttl K[2], Fuchs D[3], Winkler C[3], Hiltunen R[4], Juan H[5], Psilander N[6]

[1]Institution of Deacony, Eye Policlinic, Sibeliuksenkatu 6 C, 15110 Lahti, Finland; [2]Medical University Graz, Institute of Physiological Chemistry, Graz, Austria; [3]Innsbruck Medical University, Biocentre, Division of Biological Chemistry, Innsbruck, Austria; [4]University of Helsinki, Division of Pharmaceutical Biology, Helsinki, Finland; [5]Medical University Graz, Institute of Experimental and Clinical Pharmacology, Graz, Austria; [6]Ferrosan A/G, Sjøborg, Denmark

Background: To evaluate the quantity of oxidative stress (OS), immune activation (IA) and exfoliation material before and after four months daily ingestion of Strix® Forte in exfoliation syndrome (XFS) and exfoliative glaucoma (XFG). **Methods:** 15 XFS, 15 XFG and 15 control patients ingested one Strix® Forte tablet twice daily during four months containing 74 mg bilberry *(Vaccinium myrtillus* L.*)* anthocyanosides, 6 mg lutein, 15 mg zinc sulphate, 10 mg dl-α-tocopheryl acetate, 800 µg retinyl acetate and 50 µg selenomethionine. Exfoliation material quantity (EmQ), scale 0 – 3, was also examined. Plasma concentrations of isoprostanes, malondialdehyde, carbonyl proteins and oxidized albumin were measured as markers for OS. Neopterin and kynurenine/tryptophan ratio were measured as markers for IA. Wilcoxon Signed Ranks Tests were used for statistical analyses. **Results:** EmQ was significantly reduced after four months in both XFS and XFG group (XFS: *P*= 0.007 for right and 0.003 for left eyes, XFG: P = 014 for right and 0.005 for left eyes. Isoprostanes were reduced in XFS group (*P = 0.001)* and in controls (*P*= 0.009), but not in XFG group (*P* =0.191). Malondialdehyde levels were not diminished in any groups. Carbonyl proteins were reduced in all groups: [XFS (*P*= 0.002), XFG (*P*= 0.001), controls (*P*= 0.015)]. Oxidized albumin diminished in all groups [XFS (*P*= 0.003), XFG (*P*= 0.005), controls (*P*= 0.001)]. Neopterin levels were slightly increased in the XFG group (*P*= 0.020) and tryptophan levels were slightly elevated in the XFS group (*P*= 0.047). Kynurenine levels decreased in the XFG group (*P*= 0.012) and in controls (*P*= 0.008). Kynurenine/tryptophan levels decreased in all three groups [XFS (*P*= 0.006), XFG (*P*= 0.003), controls (*P*= 0.006)]. **Conclusion:** Regular Strix® Forte ingestion reduced besides OS and IA also EmQ in XFS and XFG patients which may be explained by the observed beneficial alterations in the OS and IA markers. *Acknowledgements:* Research supported by Ferrosan A/S, Sjøborg, Denmark **References**: 1. Koliakos, G.G. *et al.* (2003), Br. J. Ophthalmol. 87: 353 – 356. 2. Laganovska, G. *et al.* (2003), Adv Exp Med Biol 527: 367 – 74. 3. Viljanen, K. *et al.* (2004), J. Agric. Food Chem. 52: 7419 – 7424. 4. Youdim, K.A. *et al.* (2002), J. Nutr. Biochem. 13: 282 – 288. 5. Kang, J.H. *et al.* (2003), Am. J. Epidemiol. 158: 337 – 346. 6. Noaman, E. *et al.* (2002), Biol. Trace Elem. Res. 86: 55 – 64. 7. Gherghel, D. *et al.* (2005), Invest. Ophthalmol. Vis. Sci. 46: 877 – 883. 8. De la Fuente, M. (2002), Eur. J. Clin. Nutr. 56 Suppl 3: S5 – 8.

6. Other related topic

S 045

Monographic profile of Guiera, leaves a West African herbal drug

Silva O, Gomes ET

Pharmaceutical Sciences Research Centre, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Guiera senegalensis Lam (*Combretaceae*) is a major West African medicinal plant, often employed to treat venereal, diarrhoeal, respiratory and fungal diseases. Previous work confirmed *G. senegalensis* leaves antimicrobial activity against *Neisseria gonorrhoeae* (including resistant strains), *Shigella dysenteriae*, *Vibrio cholerae*, *Giardia lamblia* and *Cladosporium cucumerinum*, corroborant it's traditional uses [1 – 3]. Bioguided phytochemical studies permitted the identification of flavonoids, gallic tannins, naphthalene derivatives (naphthyl butenone and naphthopyrans) and terpenoids, among *G. senegalensis* compounds [4]. In sequence, the significant and useful markers to *G. senegalensis* leaves diagnosis were also determined [5]. Hereby we present the botanical and chemical characteristics that should be included under Characteristics, Identification and Dosage parts of a future herbal drug monograph. **References**: 1 Silva, O. *et al.* (1997), Int. J. Pharmacogn. 35: 323 – 328. 2 Silva, O. *et al.* (1996), J. Etnopharm. 50: 55 – 59. 3. Silva, O. (2004), PhD Thesis, Lisboa, Universidade de Lisboa. 4. Silva, O., Gomes, E.T. (2003), J. Nat. Prod. 66: 447 – 449. 5. Silva, O., Serrano, R., Gomes, E.T. (2005), 53rd Annual Congress of the Society for Medicinal Plant Research, Florence.

S 046

Isolation of E & Z guggulsterones from young aerial stems of Commiphora wightii without destruction of plants

Soni V

Mahatma Gandhi Institute of Applied Sceinces, Shri Ram Ki Nangal, Via: Vatika, EPIP Gate, Sitapura, Jaipur-303905, Rajasthan, INDIA

Commiphora wightii (Arn.) Bhandari is an important traditionally used plant in India. It provides oleo gum resin mentioned by Sushruta (3000 year ago) as being a valuable drug. Clinical analysis revealed that the isomers *E*- and *Z*-guggulsterone are responsible for the hypolipidemic activity of oleo gum resin [1]. Unscientific tapping methods to increase yield of oleo gum resin causes mortality of plants and danger of extinction of the species. Therefore, guggulsterones were isolated from the aerial stems of *C. wightii* plants. Dried powder of aerial stems was subjected in soxhlet apparatus followed by purification by column chromatography and quantification for *E*- and *Z*- guggulsterones by high performance liquid chromatography (HPLC).Highest extraction of guggulsterone (0.35 %) was observed when ethyl acetate was used as solvent, while the lowest in chloroform (0.06 %). Through this procedure guggulsterones can be isolated from the aerial stems thereby saving the entire plant. Though the amount of guggulsterone contents recorded was very low in the aerial stems as compared to the oleo gum resin, the yield can be enhanced using elite plant propagation method coupled with improvised extraction technique. **Reference**: 1. Urizar, N.L., Moore, D.D. (2003), Ann. Rev. Nutr. 23: 303 – 313.

S 047

Turkish Oregano: Chemistry & Biological Activities

Baser KHC, Demirci F
Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470-Eskisehir/Turkey

"Kekik" is a collective term given in Turkey to plants which smell like oregano or thyme. Several taxa belonging to the genera *Origanum* L., *Thymus* L., *Thymbra* L., *Coridothymus* L. and *Satureja* L. are variously called, used and traded as kekik in Turkey [1]. Among all, five Origanum species are widely traded and Turkey is the biggest exporter of Oregano to the world markets, with over 10500 tons in the year 2005 for a return of US$ 18 million. The main characteristic of Turkish Oregano is high yielding essential oil containing carvacrol, 2-methyl-5-isopropylphenol – isomer of thymol, as the main constituent. Carvacrol is a simple monoterpene phenol responsible for most bioactivities of Oregano. Biological activities of Oregano herb, Oregano essential oil, Oregano water (hydrosol – aromatic water), and carvacrol range from antimicrobial and antioxidant activities to the treatment of gastrointestinal disorders and even cancer. Studies using both *in vitro* and *in vivo* classical and,molecular biology techniques have provided proof to most biological activities. Other uses of Oregano and carvacrol include antiparasitic, insecticidal, herbicidal, food preservative, and as feed additive especially in poultry, etc. The paper will review recent progress in the Science of Oregano with special reference to Turkish Oregano. **Reference:** 1. Baser, K.H.C. (2002), The Turkish *Origanum* Species, In:Oregano, The Genera *Origanum* and *Lippia*, Kintzios, S.E. (Ed.) Taylor and Francis, UK.

S 048

Chemical ecology and screening for bioactivity: common and contrasting issues

Hadacek F
Department of Chemical Ecology and Ecosystem Research Faculty of Life Sciences, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

Chemical ecology deals with the elucidation of low molecular molecules in biotic interactions, such as those between plant and microbes as well as plants and herbivores. Today, industry favours high throughput screening of extracts obtained from organisms of all kinds. Dereplication in active compounds and the accession as well as false positives represent major factors affecting the success of this methodology. Conversely, many natural products may be decisive in determining the outcome of many biotic interactions. Here, the constraints in selecting for biological activity are especially high. However, many compounds may be often produced only as consequence of the stress caused by interactions with predators (microbes, plants, and herbivores) or in nutrient-limiting situations (microbes). Decomposition of tissues is accomplished by specific microbial communities that are intrinsically affected by the quality of the resource. The facts may somehow act as a constraint on the accessibility to this natural products but the quality of the recovered metabolites may justify the effort. From the pharmaceutical viewpoint result comparability and reproducibility of assay results represent central issues. In this aspect, this awareness is much less developed among ecologist utilizing biological activities in the elucidation of ecological phenomena. As example the novel weapons hypothesis will be introduced that predicts phytotoxic natural products as cause for plant invasiveness. Various assay techniques to assess phytotoxic activities of candidate natural products are compared and discussed in terms of published procedures and conclusions based on these results.

S 049

Behavioural improvements following acute guaraná administration

Haskell CF[1], Kennedy DO[1], Milne AL[1], Wesnes KA[1,2], Scholey AB[1]
[1]Human Cognitive Neuroscience Unit, Northumbria University, Newcastle upon Tyne, NE1 8ST, UK; [2]CDR Ltd, Goring-on-Thames, UK

Extracts from the plant guaraná (*Paullinia cupana* Kunth ex H.B.K.) have been largely ignored in the literature. Despite this they are added to a number of beverages with claims that they possess stimulant properties. These stimulant properties are often attributed to its caffeine content, although extracts also contain potentially psychoactive levels of tannins and saponins. A series of randomised, placebo-controlled, double-blind, balanced crossover studies assessed: effects of multi-doses of guaraná (PC-102 Pharmaton extract); effects of a single dose of guaraná compared with a matched caffeine dose (9 mg) and; effects of single doses of guaraná and *Panax ginseng* C. A. Meyer (G115), and their combination. Cognitive performance and mood were assessed pre-dose and at different times up to 6 hours post-dose. Compared with placebo, all doses of guaraná resulted in improved task performance and mood throughout the day. Comparison of guaraná with a matched caffeine dose suggests some similar effects but also some different. Combining guaraná with ginseng produced elements of each active component but provided little evidence of a synergistic effect. These studies provide the first demonstration of behavioural effects of guaraná in humans. Comparison of effects with a matched caffeine dose suggests that effects are unlikely to be attributable to caffeine content alone. No specific advantage was found for combining guaraná with ginseng.

S 050

Hydroquinone- and cinnamate-based plant phenolics in experimental contact hypersensitivity

Máñez S, Olmos A, Giner RM
Departament de Farmacologia, Facultat de Farmacia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain

Contact hypersensitivity (CHS) is characterized by the percutaneous penetration of a low molecular weight hapten and the cross-talk between lymphocytes and antigen-presenting cells. Following our investigations into the role of reactive nitrogen species (RNS) in this process, we have now studied the effect of three phenolics obtained from *Phagnalon rupestre* (Asteraceae) on CHS. The three compounds, which have previously been described as inhibitors of peroxynitrite reactivity, are 2-isoprenylhydroquinone-1-glucoside (IHG), 3,5-dicaffeoylquinic acid (DCA) and 3,5-dicaffeoylquinic acid methyl ester (DCE) [1]. The experimental design begins with the sensitization with oxazolone on abdominal mouse skin followed by the subsequent elicitation with the same agent on the ear five days later [2]. Inflammation end points include histological analyses and determination of the presence of cytokines, 3-nitrotyrosine and inducible NO synthase (iNOS). Dunnett's *t* values measures statistical significance. The most active compound was DCE, which inhibited ear swelling by 54 % 24 h after the challenge with oxazolone. Its free acid form (DCA) produced a 40 % inhibition. The levels of IL-1β were always parallel to the time course observed for swelling. IL-4 evolved similarly in a lower range. Both caffeoyl esters significantly affected the liberation of interleukins, with DCE reducing the IL-4 levels at both 24 and 96 h by 78 and 87 %, respectively. Of all the test compounds, only IGH was able to reduce iNOS expression. Taken together with our previous results, these findings suggest that the efficacy on CHS is associated with antioxidant potency rather than with the ability to inhibit RNS production. *Acknowledgements*: Ana Olmos is recipient of a grant from Generalitat Valenciana. This work was supported by the Spanish Ministry of Science and Technology (SAF 2002 – 00723). **References**: 1. Olmos, A. *et al.* (2005), Nitric Oxide 12: 54 – 60. 2. Wang, B. *et al.* (2000), J. Immunol. 165: 6783 – 6790.

S 051

Phytochemical-dependent modulation of endocytic trafficking-novel screening strategies for drug discovery from natural products

Vieira A[1,2], Chen S[1], Luong T[1], Kuo J[1]
[1]Laboratory for Nutrition & Metabolic Research, Kines-9600 Applied Sciences; [2]Institute for Health Research & Education, Simon Fraser University, Burnaby, BC, Canada, V5A 1S6

Receptor-mediated internalization and endocytic trafficking pathways are attractive targets for new drug development. Although important for delivery of therapeutics into the cell, the endocytic route has not been extensively explored as a pharmacological target *per se*. Endocytic trafficking has been implicated in some pathologies and therapies, *e.g.*, HIV infection of cells, amyloid/precursor uptake or membrane disruption, G-protein-receptors and analgesia, glutamate I receptors and neuroprotection, and generally in the control of cell signaling-mediated proliferation, death, differentiation. We report on experimental strategies for screening potential therapeutic activities of phytochemicals in this context. The receptor and transport assays considered are aimed at detecting modulators of (**i**) cell surface receptor (R) levels, (**ii**) early internalization rates of R/ligand (L), and (**iii**) recycling of internalized R/L. In particular, we are developing a cellular screen based on the use of L-enzyme conjugates for analysis of (**ii**) and (**iii**). The method is currently being tested with a human keratinocyte line. We are beginning to screen plant aq. and alc. extracts, and purified phytochemicals; in most experiments L is biotinylated-transferrin. To date, we have observed the greatest % inhibition of (**ii**) with *Capsicum annuum* (5 microL of 0.4 g/mL aq. extract): 54.2 ± 12.3 ($p < 0.05$, $n = 4$). The main phytochemical(s) responsible for this activity remain(s) to be identified. Ascorbate is one candidate; at 40 microM it exhibits strong inhibition, 49.1 ± 7.3 % ($p < 0.05$, $n = 8$). Moreover, the ascorbate level likely reaches 30 – 60 microM with *Capsicum* treatment. In conclusion, we have developed a high-throughput cellular screen for modulation of endocytic trafficking by natural products; strong inhibitors or stimulators are candidates for further analyses (*e.g.*, in context of above pathologies).

S 052

Altitudinal variation in plant secondary metabolites, recent results from the Asteraceae family

Zidorn C[1]
[1]Institut für Pharmazie, Universität Innsbruck, Innrain 52, A-6020, Innsbruck, Austria

Various factors, such as age of the plant, season, microbial attack, grazing, radiation, competition, and nutritional status, have an impact on the secondary metabolite profile in higher plants [1]. A factor rarely assessed is the altitude of the growing site. Many environmental parameters like precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow-cover, length of the vegetation period, and the intensity of radiation under clear sky conditions differ between low and high altitude sites in temperate zones [2]. The increased solar radiation at higher altitudes and the enhanced UV-B radiation in particular are assumed to have a negative impact on plant life. Moreover, an increase of the contents of phenolic compounds and carotenoids with rising altitude has been postulated as a response to increasing UV radiation [2]. In particular, phenolic acids and flavonoids are considered to possess UV-B protective properties, because they are UV-B-absorbing compounds and free radical scavengers. The induction of enzymes involved in the biosynthesis of flavonoids under experimentally enhanced UV radiation is well established [3]. However, it remains unclear whether the environmental factors correlated with altitude have an effect on plant secondary metabolism under natural conditions. Recent studies on various wild (*Leontodon helveticus* Mérat, [4]), introduced (*Crepis capillaries* (L.) Wallr., *Hieracium pilosella* L., *Hypochaeris radicata* L., [5]), and cultivated taxa (*Arnica Montana* L., [6]; *Matricaria chamomilla* L., [7]) from the Asteraceae family indicated that the factor altitude indeed has a pronounced effect on the composition of secondary metabolite profiles in flowering heads. Moreover, new data also prove that the antioxidant potential of plant extracts derived from high altitude samples is higher than that from lowland samples [7]. Implications of these findings for chemical ecology and for the cultivation of high quality medicinal plants will be discussed. **References**: 1. Harborne, G. (1982), Introduction to Ecological Biochemistry. Academic Press. London. 2. Körner, C. (1999), Alpine Plant Life. Functional Plant Ecology of High Mountain Ecosystems. Springer. Berlin. 3. Jaakola, L., Määttä-Riihinen, K. (2004), Planta 218: 721 – 728. 4. Zidorn, C., Stuppner, H. (2001), Taxon 50: 115 – 133. 5. Zidorn, C. *et al.* (2005), Biochem. Syst. Ecol. 33: 855 – 872. 6. Spitaler *et al.* (2006), Phytochemistry 67: 409 – 417. 7. Zidorn *et al.* (2006), unpublished data.

S 053

Evaluation of the effect of grapefruit juice and its components on P-glycoprotein activity

Butterweck V[1], De Castro WV[1], Mertens-Talcott S[1], Derendorf H[1]
[1]College of Pharmacy, Department of Pharmaceutics, University of Florida, Gainesville, POBox 100494, 32610, USA

Grapefruit (*Citrus paradisi* Macfad.) juice (GFJ) has been demonstrated to interact with a variety of prescription medications increasing their plasma concentrations [1]. The major mechanism for GFJ-drug interaction is the inhibition of the drug-metabolizing enzyme cytochrome P-450 3A4 (CYP450 3A4) in the small intestine [2]. GFJ also interacts with intestinal P-glycoprotein (P-gp), an energy-dependent membrane efflux-transporter which restricts the absorption of a wide range of substrates [3]. However, the modulation of P-gp activity by GFJ and its clinical relevance is still unclear. The objective of this study was to compare the contents of the specific flavonoids (naringin and naringenin) and furanocoumarins (bergamottin and 6',7'-dihydroxybergamottin) in commercially available and fresh squeezed GFJ and to assess their *in vitro* effect on P-gp activity using Caco-2 cells and talinolol (a P-gp but a non-CYP450 3A4 substrate) as P-gp substrate. From the tested compounds the furanocoumarins 6',7'-epoxybergamottin and 6',7'-dihydroxybergamottin showed the highest inhibitory effect with IC_{50} values of about 1 μmol/L and 33 μmol/L, respectively. Although not detected in any of the tested juices, naringenin showed to be three fold more potent than its glycoside naringin with IC_{50} values of about 411 and 1250 μmol/L, respectively. The *in vitro* data demonstrated that compounds present in grapefruit juice are able to inhibit the P-gp activity modifying the disposition of drugs that are P-gp substrates. **References**: 1. Bailey, D.G. *et al.* (1998), Br. J. Clin. Pharmacol. 46: 101 – 10; 2. Schmiedlin-Ren, P. *et al.* (1997), Drug metab. Dispos. 25: 1228 – 33; 3. Spahn-Langguth, H., (2001), Eur. J. Pharm. Sci. 12: 361 – 367.

S 054

Effect of Rubia cordifolia on blood glucose level and glucose utilization by isolated rat hemidiaphragm

Somani R[1], Vadnere G[2], Jain K[1], Singhai AK[3]
[1]Sinhgad College of Pharmacy, Pune-411 041 (MS), India; [2]Smt. S.S. Patil College of Pharmacy, Chopda-425 127 (MS), India; [3]Dept. of Pharm. Sci, Dr. HS Gour University, Sagar-470 003 (MP), India

The present study aims to investigate the effect of ethyl acetate fraction of roots of *Rubia cordifolia* (RCEAF) on blood glucose level and glucose utilization study to find out the mechanism of action of the extract. Recently we have reported hypoglycaemic effect of ethanolic extract of roots of *R. cordifolia* L. (RCAE). RCEAF was fractionated from RCAE by column chromatography. Single dose study of RCEAF (50,100 and 200 mg/kg, *p.o.*) was carried out in i) normal fasted ii) oral glucose tolerance test (OGTT) iii) alloxan (120 mg/kg, s.c.)- induced diabetic rats. Repeated dose study of RCEAF (100 and

200 mg/kg, *p. o.*) was carried out for two weeks. The blood glucose levels were estimated by glucose oxidase- peroxidase reactive strips (One Touch, Johnson and Johnson, India). We found that, oral pretreatment with RCEAF induced a significant ($P < 0.05$) decrease in blood glucose level in i) normoglycaemic rats at 6 h ii) OGTT at ½ h compared to control glucose fed rats iii) alloxan- induced diabetic rats at 6 h. After two weeks daily administration of RCEAF, diabetic treated rats showed significant ($P < 0.05$) reduction in blood glucose level as compared to diabetic control rats. *In vitro* experiment showed that insulin (0.05 IU/mL) increased glucose utilization by an isolated rat diaphragm. Alone RCEAF (25 mg/mL) as well as combination of RCEAF (25 mg/mL) and insulin (0.05 IU/mL) showed a marked increase ($P < 0.05$) of glucose uptake. This exhibited the extra- pancreatic effect of the RCEAF. Further studies with estimation of insulin and insulin receptor may give more insight into the mechanism of the antidiabetic activity of the *R. cordifolia.*

S 055

Hydroxylation of selected sesquiterpenes by the fungus Neurospora crassa

Demirci F[1], Akar T[2], Demir TA[2], Kiran I[2], Kirimer N[1], Baser KHC[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey; [2]Department of Chemistry, Faculty of Sciences, Eskisehir Osmangazi University, 26480 Eskisehir, Turkey

Sesquiterpenes are natural products mainly obtained from essential oils with a vast spectrum of bioactivities [1]. In recent years the use of sesquiterpenes as starting material in the microbial biotransformation of new compounds has been of great interest to chemical, cosmetic and pharmaceutical industries [1 – 3]. Within this scope, sesquiterpenes such as caryophylleneoxide, alpha-cedrol, patchoulol and diisophorone [4] were selected for the microbial transformation by the plant pathogenic fungus *Neurospora crassa*. Metabolites were screened and detected both by TLC and GC-MS. Further NMR, UV, IR and mass spectroscopic analyses showed the transformation of a variety of hydroxylated new metabolites. In addition, antimicrobial activities of each metabolite were evaluated against human pathogenic bacteria and the yeast *Candida albicans* using the broth microdilution technique. **References***:* 1. Fraga, B.M. (2005), Nat. Prod. Rep. 22: 465 – 486. 2. Garcia-Granados, A. *et al.* (2003), Org. Biomol. Chem. 1: 2314 – 2320. 3. Ishida, T. (2005), Chemistry & Biodiversity 2: 569 – 590. 4. Kiran, I. *et al.* (2005), Biotechnol. Lett. 27: 1007 – 1010.

S 056

Tissue culture and genetic engineering of an important anticancer compound producing plant Veratrum californicum Duran

Ritala A, Ma R, Nohynek L, Suortti T, Rischer H, Oksman-Caldentey KM
VTT Technical Research Centre of Finland, P.O.Box 1000, Tietotie 2, Espoo, FI-02044 VTT, Finland

Veratrum californicum Duran (Liliaceae) is an important monocotyledonous medicinal plant which is the only source of the anticancer compound cyclopamine. The *in vitro* platform is needed for utilization of *Veratrum* cells in the production of the important secondary metabolites. Tissue culture, green plant regeneration and genetic engineering of *V. californicum* were developed. Fine suspension cell lines were established from germinated mature embryos by embloying friable embryogenic calli, AA- and L2-medium as culture media. The suspension cells were cryopreserved successfully and recovered at a high rate. Green plants were regenerated from embryogenic calli maintained on solid medium with 73 % regeneration ability (green plants/100 calli) in 27 months old culture. The *in vitro* plantlets contained the steroid alkaloids cyclopamine and veratramine. In addition, *Agrobacterium*-mediated and protoplast-based transformation methods were developed. For the first time, the basic tools for the metabolic engineering and biotechnological production of secondary metabolites of *V. californicum* are now available.

S 057

Integrated transcript and metabolite profiling of the medicinal plant Catharanthus roseus

Rischer H[1], Goossens A[2], Orešič M[1], Inzé D[2], Oksman-Caldentey KM[1]
[1]VTT Technical Research Centre of Finland, Tietotie 2, Espoo, FIN-02044 VTT, Finland; [2]Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, B-9052 Gent, Belgium

Plant-derived secondary metabolites still constitute important resources for currently prescribed drugs and for the discovery of active principles which are effective in new indication areas. The medicinal plant *Catharanthus roseus* (L.) G. Don has been extensively investigated during the last decades in order to utilize cell culture systems for the biotechnological production of important anticancer compounds *e. g.* vinblastine and vincristine. However, rational engineering of complicated metabolic networks such as the pathways leading to terpenoid indole alkaloids (TIAs) has been greatly impeded by our poor understanding of the regulation and structural organization underlying the biosynthesis. We have applied a comprehensive profiling approach based on functional genomics, which is independent of prior sequence knowledge, to monitor jasmonate-induced changes on the transcript and metabolite profiles of cell cultures. The behaviour of most of the currently known genes and metabolites involved in TIA biosynthesis plus hundreds of unknown elements could be observed in a single experiment. The integration of the expression profiles of 417 gene tags and the accumulation profiles of 178 metabolite peaks through correlation network analysis resulted in novel gene-to-metabolite networks revealing that the different branches of TIA biosynthesis as well as various other metabolic pathways are subject to differing hormonal regulation. These networks served also to identify a select number of genes and metabolites likely to be involved in the biosynthesis of TIAs. This study sets the base for a better understanding of periwinkle secondary metabolism and increases the practical potential of metabolic engineering of this important medicinal plant.

S 058

Pro-secretory effects in the human small and large intestine as a mechanism of action of STW 5 (Iberogast®) in irritable bowel syndrome (IBS)

Kelber O[3], Krüger D[1], Zeller F[2], Okpanyi SN[3], Frieling T[4], Schemann M[1]
[1]Human Biology, TU Munich, Hochfeldweg 2, 85350 Freising, Germany; [2]Department Surgery, Clinic Freising, Mainburger Straße 29, 85356 Freising, Germany; [3]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany; [4]Medical Clinic II, Clinic Krefeld, Lutherplatz 40, 47805 Krefeld, Germany

Phytotherapy is a successful approach to treat functional gastrointestinal diseases. The indications of the fixed herbal combination STW 5, consisting of hydroethanolic extracts from *Iberis amara*, chamomile flower, peppermint leaves, caraway fruit, liquorice root, melissa leaves, angelica root, greater celandine herbs, and milk thistle fruit, include functional dyspepsia and irritable bowel syndrome (IBS). Clinical data show the efficacy in these indications [1 – 3]. We therefore investigated the effect of STW 5 on secretory activity of mucosa/submucosa preparations from human ileum and colon using the Using chamber technique. Experiments were performed on normal tissue from surgical specimens (59 preparations from 29 patients, age: 69.8 ± 11.1), using an ethanol-free lyphilisate of the drug. Serosal application of STW 5 (256 µg/mL-1024 µg/mL) concentration dependently increased the short circuit current. Mucosal application had no effect. The response was similar in small and large intestine and the data were therefore pooled. The increase was 9.7 ± 2.9 µA/cm^2 for 256 µg/mL, 22 ± 7.9 µA/cm^2 for 512 µg/mL and 29 ± 8.1 µA/cm^2 for 1024 µg/mL ($p < 0.05$ at all concentrations).

The STW 5 evoked secretory effect was bumetanide (100 μM) sensitive and therefore due to increased chloride secretion. Blockade of nerves by tetrodotoxin (1 μM) and electrical field stimulation of nerves did not influence the effect, indicating a direct epithelial action of the drug. Our results indicate that STW 5 has a significant pro-secretory effect in the human intestine *in vitro*. It does not interfere with neurally mediated secretion but appears to stimulate chloride secretion at the level of the epithelial cell. Decreased secretion is discussed as a relevant factor in the aetiology of IBS, in particular in its constipation-predominant form. So this mechanism of action may be of special relevance in the clinical effect of STW 5 (Iberogast®) in patients with IBS. **References**: 1. Gundermann, K.J. *et al.* (2003), Advances in Therapy 20: 2 – 7. 2. Von Arnim, U. *et al.* (2004), Gut 53: A284. 3. Madisch, A. *et al.* (2004), Aliment. Pharmacol. Ther. 19: 271 – 279.

S 059

Evaluation of EndoTrap® blue for removing endotoxin contamination from Echinacea extracts

Gusenleitner S[1], Woelkart K[1], Barth S[2], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitätsplatz 4/I, A-8010 Graz, Austria; [2]Institute of Hygiene, Medical University of Graz, Universitätsplatz 4, A-8010 Graz, Austria

Endotoxins (lipopolysaccharides, LPS) are part of the outer membrane of the cell wall of Gram-negative bacteria. It is known that LPS causes cytokine production in immune cells. Therefore contamination of plant extracts with traces of LPS severely influences measurements of immune reactions in cell based assays. For that reason efficient endotoxin removal is necessary before studying immunmodulating actions of plant extracts. We determined the ability of EndoTrap® blue (Profos) to remove LPS from an extract of Echinacea angustifolia roots (60 % ethanol), and checked whether the content of active plant constituents like alkamides, caffeic acid-derivatives and polysaccharides is influenced. EndoTrap® blue is an endotoxin removal system based on high affinity chromatographic separation. The content of active constituents was determined before and after endotoxin removal: alkamides and caffeic acid-derivatives by a HPLC method [1] and polysaccharides with a colorimetric method [2]. The efficiency of endotoxin removal was measured by the Limulus-Amebocyte-Lysat Test (Charles River Endosafe®). EndoTrap® blue removed 76 % of the LPS. From the active compounds only the polysaccharides could be recovered by 99.0 %. Alkamides and caffeic acid-derivatives were retrieved only by 7.4 % and 13.6 % respectively. According to these results EndoTrap® blue seems to be an inappropriate tool for endotoxin removal from Echinacea extracts. **References**: 1. Turner, R.B., *et al.* (2005), NEJM 353: 341 – 348 (Supp. Materials). 2. Dubois, M. *et al.* (1956), Anal. Chem. 28: 350 – 356.

S 060

A plant antifungal product from Melianthus comosus (Melianthaceae) leaf extracts

Eloff JN, Angeh I, McGaw L
Phytomedicine Programme, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa

Melianthus comosus Vahl growing widely in southern Africa is used traditionally to treat bacterial infections. A company was interested in developing an antibacterial product for the veterinary market. The antibacterial activity of extracts was not high enough to pursue any further. It had excellent antifungal activity against animal pathogens, but the toxicity would have complicated the development of a product. Extracts had an excellent activity against 10 plant fungal pathogens investigated (*Rhizoctonia solani, Fusarium oxysporum, Penicillium janthinelum, Penicillium expansum, Colletotrichum glocosponicales, Trichoderma harzianum, Pythium ultimum, Phytophthora nicotiana, Aspergillus niger,* and *Aspergillus parasiticus)*. The extract contained one major antifungal compound and this compound was isolated and characterized as 3-hydroxy-12-oleanen-30-oic acid. By selective extraction and solvent fractionation an extract with an average MIC of 0.066 mg/mL against all ten fungal pathogens was obtained. Ignoring MIC values of 0.16 mg/mL against *Penicillium expansum* and *Aspergillus niger*, the average MIC for the other fungi was 0.04 mg/mL. The acetone extract did not lose activity at room temperature for a month. The dried extract was slightly soluble in water and ethanol, reasonably soluble in ethyl acetate and highly soluble in acetone. The potentised extract had a higher antifungal activity than six commercially used fungicides against some important plant fungal pathogens. In a limited field trial it gave a much better result than a commercial fungicide even though it was used at a quarter of the dose of the commercial fungicide. The results have been patented and a product is under development. *Acknowledgements:* Healthtechlaboratories and THRIP provided funding.

S 061

Characterization and partial purification of cystatins from Malian medicinal plants

Bah S[1,2], Diallo D[2], Paulsen BS[1], Johansen HT[3]
[1]University of Oslo, School of Pharmacy, Department of Pharmaceutical Chemistry, PO Box 1068 Blindern, N-0316 Oslo, Norway; [2]Institut National de Recherche en Santé Publique, Département de Médecine Traditionnelle, BP 1746, Bamako, Mali; [3]University of Oslo, School of Pharmacy, Department of Pharmaceutical Biosciences, PO Box 1068 Blindern, N-0316 Oslo, Norway

Cysteine proteases (CPs) expressed by *Schistosoma mansoni* (Sm) participate in the hydrolysis of host hemoglobin [1]. Cystatins are proteinaceous CP inhibitors (CPIs) present in humans as well as in plants. Two Malian medicinal plants used against schistosomiasis, *Securidaca longepedunculata* Fres (root: SR and leaf: SL) and *Stylosanthes erecta* Beauv. (aerial part: SE), were investigated for presence of cystatins. Cystatins were purified by extraction of powdered plant material with Tris-HCl, followed by affinity chromatography, gel filtration (GF) and anion exchange chromatography (AEC). *S. mansoni* CPs activity and protease inhibitory assays were performed using fluorogenic substrates according to [2]. High papain inhibition observed in all crude extracts indicated presence of CPIs. The papain inhibitory activity in the three extracts eluted into one single peak each, after affinity chromatography. These fractions were resolved by GF into papain inhibitory activities consistent with the presence of cystatins. SmCP activities were also inhibited by these cystatins. The strongest papain and SmCPs inhibitory activities were observed in SR. After AEC, one papain inhibitory peak which weakly inhibited SmCP was obtained from SR. On SDS-PAGE (under reduction), this peak appeared as a single 88kDa- band. The purified cystatins were characterized with respect to their papain and SmCPs inhibitory activities and by Mr. Cystatins with strong papain and moderate SmCPs inhibitory activity are isolated from the extracts and could participate into the antischistosomal activity of the studied plants. **References**: 1. McKerrow, J.H., Engel, J.C., Caffrey, C.R. (1999), Bioorg. Med. Chem. 7: 639 – 644. 2. Bah, S., Paulsen, B.S., Diallo, D., Johansen, H.T. (2006), Characterization of cysteine proteases in Malian medicinal plants. J. Ethnopharmacol., in press.

S 062

New Insights in the bioavailability and molecular mode of action of Echinacea preparations

Woelkart K, Bauer R
Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitätsplatz 4/I, A-8010 Graz, Austria

Echinacea is a widely used herbal remedy for prevention and treatment of the common cold. Recently a lot of new insights concerning the molecular mode of action of the main lipophilic constituents,

the alkamides, have renewed interest in this plant [1 – 3]. Alkamides have recently been shown to be quite fast absorbed and nanomolar quantities have been detected by LC-MS/MS in the blood after oral application of different Echinacea preparations in randomized open, single-dose, crossover studies. Due to the structural similarity of the alkamides with anandamide, an endocannabinoid, we have evaluated their ability to bind to cannabinoid receptors CB1 and CB2. Each of the alkamides was recognized by both the CB1 and CB2 receptors and can therefore be considered as a new class of cannabinomimetics. There is also evidence that alkamide containing Echinacea preparations trigger effect on the pro-inflammatory cytokine TNF-α and chemokine IL-8 in an *ex vivo* study and therefore not only bind but also activate these CB2 receptors. However, due to a lot of new cognitions the effects are not exclusively related to CB binding. **References**: 1. Woelkart, K. *et al.* (2005), Planta Med. 71:701 – 705. 2. Gertsch J. *et al.* (2004), FEBS Lett. 577:563 – 569. 3. Raduner, S. *et al.* (2006), J. Biol. Chem. 281:14192 – 14206.

Workshops

WS 001

Workshop of the Permanent Committees (PCs) on Herbal Medicinal Products (HMPs)

Chair: Prof. Winterhoff H[1]
Panellists: Prof. Pelkonen O[2], Prof. Schrenk D[3], Dr. Abeld G[4]
[1]Institute for Pharmacology and Toxicology University of Münster, Germany; [2]University of Oulu, Finland; [3]Department of food chemistry and environmental toxicology, University of Kaiserslautern, Germany; [4]Bionorika, Germany

Discussion on the Guideline on non-clinical documentation for herbal medicinal products in applications for marketing authorisation (bibliographic and mixed applications) and in applications for simplified registration.
A synopsis of the different proposals worked out in a presession will be presented as a modified form of the Guideline. These comments shall serve as a basis for the comments at the workshop and a final proposal.

WS 002

Plant Intellectual Property Rights (Workshop Perm. Comm. Breeding and Cultivation)

Franz C[1], Krück C[2]
[1]Institute for Applied Botany and Pharmacognosy, VU-Wien, Veterinaerpl. 1, A-1210 Vienna; [2]ProBenefit, Graf-Recke-Str. 84, D-40002 Duesseldorf

Medicinal Plants are either systematically cultivated, or collected from the wild. In both cases there is a large discussion on intellectual property of the starting material since neither "*natural nature*" nor "*essentially biological processes*" are patentable. But investments in research and development of new pharmaceutical drugs derived from natural products or especially for herbal medicinal products depend heavily on distinguishing features and on the possibility of legal product protection. This Workshop will therefore deal on one hand with the Convention on Biological Diversity (CBD) regulating the legal access to genetic resources as well as benefit-sharing and on the other hand with plant variety rights (PVR) versus plant patents. **References:** Ten Kate, K., Laird, S.A. (2000), The commercial use of biodiversity. Earthscan Publ., London. Llewelyn, M., Adcock M., Goode, M-J. (ed.) (2001), PIPWEG 2001: Proceedings of the Conference on Plant Intellectual Property within Europe and the Wider Global Community. Sheffield Academic Press.

WS 003

Workshop of the Permanent GA-committee of Manufacturing and Quality Control of Herbal Medicinal Products – Reference Compounds

Chair: Prof. Dr. Meier B[1]
Panelists: Dr. Reif K[2], Dr. Rose U[3], Prof. Dr. Verpoorte R[4]
[1]University of Applied Sciences, Grüental, CH-8820 Wädenswil, Switzerland; [2]PhytoLab GmbH & Co. KG, Dutendorferstrasse 5 – 7, D-91487 Vestenbergsgreuth, Germany; [3]European Directorate for the Quality of Medicines EDQM, Council of Europe, 225, Avenue de Colmar, F-67029 Strasbourg, France; [4]Division of Pharmacognosy, Institute of Biology, Leiden University, PO Box 9502, NL- 2300 RA Leiden, The Netherlands

Topics: Reference compounds and Herbal Medicinal Products – how much of identity and quality do we need? European Pharmacopeia Reference Standards – the new policy of the European Directorate for Herbal Medicinal Products. Is there a near future without reference compounds? Metabolomic type of approaches in quality control. The quality criteria for reference standards used in the quality control of pharmaceutical products are described in several documents like in NOTE FOR GUIDANCE ON GOOD MANUFACTURING PRACTICE FOR ACTIVE PHARMACEUTICAL INGREDIENTS (**CPMP/ICH/4106/00**, Glossary: Reference Standard), NOTE FOR GUIDANCE ON QUALITY OF HERBAL MEDICINAL PRODUCTS / TRADITIONAL HERBAL MEDICINAL PRODUCTS (**CPMP/QWP/2819/00 Rev 1**) and others. Also the Federal Institute for Medicinal Products and Medical Devices in Berlin (BfArM) released requirements on the quality of reference standards back in 1996. In practice QC labs in pharmaceutical companies try to follow these guidelines. For herbal reference standards it is not always possible to adopt these guidelines due to availability of the compounds, price and reasonableness of the test procedures. In the workshop we will discuss how to combine official requirements and the practicability of the test procedures on the basis of practical examples. The quality requirements for many herbal drugs and drug preparations are described in a growing number of monographs of the European Pharmacopoeia. Increasingly, physico-chemical methods are described therein to enable a more objective and reproducible control of the products concerned. For instance, HPLC-assays are often used to quantify a constituent with known therapeutic activity or an inactive „ marker " for which there is a minimum content given in the definition section of the monograph. The former policy using reagents of a defined minimum purity as „ reference standards " in these HPLC-assays was not always satisfactory for different reasons. With the intention of having a harmonised approach for monographs for synthetic compounds and herbal products, the European Pharmacopoeia Commission decided to introduce the use of chemical reference substances with assigned content into the monographs for herbal drugs and their preparations. This was also in agreement with recent guidelines of the EMEA (CPMP/QWP/2820/00). Depending on the characteristics of the individual herbal product, different types of reference standards may be chosen, such as the „ active constituent ", a marker substance or an extract with a defined content of the constituent. This presentation describes the strategy for the choice of a suitable material and the ways of establishment and value assignment for this type of reference standards. A totally different approach to total quality control of botanicals is the metabolomics approach. In this approach the aim is to determine all metabolites in a biological sample both qualitatively and quantitatively. LC-MS, GC-MS, MS(-MS) and NMR could be used for this purpose. The latter has the great advantage that signal intensities of all kind of compounds are only dependent on molar concentrations. This allows the quantitation without the need of standards and calibration curves. By combining such a holistic approach with chemometric methods such as multivariate and principle component analysis, it is possible to define a quality profile for a botanical, without the need of any chemical standard. The fact that depending on the type of equipment an ^{1}HNMR spectra can be obtained in less than 1 minute to 10 minutes makes it also suitable for a high throughput method. The high degree of reproducibility (independent of chromatographic col-

umns of variable quality, or matrix effects in ionization in case of MS) is a further major advantage of an NMR-metabolomics based approach for quality control of herbal products. *Acknowledgements*: The Workshop is sponsored by **Zeller AG**, Herbal Medicinal Products, CH-8590 Romanshorn und by **PhytoLab GmbH**, D-914867 Vestenbergsgreuth.

WS 004

Implementation of the Guideline on non-clinical documentation for HMPs in applications for marketing authorisation and simplified registration

Chair: Vlietinck AJ[1]
Panellists: Pelkonen O[2]*, Claeson P*[3]*, Abel G*[4]
[1]*Department of Pharmaceutical Sciences, University of Antwerp (UA), Antwerp, Belgium, Member HMPC, EMEA, London;* [2]*University of Oulu, Finland, Member HMPC, EMEA, London;* [3]*Swedish Medical Products Agency, Uppsala, Sweden, Member HMPC, EMEA; London;* [4]*Bionorika, Germany*

Half April 2006 the deadline for comments on the guideline on non-clinical documentation for herbal medicinal products in applications for marketing authorisation (bibliographic and mixed applications) and in applications for simplified registration (Doc. Ref. EMEA/HMPC/321 16/2005) expired. The rapporteur will address the many comments which were sent to the Herbal Medicinal Products Committee of the EMEA and it is expected that the guideline will be finalised in one of next meetings of that Committee. It is therefore appropiate in this workshop to explain the scope, legal basis and the different aspects of this guideline and to discuss its implementation at the level of the national authorities and the manufacturers of herbal medicinal products, especially in terms of legal basis, rational and feasibility.

WS 005

Rethinking the new role of nasal epithel – more than a simple barrier?

Chair: Stierna P[1]
Speakers: Maune S[2]*, Neher A*[3]*, Pahl A*[4]*, Stecher G*[5]*, Szelenyi I*[4]
[1]*Karolinska Intitute, E.N.T. Research Laboratory, Huddinge University Hospital, Huddinge, Sweden;* [2]*E.N.T Hospital, University Hospital Schleswig-Holstein, Kiel, Germany;* [3]*University Hospital for E.N.T, Innsbruck, Austria;* [4]*Institute of Experimental and Clinical Pharmacology and Toxicology, Friedrich-Alexander-University of Erlangen, Germany;* [5]*Leopold-Franzens-University, Innsbruck, Austria*

It is important that the cell is able to transport molecules in and out of itself. The transport mechanisms can be divided in simple diffusion, facilitated diffusion, and active transport. Simple **diffusion** means that the molecules can pass directly through the membrane corresponding to a concentration gradient. **Facilitated diffusion** utilizes membrane protein channels to allow charged molecules to freely diffuse in and out of the cell. **Active transport** requires energy to transport the molecule from one side of the membrane to the other, but active transport is the only type of transport that can take molecules up their concentration gradient as well as down. Similarly to facilitated transport, active transport is limited by the number of protein transporters. Drug transport through the nasal epithelium can be classified as either paracellular or transcellular. In the absence of active transport components, most drugs cross the nasal epithelium by the **paracellular route**, driven by passive diffusion. The **transcellular route** is relevant for carrier or receptor mediated transport processes or for transcytosis. Both transcellular routes are energy-dependent and are therefore designated as active transport processes. Additionally, the nasal epithelium is also rich in many cell-surface located enzymes. Apart from the **"transport-barrier"** we have to consider the **"metabolic barrier"**. The **"mucus barrier"** in the nasal epithelium is important for hydrophobic drugs, but relatively permeable to hydrophilic compounds. Influence of various drugs on the ciliary beat activity has been investigated and the results will be presented. A separate lecture will deal with the highly important role of the immunologically active cells in the epithelium. An additional defence mechanism of the nasal mucosa will be discussed. The effects of herbal drugs on the immunological function of the epithelial cells will be revealed. Special interest has been focussed on the compartmentalisation of a herbal drug using high-sophisticated analytical methods. *Szelenyi I.*: Transport mechanisms and function of nasal epithelial cells. *Stecher G.*: What is to be found in different parts of the nasal epithelium? – Analytical profiles of a herbal drug. *Maune S.*: Antibacterial activity of the nasal epithelium. *Neher A.*: Influence of ciliary beating activity by different compounds. *Pahl A.*: Is the nasal epithelium a simple barrier or an immunologically important organ?

Posters

1. Drug Discovery from Natural Products

P 001

Blood Pressure Lowering Action of Active Principle from Ocimum basilicum

Aftab K
Hamdard Institute of Pharmaceutical Sciences, Hamdard University, Islamabad Campus & H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

Ocimum basilicum (L.) belongs to the family Labiaceae and commonly has known as Basil (Tulsi). It is a widespread plant cultivated in the world. In Indo-China, the ashes of the roots are suggested as a remedy for skin disease. The plant is used as aromatic, anti-microbial, astringent in dysentery, while the leaves are antipyretic. The seeds are laxative, particularly in case of habitual constipation. The juice of the leaves and flowers are a treatment of cough. A decoction may be given after parturition as emmenagogue and febrifuge. The leaves are carminative, antispasmodic and sedative. Preparations of basil are used fir supportive therapy for feeling of fullness and flatulence, for the stimulation of appetite and digestion, and as diuretic. In anaesthetized rats, methanolic extract, fractions, and pure compound eugenol (0.3 – 3.0 mg/kg) produced dose-dependent fall in blood pressure and heart rate. These effects were not blocked by atropine (1 mg/kg) and eugenol did not modify presser response of norepinephrine which rules out the possibility of cholinergic stimulation or α-adrenergic blockade. In spontaneously beating atria, Eugenol caused decrease in force and rate of contractions. These effects remain unaltered in presence of atropine. In rabbit aorta, eugenol caused relaxation of norepinephrine and potassium induced contractions in a concentration-dependent manner. These results suggest that the direct relaxant action of Eugenol on myocardium and on blood vessels may be responsible for its hypotensive and bradycardiac effects observed in the *in vivo* studies.

P 002

Bioassay guided purification of an immunomodulatory polysaccharide from roots of Tinospora cordifolia

Verma R[1]*, Juvekar AR*[1]*, Gopalkrishnan R*[2]
[1]*Department of Pharmaceutical Sciences and Technology, Mumbai University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019, India;* [2]*Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Center,Trombay,Mumbai-400085,India*

The interest of researchers in medicinal plants as natural sources has noticeably increased in the past 20 years. Further particular attention has been given to substances, which are used as folklore medicines. Plant polysaccharides have been extensively studied for their antitumor, antibacterial and antifungal properties. Certain polysaccharides from herbs possess immuno-enhancing effects,

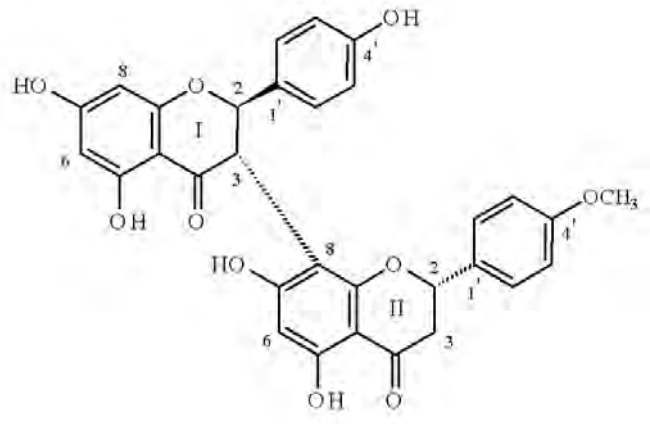
such as the augmentation of spleen lymphocyte functions. Hence the present investigation was carried out towards isolation and activity based purification of one such immunologically active polysaccharide (arabinogalactan) from the roots of *Tinospora cordifolia* (Menispermaceae). Fractionation of crude isolated polysaccharide was carried out using sephacryl S-400 GPC column followed by *in vitro* mitogenic stimulation of mice spleen lymphocytes. The cells were pulsed with ^{3}H Thymidine (1 μCi/well) and the amount of radioactivity incorporated into DNA was measured using liquid scintillation counter. Results revealed that fraction-II exhibited a higher stimulation index (S.I.= 17) as compared to that of both fraction-I (S.I.= 12) and crude (S.I.= 11). Further, on structure elucidation of fraction-II by qualitative sugar analysis, ^{1}HNMR-spectroscopy and partial acid hydrolysis revealed predominantly, the presence of arabinogalactan in the pure fraction. **References**: 1. Kapil A., Sharma S. (1997), J. Ethnopharmacol., 50: 89 – 95. 2. Vogel G. H., Vogel W. H. (1997), Drug Discovery and Evaluation: Pharmacological assays, Springer – Verlag, Berlin. 3. Sendl, A. *et al.*,(1993), Phytochemistry, 4**:** 1357 – 1362.

P 003

Antiparasitic Activity of Some Xanthones and Biflavonoids and Identification of a New Biflavanoid from the Root Bark of Garcinia livingstonei

Pieters L[2], Mbwambo ZH[1], Kapingu MC[1], Moshi MJ[1], Machumi F[1], Apers S[2], Cos P[3], Ferreira D[4], Marais JPJ[4], Vanden Berghe D[3], Maes L[3], Vlietinck A[2]
[1]Institute of Traditional Medicine, Muhimbili University College of Health Sciences, P.O. Box 65001, Dar es Salaam, Tanzania; [2]Laboratory of Pharmacognosy and Phytochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium; [3]Laboratory of Microbiology, Parasitology and Hygiene, Departments of Pharmaceutical and Biomedical Sciences, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium; [4]Department of Pharmacognosy and National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, University, MS 38677, USA

A new biflavanoid, *ent*-naringeninyl-(I-3,II-8)-4'-*O*-methylnaringenin (**1**), along with five known xanthones and two known biflavonoids, (+)-volkensiflavone and (+)-morelloflavone, was isolated from the root bark of *Garcinia livingstonei* (Clusiaceae) T. Anders., collected in Tanzania. The absolute configuration of **1** was established by CD spectroscopy. This compound showed a moderate activity against *P. falciparum* (IC_{50} 6.0 ± 1.7 μM). Antitrypanosomal activity (IC_{50} 0.87 ± 0.23 μM) was observed for 1,4,5-trihydroxy-3-(3-methylbut-2-enyl)-9*H*-xanthen-9-one. The dimeric xanthone garcilivin A showed a higher and non-selective antiparasitic activity and cytotoxicity (IC_{50} 2.0 ± 0.1 μM against MRC-5 cells) than its diastereoisomer garcilivin C (IC_{50} 52.3 ± 5.5 μM).



P 004

Flavonoids from Acacia saligna leaves and Evaluation of Antihyperglycaemic Effect of Aqueous Extract

El-Toumy SAA
Chemistry of Tannins Department, National Research Center, Cairo, Egypt

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 flavonoids from *Acacia saligna* (Labil L.) H.L. Wendel. leaves and evaluation of antihyperglycaemic effect of aqueous alcoholic extract. The aqueous alcoholic extract (MeOH: H_2O, 7: 3) of *Acacia saligna* leaves was subjected to extensive repeated Column chromatography on polyamide, cellulose and Sephadex LH-20 resulted in myricetin 3-*O*-β-arabinopyranoside, quercetin 3-*O*-β-arabinopyranoside, luteolin 7-*O*-β-arabinopyranoside, myricetin 3-*O*-α-L-rhamnopyranoside, quercetin 3-*O*-α-L- rhamnopyranoside, myricetin 3-*O*-β-glucopyranoside, quercetin 3-*O*-β-glucopyranoside, luteolin 7-*O*-β-glucopyranoside, luteolin, quercetin and myricetin. 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 saligna* leaves (30 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 saligna* leaves from an initial level of (255.6 ± 20.8) to (117.8 ± 10 mg/dL). The extract failed to produce hyperglycemic activity in normal treated rats. The chemical constituents of plant especially flavonoids and other compounds present in the plant may be involved in the observed hypoglycemic effect of the plants extract [1]. The results show that the oral administration of *Acacia saligna* leaves extract on the diabetic state reducing hyperglycemia. **Reference**: 1. Resurreccion-Mago, M.H., Villasenor I.M. *et al.* (2005), Phytother. Res. 19: 246 – 251.

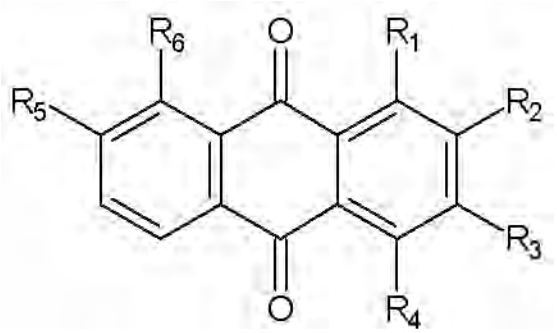
P 005

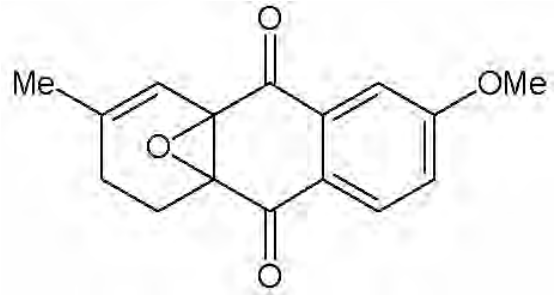
Isolation and Structure Elucidation of Anthraquinones from Barleria eranthemoides (Acanthaceae)

Pieters L, Maregesi S, Apers S, Vlietinck A
Laboratory of Pharmacognosy and Phytochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium

Barleria eranthemoides R. Br. (Acanthaceae) is used in traditional medicine in the Bunda district, Tanzania. Root decoctions or infusions of pounded leaves are drunk for treatment of dysentery and against infectious diseases. Whereas phytochemical and biological investigations on the 80 % methanolic extract of roots of *B. eranthemoides* are still in progress, a series of anthraquinone derivatives have been obtained from the *n*-hexane extract, which was investigated for its antiprotozoal activity. In addition to barleriaquinone (**1**), reported before from *Barleria buxifolia*, and the other known anthraquinones chrysophanol (**2**), isochrysophanol (**3**), digitopurpone (**4**), and 2-methoxy-7-methylanthraquinone (**5**), a new unusual anthraquinone derivative containing an epoxide moiety (**6**) was

obtained. Structures were elucidated by means of 1D (^{1}H, ^{13}C, DEPT) and 2D (COSY, HSQC and HMBC) NMR and mass spectroscopy.





	R_1	R_2	R_3	R_4	R_5	R_6	6
1	OH	H	H	H	Me	H	
2	OH	H	Me	H	H	OH	
3	OH	Me	H	H	H	OH	
4	OH	Me	H	OH	H	OH	
5	H	OMe	H	H	Me	H	

P 006

Evaluation of hepatoprotective activity of the Acacia nilotica (L.) Wild. ex Delile. leaves on carbon tetrachloride-induced liver damage in rats

Abdel-Razik HF[1], Enayat AO[1], El-Toumy SAA[2], Wafaa EAA[1]
[1]Pathology Department, National Research Center, 33 El-Bohouth Street, Dokki, PO: 12622- Cairo, Egypt; [2]Chemistry of Tannins and Leather Technology Department, National Research Center, 33 El-Bohouth Street, Dokki, PO: 12622- Cairo, Egypt

In this study, the hepatoprotective effect of the methanolic extract of *Acacia nilotica* leaves was investigated against CCl_4-induced liver damage in rats. The extract was tested in two different treatments (15 and 30 mg/kg/b.w) and three different durations (1, 2 and 3 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 (15 and 30 mg/kg/b.w for 1, 2, or 3 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 *Acacia nilotica* leaves 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 (30 mg/kg b. wt.) of the extract and the effect of *Acacia nilotica* seems dose- and time-dependant. In conclusion, the results suggest that *Acacia nilotica* exerts hepatoprotective effects against CCl_4-induced liver injury.

P 007

Antiprotozoal activity of saponins from Anogeissus leiocarpus (Combretaceae)

Chaabi M[1], Benayache S[2], Vonthron-Sénécheau C[1,3], Weniger B[1], Anton R[1], Lobstein A[1]
[1]Laboratoire de Pharmacognosie, LC1 UMR 7175, Faculté de Pharmacie de Strasbourg,78 route du Rhin, 67401 Illkirch, France; [2]Laboratoire de Valorisation des Ressources Naturelles, Route Ain El-Bey. 25000 Constantine. Algérie; [3]Laboratoire de Biologie et Biotechnologies Marines, Ifremer UMR 100, Université de Caen Basse Normandie, Esplanade de la Paix, 14032 Caen, France

In the frame work of our research on African species presenting antiparasitic activities, we reported previously the good antiprotozoal activity of *Anogeissus leiocarpus* (DC.) Guill. & Perr. (Combretaceae) [1]. In continuation of our work, we examined the constituents of the bark of this species. Fractionation of the ethanolic bark crude extract was carried out by combination of gel filtration on Sephadex LH-20 and preparative TLC. Two saponins of oleane type were isolated for the first time from this genus and their structures were established by spectroscopic methods, including 2D-NMR heteronuclear correlation experiments. They were identified as olean-12-en-28-oic acid 2α,3β,19α,23,24-pentahydroxy-β-D-glucopyranosyl ester (trachelosperoside E1) (**1**) and olean-12-en-28-oic acid 2α,3β,19α,23-tetrahydroxy-β-D-glucopyranosyl ester (arjunglucoside I) (**2**), both previously isolated from *Rudgea viburnioides* [2]. A comparison of their antiprotozoal activity shows that (**1**) has a good antitrypanosomal activity (IC_{50}= 1.24 μM), without significant cytotoxicity (SI > 100). Structure elucidation of three other saponins and three ellagic acid derivatives are currently under progress.

Samples	In vitro antiprotozoal activity IC_{50} (μM)				Cytotoxicity[e]	
	Antiplasmodialactivity [a]	Leishmanicidalactivity [b]	Antitrypanosomalactivity [c]	Antitrypanosomalactivity [d]	IC_{50} (μM)	SI [f]
1	> 5	> 5	> 5	1.24	> 150	> 100
2	> 5	> 5	> 5	> 5	> 150	> 5
Chloroquine	0.19	-	-	-	-	-
Artemisinin	0.007	-	-	-	-	-
Miltefosin	-	0.47	-	-	-	-
Benznidazole	-	-	1.69	-	-	-
Melarsoprol	-	-	-	0.005	-	-
Podophyllotoxin	-	-	-	-	0.048	-

Data shown are values of duplicate

[a] *Plasmodium falciparum* K1 resistant strain; [b] *Leishmania donovani* amastigotes; [c] *Trypanosoma cruzi Talahuen* strain trypomastigotes; [d] *Trypanosoma brucei rhodensiense* STIB 900 strain trypomastigotes; [e] L6 cells, [f] SI: selectivity index, ratio of cytotoxic activity on L6 cells to antitrypanosomal activity against STIB 900 strain of *T. brucei rhodensiense* trypomastigotes.

References: 1. Vonthron-Sénécheau, C. *et al.* (2003), J. Ethnopharmacol. 87: 221 – 225. 2. Young, M.C. *et al.* (1998), J. Nat. Prod. 617: 936 – 938.

P 008

Antimalarial and antitrypanosomal activities of West Cameroon medicinal plants

Ndjakou BL[1], Weniger B[2], Tantangmo F[3], Chaabi M[2], Ngouela S[3], Tsamo E[3], Anton R[2]
[1]Department of Chemistry, Higher Teacher's Training College, University of Yaounde 1, BP 47, Yaounde, Cameroon; [2]Laboratoire de Pharmacognosie, LC1 UMR 7175, Faculté de Pharmacie de Strasbourg,78 route du Rhin, 67401 Illkirch, France; [3]Department of Organic Chemistry, Faculty of Science, University of Yaounde 1, BP 812, Yaounde, Cameroon

Fourteen extracts from seven Cameroon medicinal plants [1, 2], traditionally used to treat malaria and other parasitic diseases were screened for their antiprotozoal activities against *Plasmodium falciparum* K1 chloroquine resistant strain and *Trypanosoma brucei rhodesiense*, protozoa responsible for malaria and trypanosomiasis, respectively. The most active extract against *P. falciparum* was the

methanolic extract of *Albizia zygia* stembark, with an IC_{50} value of 1.04 μg/mL. Three of the tested extract showed IC_{50} below 7.15 μg/mL against *T.b. rhodesiense*, with *Albizia zygia* methanolic extract showing again the best activity (IC_{50} = 0.18 μg/mL). These results contribute to the validation of the traditional antiprotozoal use of these medicinal species in Cameroon. **References**: 1. Vivien J., Flaure J.J. (1973), *Arbres des forêts denses d'afrique centrale: Espèces du Cameroun.* République Francaise. Agence de coopération culturelle et technique. 2. Laird S.A. *et al.* (1997), Medicinal Plants of the Limbe Botanic Garden, Cameroon.

P 009

Bio-guided isolation of anti-salmonellae compounds of Thonningia sanguinea, an Ivorian medicinal plant

Chaabi M[2], N'guessan JD[1], Weniger B[2], Ramanou A[1], Andre P[3], Guedeguina F[1]

[1]Laboratoire de Pharmacodynamie Biochimique, Université de Cocody Abidjan, 22 BP 582 Abidjan 22; [2]Laboratoire de Pharmacognosie, LC1 UMR 7175, Faculté de Pharmacie de Strasbourg,78 route du Rhin, 67401 Illkirch, France; [3]Laboratoire de Bactériologie et de Cryptogamie, Faculté de Pharmacie, Université Louis Pasteur Strasbourg BP 60024 – 67401 ILLKIRCH cedex

Salmonella enterica ssp. *enterica* is a leading cause of bacterial foodborne outbreaks in developed countries and is also a public-health concern in developing countries. Diarrhoea, a common symptom of human salmonellosis, kills 3-million children each year in developing countries [1]. The emergence of strains of *S. enterica* with multiple drug resistance is of great concern worldwide. Our preliminary work showed that the aqueous extract of *Thonningia sanguinea* (Balanophoraceae), an Ivorian plant used traditionally for the treatment of diarrhoea [2] demonstrate growth inhibitory effect *in vitro* against different strains of *Salmonella* such as *S. Typhi* (CMI = 4.16 mg/mL), *S. Typhimurium* (CMI = 4,16 mg/mL), *S. Hadar* (CMI = 3.33 mg/mL), *S. Essen* (CMI = 4.16 mg/mL). In order to isolate the antibacterial compounds, the aqueous extract was successively fractionated with cyclohexane, ethyl actetate and butanol. All these fractions were evaluated for their antibacterial activity using the disc-diffusion assays. The butanolic extract (5 mg/disc) was the most active extract according to the inhibition zone diameter (12 ± 0.8 mm). Fractionation of the butanolic extract lead to the isolation of two polyphenolic derivates which structure elucidation is under process. **References**: 1. White, P.L. *et al.* (1997), Rev. Sci. Tech. 16: 525 – 541. 2. Vangah-Manda, M. *et al.* (1994), Rev. Med. Pharm. Afr. 8: 154 – 157.

P 010

Antioxidant and lipoxygenase inhibitory activities of boropinic acid, active principle of Boronia pinnata

Genovese S[1], Curini M[1], Epifano F[2], Menghini L[2], Ricci D[3], Fraternale D[3], Giamperi L[3], Bucchini A[3],Bellacchio E[4]

[1]Dipartimento di Chimica e Tecnologia del Farmaco, Sezione di Chimica Organica, Via del Liceo, 06123 Perugia, Italy; [2]Dipartimento di Scienze del Farmaco, Via dei Vestini 31, 66013 Chieti Scalo, Italy; [3]Istituto di Botanica e Orto Botanico, Via Bramante 28, 61029 Urbino, Italy; [4]CSS Hospital, IRCCS, San Giovanni Rotondo and CSS Mendel Institute, Viale Regina Margherita 261, 00198 Roma, Italy

Boropinic acid is a prenyloxy-cinnamic acid recently isolated from *Boronia pinnata* Sm., an Australian shrub belonging to the family of Rutaceae [1]. Like other prenyloxy-cinnamic and benzoic acids isolated from natural sources, few data about biological activity have been reported in the literature. Boropinic and other natural prenyloxy-cinnamic and benzoic acids, namely 4'-geranyloxy-*p*-coumaric acid, 4'-geranyloxy-ferulic acid, isolated from *Acronychia baueri* Schott [2], valencic acid, isolated from *Citrus sinensis* L. and *Aegle marmelos* Corr. [3] and 4-isopentenyloxy-vanillic acid, isolated from the liverwort *Trichocolea lanata* (Ehrh.) Dum. [4] were easily synthesized by a two-step sequence in high yield from the corresponding *p*-hydroxy aromatic acid and were assayed for radical scavenging activity using the DPPH test and for inhibition of enzymatic lipid peroxidation mediated by soybean 5-lipoxygenase. Compared to other acids and to known antioxidant compounds like BHT, Trolox and ascorbic acid, boropinic acid was by far more active in the lipoxygenase test (IC_{50}= 7.6 ng/mL, $p < 0.05$). The inhibition value recorded suggested that boropinic acid acted as an enzyme inhibitor rather than a mere radical or peroxide scavenger. This hypothesis was confirmed by studying the interaction between boropinic acid and soybean 5-lipoxygenase by molecular modeling techniques. **References**: 1. Ito, C. *et al.* (2000), J. Nat. Prod. 63: 1344 – 1348. 2. Prager, R.H., Thredgold, H.M. (1966) Aust. J. Chem. 19: 451 – 454. 3. Ali, M.S., Pervez, M.K. (2004), Nat. Prod. Res. 18: 141 – 146. 4. Perry, N.B. *et al.* (1996), J. Nat. Prod. 59: 729 – 33.

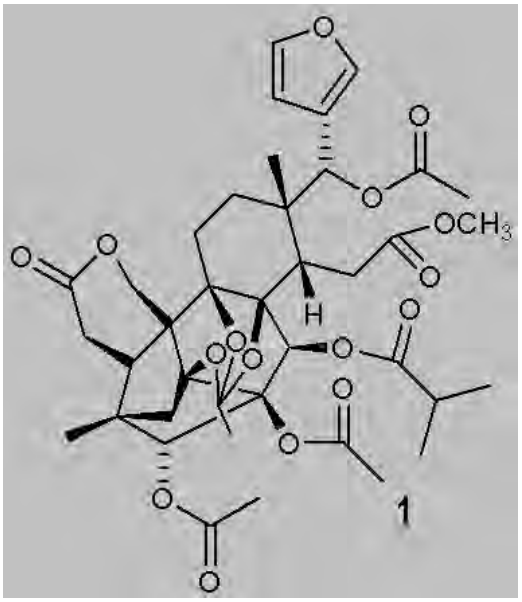
P 011

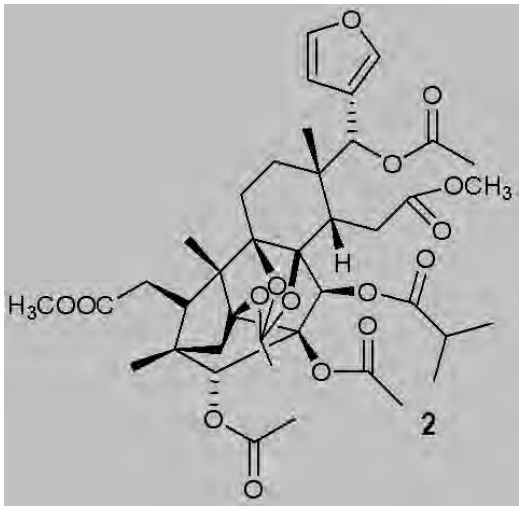
New limonoid orthoacetates and antiprotozoal compounds from Pseudocedrela kotschyi (Schweinf.) Harms

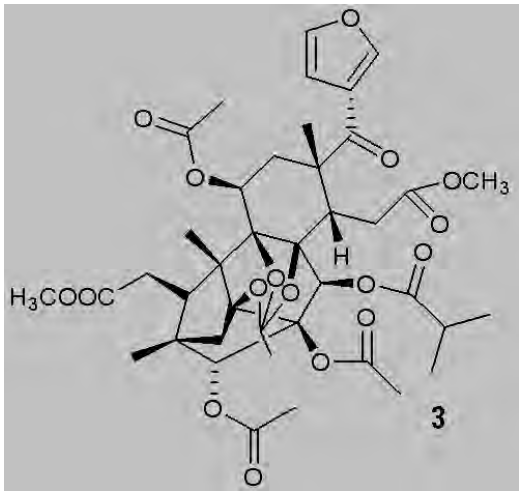
Hay AE[1], Ioset JR[1], Ahua KM[1], Diallo D[2], Brun R[2], Hostettmann K[1]

[1]Laboratory of Pharmacognosy and Phytochemistry, Geneva-Lausanne School of Pharmacy, University of Geneva, CH-1211 Geneva 4, Switzerland; [2]Department of Tropical Medicine, National Institute of Research of Public Health, BP 1746, Bamako, Mali; [3]Parasite chemotherapy, Swiss Tropical Institute, CH-4002 Basel, Switzerland

In the course of an antiprotozoal screening of extracts issued from plant species commonly used in the Malian traditional medicine, the dicholoromethane extract of *Pseudocedrela kotschyi* (Meliaceae) demonstrated a marked activity against the intracellular form of *Leishmania major. P. kotschyi* is commonly used in the Sub-Saharan region to treat various skin affections, yaws, syphilis chancres, sleeping sickness and treat malaria. Its phytochemical investigation permitted to isolate three novel phragmalin-type limonoid orthoacetates named kotschyin A-C (**1**-**3**) besides the known compounds 7-deacetylgedunin, 7-deacetyl-7-oxogedunin [1] (-)-catechin, and (-)-epicatechin. The relative configurations of kotschyin A-C were assigned on the base of NOE correlations. The extract and some pure compounds have then been tested for their cytotoxicity and antiparasitic activity against *Leishmania donovani*, *Trypanosoma brucei rhodesiense, Trypanosoma cruzi,* and *Plasmodium falciparum*. The raw extract exhibited good antiplasmodial, antileishamanial and trypannocidal activities that could be attributed to 7-deacetylgedunin and 7-deacetyl-7-oxogedunin. Kotschyin A-C remained inactive in the same assays.







Reference: 1. MacKinnon, S., Durst, T. *et al.* (1997), J. Nat. Prod. 60: 336 – 341.

P 012

Flavonoids and insecticidal activity of Teucrium zanonii

Abdelshafeek KA[1], Ismail IA[2], Alwahsh MA[1]
[1]ALTahady University, Faculty of Science, Chemistry dept, Sirt, Libya p. o.674; [2]National Research Center, pests and plant protection dept, Dokki, Cairo, Egypt

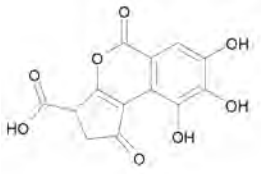
In frame of our investigation for medicinal Libyan plants we choose Teucrium zanonii Pamp. (Family Labiatae) which is an endemic plant growing in Abofakhra region (25 Km from Benghazi City) [1]. The plant was used in folk medicine for gastrointestinal troubles, tonic, renal inflammatory and antidiabetic [2]. Investigation of the flavonoidal constituents lead to the identification of ciriliol, luteolin, chrysoeriol, and xanthomicrol from the ethyl acetate fraction while apigenin – 6,8 – di-*O*-glucoside and Luteolin – 7 – *O* – rutinoside from the butanol fraction. All structures were established using different chromatographic and spectroscopic (UV,MS, FAB-MS, ^{1}H,^{13}C-nmr) measurements [3]. The insecticidal activity measurements of different extracts against *Phloeotribus oleae* on olive trees showed that, the aqueous extract exhibit the highest mortality in the lab. and field experiments (86.67 % and 70.82) respectively [4].
References: 1. Siddiqi, M.A. (1985), Flora of Libya, Vol. 118: Lamiaceae. Revolution printing press, Tripoli, Libya. 2. Assem, M.E., Karam, T.H. (2004), Biochem. Syst. Ecol. 32: 665 – 674. 3. Savona, G., Paternostro, M.P, *et al.* (1979), An. Quim. 75: 433 – 436. 4. Bruno, M., Piozzi, F. *et al.* (2002) Biochem. Syst. Ecol. 30: 595 – 599.

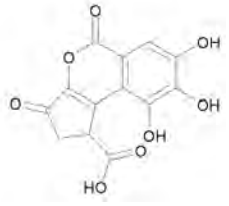
P 013

Brocchiana carboxylic acid; the analogue of brevifolin carboxylic acid, Isolation and identification from Chrozophora brocchiana

Hawas UW
Phytochemistry and Plant Systematic Dept, National Research Centre, Dokki, Cairo, Egypt

The *Euphorbiaceae* is a large family with close to 300 genera and 5000 – 7500 species. In Egypt, this family is represented by only seven genera [1]. The chemosystematics of Euphorbia species in Egypt have recently been investigated and the current study of *Chrozophora* species is a continuation of our research on the phenolic constituents of this family. Previous phytochemical investigation of the genus *Chrozophora* resulted in the isolation of several types of chemical constituents including essential oils, terpenes, sterols, phenylpropanoid glycosides, xanthones, chromone and flavonoids [2]. The present study deals with the isolation and identification of phenolic constituents from the aerial parts of *Chrozophora brocchiana* Vis. Brocchiana carboxylic acid, the analogue of brevifolin carboxylic acid [3] was isolated and identified from the aqueous methanol extract of the aerial parts of *Chrozophora brocchiana* in addition to eight known compounds identified as gallic acid, methyl gallate, ethyl gallate, ellagic acid, mono- and di-methoxy ellagic acid, apigenin and luteolin 7-*O*-glucoside. The structures were determined primarily by ESI-MS spectrometry and NMR spectroscopy. The assignment of NMR signals was performed by means of ^{1}H–^{1}H COSY, HMQC and HMBC experiments.





Brocchiana carboxylic acid
Brevifolin carboxylic acid

References: 1. Taeckholm, V. (1974), Student's Flora of Egypt, 2nd ed., Cairo University Press, Cairo. 2. Hashim, O.K, Abou–Zaid, M.M. *et al.* (1990), Biochem. Syst. Ecol. 18: 151 – 152. 3. Nawwar, M.A.M. *et al.* (1994), Phytochemistry 36: 793 – 798.

P 014

Helichrysums: antibacterial and monoamine oxidase inhibitory activity of South African summer-rainfall species

van Staden J[1], Stafford GI[1], Pedersen PD[2], Chukwujekwu JC[1], Jäger AK[2]
[1]Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa; [2]Department of Medicinal Chemistry, Pharmacognosy, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark

A large number of *Helichrysum spp.* (Asteraceae) is used traditionally in southern Africa to treat a variety of ailments. It is a popular ingredient in wound dressings [1] and is an important plant culturally as it is burned at almost all traditional gatherings. Several species have been shown to have a sedative effect [2] and antibacterial activity both against Gram-positive and Gram-negative bacteria [3]. Antibacterial activity was detected using MIC values of crude extracts ranging from 6.25 to 0.049 mg/mL. Of the 9 *Helichrysum* species assayed for activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis*, 2 species, *H. ruderale* and *H. argyolepis* showed broad spectrum activity. Besides *H. hesbaceum* and *H. adenocarpum* that did not display antibacterial activity, the rest were active either against one or two bacteria. MAO-B inhibitors are known to increase the basal dopa-

mine levels in the nigrostriatal dopaminergic input pathway, a fact that is utilised in the symptomatic therapy for Parkinson's disease [4].Seven species were assayed for MAO-B inhibitory activity using a peroxidase-linked photometric assay. *H. agyrolepis* (IC_{50}= 0.1 μg/mL), *H. umbraculigerum* (IC_{50}= 4.3 μg/mL) and *H. ruderale* (IC_{50}= 3.3 μg/mL) were the most active species, although all species tested exhibited some MAO-B inhibition activity. **References**: 1. Watt, J.M., Breyer-Brandwijk, M.G. (1962), The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingston. London. 2. Stafford, G.I. *et al.* (2005), J. Ethnopharmacol. 100: 210 – 215. 3. Afolayan, A.J., Meyer, J. J. M. (1997), J. Ethnopharmacol. 57: 177 – 181. 4. Cesura, A.M., Pletscher A. (1992), Prog. Drug. Res. 38: 171 – 297.

P 015

In vitro antiplasmodial activity and cytotoxicity of ethnobotanically selected East African plants used for the treatment of malaria

Froelich S[1], Onegi B[2], Kakooko A[2], Schubert C[1], Jenett-Siems K[1]*
[1]Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Königin-Luise-Str.2 – 4, D-14195 Berlin, Germany. Fax: +49 – 30 – 83853729, E-mail: kjsiems@zedat.fu-berlin.de; [2]Department of Pharmacy (Pharmacognosy Unit), Makere University, Kampala, Uganda

In Uganda, a variety of plant remedies is used by traditional healers to treat symptoms of malaria. Due to oral interviews, *Vernonia amygdalina* (Asteraceae), *Aspilia africana* (Asteraeae) and *Momordica foetida* (Cucurbitaceae) were selected for further evaluation. Extracts were obtained by macerating air dried plant material collected near Kampala, Uganda, in equal volumes of petroleum ether/ethyl acetate and methanol (herb-solvent ratio 1:3, repeated twice), respectively. The crude extracts from roots and leaves, characterized by HPLC fingerprint chromatograms, were tested in vitro against the chloroquine-sensitive strain PoW (IC_{50} value for chloroquine = 0.011 μM) and a chloroquine-resistant strain Dd2 (IC_{50} value for chloroquine = 0.12 μM) of *Plasmodium falciparum*. The antiplasmodial activity was determined according to Desjardins et al. [1]. *M. foetida* showed significant antimalarial activity (IC_{50} values ranging from 7.3 to 13.0 μg/mL), whereas the root extract of *V. amygdalina* and the leaf extract of *A. africana* displayed lower activities (IC_{50} values: 19.0 and 30.3 μg/mL [PoW]). Cytotoxicities of all extracts were determined against human hepatocellar carcinoma (HepG2) and human urinary bladder carcinoma (ECV-304) (derivative of T-24) cells [2]. The petroleum ether/ethyl acetate leaf extract of *Vernonia amygdalina* showed the highest cytotoxicity. *Acknowledgements:* Mrs. Ursula Friedrich (Institut für Pharmazie) for technical assistance in the cell-laboratory. **References**: 1. Desjardins, R.E. *et al.* (1979), Antimicrobial Agents Chemother. 16: 710 – 718. 2. Mosmann, T. *et al.* (1983), J. Immunol. Methods 65: 55 – 63.

P 016

Cytotoxic stilbenes from Cajanus cajan (L.) Millsp. leaves

Ashidi JS, Houghton PJ, Hylands PJ
Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom

Cancer remains one of the leading causes of death in the world especially in the developed countries [1]. We now report a biologically monitored phytochemical separation of the leaves of *Cajanus cajan* against a panel of human cancer and non- cancer cell lines *in vitro*. *Cajanus cajan,* commonly called Pigeon pea, is grown for food and medicinal purposes in the tropics, especially Nigeria. The air dried leaves were extracted with methanol continuously for five days. The extract was then concentrated under reduced pressure. 60 g of the extract was adsorbed on Silica Gel GF_{254} and separated into fractions by vacuum liquid chromatography (VLC). The SRB assay was used to evaluate the cytotoxicity of the extracts and the isolated compounds. The dichloromethane (DCM) fraction of the leaves exhibited modest cytotoxicity against human amelanotic melanoma – C32, human breast adenocarcinoma – MCF-7 and human large cell lung carcinoma cell lines – COR-L23 and human fetal lung fibroblast – MRC-5 (IC_{50} = 12.0, 10.0, 10.0 and 15.0 μg/mL, respectively). This finding prompted further activity-guided fractionation of the DCM fraction by flash chromatography and subsequent purification on preparative thin-layer chromatography which led to the identification of two prenylated stilbenes, longistylin A and C. These compounds have previously been reported to have antiplasmodial activity [2]. This is also the first time that these prenylated stilbenes are shown to exhibit *in vitro* cytotoxic activity against human amelanotic melanoma, C32, human breast adenocarcinoma, MCF-7, and human large cell lung carcinoma, COR-L23, cell lines. The IC_{50} of the compounds ranges between 20 and 35 μM. These compounds could explain the rational inclusion of *Cajanus cajan* in traditional herbal medicines used for the treatment of cancer in south- western Nigeria. Further study to establish the mechanism of action of these two stilbenes is in progress. *Acknowledgement:* JSA thanks the Association of Commonwealth Universities, UK, for financial support. **References**: 1. Anonymous, http://health.yahoo.-com/news/58569. 2. Duker-Eshun, G, *et al.* (2004), Phytotherapy Research, 18:128 – 130.

P 017

Antibacterial and resistance –modifying effects of Mezoneuron benthamianum

Dickson RA, Houghton PJ, Hylands PJ
Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NN, UK

The ever increasing resistance of human pathogens to current antimicrobial agents is a serious medical problem, and has resulted in the need for novel antibiotic prototypes. The root bark of *Mezoneuron benthamianum* Baill.(Caesalpinaceae) is used in Ghanaian traditional medicine for the treatment of wounds and other dermal infections. Bioactivity-guided fractionation of the pet. spirit extract led to the isolation of cassane-type diterpenes active against various bacteria and two strains of *Staphylococcus aureus* possessing the multidrug efflux pumps NorA and TetK (SA1199B and XU212) [1]. Addition of R2 and R3 in the growth medium at 10 μg/mL resulted in a 16-fold and 8-fold (Norfloxacin) and 8-fold and 4-fold (Tetracycline) potentiation of activities in both compounds respectively (Table 1). The other three compounds however were not active against the efflux pumps but showed various degrees of activities against other bacteria.

Table1 Antibacterial susceptibility of test strains in the absence and presence of 10 μg/mL of R2 and R3 and 20 μg/mL reserpine, a naturally occurring MDR efflux inhibitor serving as a standard modulator, n = 3.

Antibacterial agent	MIC of test strain expressing the indicated efflux protein SA 1199B(NorA)	XU 212(TetK)
Norfloxacin	32	NT
+R2	2	
+R3	4	
+Reserpine	32	
Tetracycline	NT	128
+R2		16
+R3		32
+Reserpine		32

Ackowledgement: Rita Akosua Dickson is funded by the Commonwealth Scholarship Commission, UK. **Reference**: 1. Gibbons, S. *et al.* (2004), Phytochem. 65: 3249 – 3254.

P 018

Chemical composition and cytotoxic activities of essential oils of leaves and berries of Juniperus phoenicea L grown in Egypt

El-Sawi S, Motawae H
National Research Centre, Dokki, 12622,Cairo, Egypt

Hydrodistillation of berries and leaves of Juniperus phoenicea grown in Sinai yielded volatile oils in the yield of 0.36 and 1.96%, respectively. Using gas chromatography/mass spectrometry technique, fifty eight compounds were identified in berry oil representing 99.2% of the oil composition. Alpha-pinene was the major compound in berry oil (39.30%) followed by sabinene (24.29%). Berry oil composed mainly of monoterpenoids which amounted to 90.58%, of which 72.85% was monoterpene hydrocarbons. The sesquiterpenoids accounted for about 8% of the total oil composition. Leaf oil was composed of about 66 compounds representing 99.16% of the total composition of the oil. Alpha-pinene was the major constituent of leaf oil at concentration of 38.22%, followed by alpha-cedrol (31.23%). The monoterpene hydrocarbon was the predominant chemical group (41.29%) followed by the oxygenated sesquiterpenes (32.21%). Both oils showed very high cytotoxic activities against all cell line tested. Both oils showed equal activities against brain (0.6 microgram/mL) and cervix (5.0 microgram/mL) human cell lines, while berry oil was slightly more active than leaf oil against lung (0.6 and 0.7 microgram/mL, respectively), liver (0.7 and 0.9 microgram/mL, respectively) and breast human cell lines (0.8 and 1.0 microgram/mL, respectively). **References**: 1, Adams, R. (1995), Identification of Essential Oils Components by Gas Chromatography/Mass Spectrometry. Allured, Carol Stream, Illinois. 2, Afifi, M. *et al.* (1992), Mans. J. Pharm. Sci. 8: 37 – 46.

P 019

Effects of different carbon sources on production of polysaccharides by Agaricus blazei

Vahidi H, Hamedi A
School of Pharmacy Shaheed Beheshti University of Med. Sci. Tehran, Iran

Mushroom polysaccharides offer a lot of hope for cancer patients and sufferers of many devastating diseases. A variety of polysaccharides from a number of mushroom varieties have been demonstrated to enhance the immune system. Yield and functionally of polysaccharides by fermentation are highly dependent on their culture conditions, such as different microorganisms, medium compositions and environmental parameter. Carbon source is one of the most important parameter affecting polysaccharide fermentations. [1]. In this study the effects of different carbon sources including glucose, lactose, sucrose, manitol, starch, galactose, maltose and fructose in two different media (Complex and synthetic) were investigated. For the determination of polysaccharides produced by the fungus the total polysaccharides which precipitated by absolute alcohol were weighed. The experiments showed that the highest growth and polysaccharide production were obtained when galactose and starch were used as carbon source. The concentration of polysaccharide in both complex and synthetic media were similar. The lowest growth and productivity were also seen in medium containing sucrose. **Reference**: 1. Chin–Hang, S. *et al.* (2004), J. Chem. Technol. Biotechnol. 79: 998 – 1002.

P 020

Activity of a compound isolated from Senna villosa against Trypanosoma cruzi

Pérez MS[1], Guzmán E[2], González MR[2], Pérez C[1], Zavala MA[1], Acosta K[2]
[1]Universidad Autónoma Metropolitana-Xochimilco, Calz. del Hueso 1100 CP 04960 México D.F. A.P. 23 – 181 México; [2]Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autónoma de Yucatán, Merida Yucatán, México

Previously we reported that chloroform extract of *S. villosa* (Miller) Irwin & Barneby possess activity against epimastigotes and trypomastigotes of *T. cruzi* [1]. From this extract was isolated a compound, which was identified as 4-hydroxymethylen-2-pentaeiconanone mp 76 – 77 °C, (KBr/cm): 3451, 2919, 2850, 1735. ^{1}HNMR (400 MHz $CDCl_3$) δ (ppm): 0.88(t,3H), 1.25(m,38H), 1.57(s,3H), 1.61(m,3H), 2.29(m,2H), 4.05(m,2H). ^{13}CNMR: 14.0(CH_3), 22.7(CH_2), 25.0(CH_2), 25.9(CH_2), 28.6(CH_2), 29.4(CH), 29.5(CH_3), 29.6(CH_2), 31.0(CH_2), 34.3(CH_2), 64.3(CH_2), 173.6(C=O), EIMS (m/z): 396(1), 97(54), 83(60), 57(100). Analysis combustion calcd for C_{26}, $H_{52}O_2$, C 78.35%, H 13.44%, O 8.21%. Activity was assayed on epimatigotes and trypomastigotes of *T. cruzi* strain Y isolated from human, cultured in liver infusion tryptose medium supplemented with 10% of heat-inactived fetal calf serum and cultured at 28 °C. 1.65, 3.3 and 6.6 μg/mL concentration of this compound was tested. Gentian violet (17.5 mg/mL) was used as positive control. Parasites were counted daily for 10 days. At concentration of 1.65 μg/mL the growth inhibition of trypomastigotes was 60%, with 3.3 μg/mL 72% and with 6.6 μg/mL was 82%, similar results were obtained with epimatigotes. The activity was doses-dependent in both forms of parasite. The susceptibility of parasite to the compound is similar to that observed with gentian violet. **Reference**: 1. Guzmán, E. *et al.* (2004), Pharm. Biol. 42: 1 – 4.

P 021

Partial chemical structure and immunomodulating activities of RGAP (Red Ginseng acidic Polysaccharide) from Korean red ginseng (Panax ginseng C. A. Meyer)

Park JD[1], Shin HJ[1], Kwak YS[1], Wee JJ[1], Song YB[1], Kyung JS[1], Kiyohara H[2], Yamada H[2]
[1]Div. Of Ginseng Efficacy, KT&G Central Research Institute, Daeduk Science Town, 305 – 345, KOREA; [2]Laboratory of Biochemical Pharmacology for Phytomedicine, Kitasato Institute for Life Science, Kitasato University, Tokyo 108 – 8642, JAPAN

A red ginseng acidic polysaccharide (RGAP) with immunomodulating antitumor activities was isolated from Korean red ginseng, steamed and dried ginseng (*Panax ginseng* C. A. Meyer). The molecular weight of RGAP was estimated to be 12 – 450 kDa by gel filtration chromatography. RGAP has been found to increase survival rate and to inhibit of tumor growth significantly in a dose dependent manner in mice transplanted with tumor cells. RGAP significantly promoted nitric oxide (NO) production from peritoneal macrophages both *in vivo and in vitro*. Western blot analysis exhibited a newly synthesized inducible nitric oxide synthase (iNOS) protein band in the RGAP treated group. It seems likely that immunomodulating antitumor activities of RGAP are mainly mediated by NO production of macrophage. RGAP was further purified by ultrafiltration and anion exchange chromatography on DEAE-sepharose, followed by gel filtration on Sephacryl S-300 to give an active fraction (GFP) with stronger NO production in murine macrophages. GFP increased survival rate ten times compared to RGAP in male ICR mice transplanted with sarcoma 180 and also showed more potent tumoricidal activities of natural killer cells than RGAP. Sugar composition(mol %) of GFP was found to be arabinose: rhamnose: xylose: galacturonic acid: mannose: galactose: glucose (10:9:1:25:8:20:27) by GC/MS. Partial acid hydrolysis of GFP resulted in acidic oligosaccharides, and a combination of enzymatic digestion(Endo-α-D-(1→4)-polygalacturonase) and methylation analysis, MALDI-TOF-MS, PSD analysis of the acidic oligosaccharides suggested that GFP comprised a ga-

lactogalacturonan core such as –α-D-GalA-(1→4)-α-D-GalA-(1→4)-α-D-GalA-(1→6)-α-D-Gal as the acidic moiety. The results suggest that certain galactogalacturonan core chains are responsible for immunomodulating antitumor activities as well as nitric oxide (NO) production. *Acknowledgement*: This work was supported by a grant(GP-303) from Korea Ginseng Corporation.

P 022

Effects of QKL on the level of TNF-α, IL-1β, ICAM-1 after intracerebral hemorrhage

Yi L[1,2], Ming L[1], Pengtao L, Qingguo W[1]
[1]School of preclinical Medicine, Bei San Huan Dong Lu 12, Beijing University of Chinese Medicine, 100029, Beijing, China; [2]Institut für Pharmazie (Pharmazeutische Biologie), FU Berlin, Königin-Luise-Str. 2 – 4. 14195 Berlin, Germany

Aims: Inflammatory cytokines release and action are central in the pathogenesis of the inflammatory response that occurs after intracerebral hemorrhage (ICH). Among the inflammatory cytokines, TNF-α, IL-1β, intercellular adhesion molecule (ICAM)-1 is the key factors which lead to secondary brain damages e. g. hydrocephalus and neuron death. QKL is an injection of natural products (consisting of radix scutellariae, fructus gardeniae, cholic acid, radix notoginseng, concha margaritifera). The aim here was to detect the function of anti-inflammation of QKL after the ICH. Method: We established a rat model with collagenase-induced intracerebral hemorrhage. The QKL was injected from the tail vein 3mL every day. The ELISA and RIA were used to detect the level of TNF-α, IL-1β, ICAM-1 in the cerebral homogenate in different periods after ICH. Result: The level of the TNF-α, IL-1β, ICAM-1 is markedly decreased with treating in the period of 48 hours after occurrence of the ICH. But in the period of 24 hours and 72 hours it shows no visible effect. Therefore, QKL has a good effect to inhibit the inflammatory cytokines TNF-α, IL-1β, ICAM-1 in the period of 48 hours after the ICH and consequently protects the neurons from secondary damages.

P 023

Indirubin-3'-monoxime inhibits rat vascular smooth muscle cell proliferation induced by plateled-derived growth factor via the Jak/STAT-pathway

Schwaiberger AV, Heiss E, Dirsch VM
Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria

Indirubin, a constituent identified in the traditional Chinese antileukaemic recipe Danggui Longhui Wan, and its derivatives have been shown to be potent cyclin-dependent kinase (CDK)-inhibitors in vitro [1]. CDKs as key regulators of cell cycle progression are promising targets in the treatment of vasculoproliferative disorders, for instance atherosclerosis and restenosis [2]. The aim of the study was therefore to investigate the antiproliferative effect of indirubin-3'-monoxime (I3MO) on plateled-derived growth factor (PDGF-BB)-induced rat vascular smooth muscle cell (RVSMC) growth. Effects on DNA-synthesis were assessed via BrdU-incorporation after 23 h of treatment with PDGF-BB (20 ng/mL) and increasing concentrations of I3MO (0.1 – 10 μM). At a concentration of 3 μM, the BrdU-positive labeling index was reduced to control level. Cell cycle analysis in the presence of I3MO showed a significant arrest of RVSMCs in the G0/G1 phase after 16 h of stimulation with PDGF-BB. Ongoing treatment over 48 h led to DNA strand breaks at high concentrations (10 μM), as shown by the detection of propidium-iodide stained nuclei with a sub-diploid DNA-content by flow cytometry. Focusing on the involved signaling pathways for these effects, activation of molecules evidentially participating in proliferation was examined. Western blot analysis revealed that the kinases Akt, Erk1/2 and p38 were activated after 10 minutes of stimulation with PDGF-BB, but the effects were not blocked by I3MO at concentrations of 3 and 5 μM. Further investigation of influence on the Jak/STAT pathway, however, indicated a significant inhibition of STAT1 and STAT3(Y705) phosphorylation. These results demonstrate that I3MO inhibits STAT1 and STAT3(Y705) phosphorylation, suggesting that blocking of the Jak/STAT pathway is at least partially responsible for the antiproliferative activity of the compound. *Acknowlegdements:* CNRS, Station Biologique, Amyloÿds and Cell Division Cycle, Meijer L. **References**: 1. Hoessel, R. *et al.* (1999), Nat. Cell Biol. 1: 60 – 67. 2. Dzau, V.J. (2002), Nat. Med. 8: 1249 – 1256.

P 024

Wound healing activity of Alocasia odora (Roxb.) Koch

Viet LD, Houghton PJ, Forbes B, Corcoran O, Hylands PJ
Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NH, United Kingdom

Introduction: During the twenty years of the Vietnam war, the stem of *Alocasia odora* has been used in Vietnam for the treatment of wounds. However, there is little literature about this activity of the plant or its chemical composition. The present study investigates some possible modes of action of the plant in wound healing process and attempts to identify the constituents responsible. **Methods:** Skin fibroblast proliferation assay-guided fractionation and HPLC isolation were used to study bioactivity in extracts of the stem and to locate the active moieties. In addition, an assay measuring protection against H_2O_2-induced damage to skin fibroblasts (142-BR) [1] and the DPPH radical scavenging assay were used to supplement the results of the proliferation assay [2]. **Results:** A total of 10 compounds (1 triterpenoid glycoside, 2 flavonoid C-glycosides, 5 lignan glycosides, 1 lignan and 1 alkaloid) were isolated from the active fractions and tested for bioactivities. The structures of the isolated compounds were determined by the joint application of spectroscopic methods. The lignans, the triterpenoid and the alkaloid showed a slight stimulation of cell proliferation. No compound was shown to possess a protective effect against H_2O_2-induced damage on fibroblasts. Only lignans were shown to have DPPH radical scavenging activity. **Conclusions:** Extracts and some isolated compounds from *Alocasia odora* stems were shown to possess modest activity in the bioassays. It is possible that proliferation of skin fibroblasts and antioxidant activities partly contribute to wound healing activity of *Alocasia odora*. *Acknowledgements:* Vietnamese Ministry of Education and Training, Prof. P K Man, Dr. P V Hien **References**: 1. Tran, V.H. *et al.* (1997), Wound Rep. Reg. 5: 159 – 167. 2. Kyong Soon, K. *et al.* (2003), *J. Ethnopharmacol.* 85: 69 – 73.

P 025

Evaluation of Picralima nitida hypoglycemic activity, toxicity and analytical standards

Inya-Agha SI[1], Ezea SC[1], Odukoya OA[2]
[1]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria; [2]Department of Pharmacognosy, Faculty of Pharmacy University of Lagos, Nigeria

WHO Expert Committee on diabetes encourages further investigation into traditional methods of treatment and also emphasizes the need to ensure safety and quality control of ingredients used. *Picralima nitida* Stapf (Apocynaceae) seeds used in the treatment of hepatitis, worms, sleeping sickness and malaria in Ihiala town, Anambra state of Nigeria, have been shown to have hypoglycemic effects [1]. Hypoglycemic activity was confirmed, recorded as an index of blood glucose with a glucometer in normal and intraperitonially induced alloxan diabetic albino rats with glibenclamide as reference standard and normal saline as control. Toxicity studies included the evaluation of acute and sub-acute (15 days) tests. The animals were observed for toxic signs and symptoms, body weight changes recorded and LD_{50} calculated. Analytical standards were moisture content, ash and extractive values for quality assurance. 100 mg/kg, 300 mg/kg and 900 mg/kg of the extracts to normal rats resulted in

significant ($P < 0.01$) lowering of fasting blood sugar after eight hours. Extract maintained hypoglycemic action throughout the 24hours of study indicating a long duration of action. In normal rats, pulp extract (100 mg/kg) produced a maximum percentage reduction of 38.35 %, rind extract (900 mg/kg) 46.19 % and seed extract (100 mg/kg) 36.81 %. Alloxan induced rats were pulp 85.85 % (300 mg/kg), seed 83.26 % (300 mg/kg) and rind 80.25 % (900 mg/kg) respectively. Order of activity recorded as pulp > seed > rind. Acute toxicities (LD_{50}) of pulp, seed and rind were 7071.06 mg/kg, 948.68 mg/kg and 1364.91 mg/kg respectively. **Reference**: 1. Aguwa, C.N. *et al.* (2001), J. Natural Remed. 1: 135 – 139.

P 026

Effects of Leuzea carthamoides DC. on human breast cancer MCF-7 cells detected by gene expression profiling

Hamburger M[1,5], Gaube F[1], Wölfl S[2,3], Pusch L[2], Kroll T[2], Riese U[1], Schrenk D[4]

[1]Institute of Pharmacy, Department of Pharmaceutical Biology, Friedrich-Schiller-University Jena, D-07743 Jena, Germany; [2]Clinic of Internal Medicine II, Friedrich-Schiller-University Jena, D- 07740 Jena, Germany; [3]Institute of Pharmacy and Molecular Biotechnology, Ruprecht-Karls-University Heidelberg, D-69120 Heidelberg, Germany; [4]Department of Food Chemistry and Environmental Toxicology, University of Kaiserslautern, D-67663 Kaiserslautern, Germany; [5]Department of Pharmaceutical Sciences, Institute of Pharmaceutical Biology, University of Basel, CH-4056 Basel, Switzerland

Products derived from roots of *Leuzea carthamoides* DC. (Maral root) are being promoted as anti-aging and adaptogenic. The phytoecdysteroids are considered as active principles with numerous beneficial effects [1], but little is known about the pharmacological properties of *Leuzea* extracts. We, therefore, investigated the effects of a lipophilic *Leuzea* root extract on the human breast cancer cell line MCF-7. Cell proliferation was inhibited by the extract (IC_{50}= 28 μg/mL) but not by the major phytoecdysteroid, 20-hydroxyecdysone. Genome-wide expression profiling using Affymetrix HG U133 Plus 2.0 microarrays was carried out to analyze effects at the mRNA level. 241 genes appeared to be significantly regulated. Transcripts of gene products involved with cell cycle progression and DNA replication were decreased, while mRNAs coding for inhibitory products were increased. This was in agreement with the antiproliferative activity of the extract. Upregulation of several pro-apoptotic genes provide evidence that the extract may sensitize the cells for apoptotic events. Downregulation of estrogen receptor α could be confirmed by real-time RT-PCR and Western blot. Additionally, expression levels of several transcripts of enzymes with oxidoreductase activity were induced, including a strong increase of CYP1A1 transcript which is known to be regulated via the aryl hydrocarbon receptor (AhR). AhR-agonistic activity of the *Leuzea* root extract, but not 20-hydroxyecdysone was confirmed by a XRE-dependent reporter gene assay. This suggests that at least a part of the effects could be due to AhR activation. However, the phytoecdysteroids are not active principles in *Leuzea* root. **Reference**: 1. Sláma, K., Lafont, R. (1995), Eur. J. Entomol. 92: 355 – 377.

P 027

Antibacterial activity of the essential oils of catnip (Nepeta cataria L.) and lemon balm (Melissa officinalis L.) against clinical isolates from the respiratory tract

Suschke U[1], Geiss HK[2], Reichling J[1]

[1]Institute of Pharmacy and Molecular Biotechnology, Dept. Biology, University of Heidelberg, INF 364, 69120 Heidelberg, Germany; [2]Hygiene Institute, Dept. Medical Microbiology, University Hospital Heidelberg, INF 324, 69120 Heidelberg, Germany

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils of catnip (*Nepeta cataria*), lemon catnip (*N. cataria* var. *citriodora*) and lemon balm (*Melissa officinalis*), whose composition has been analyzed by GC-MS, were determined *in vitro* by a modified broth microdilution method according to the German DIN-regulation 58940 – 8 [1] against clinical isolates of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. These bacteria are frequently involved in respiratory tract infections like sinusitis, tonsillitis, otitis media, bronchitis and pneumonia. Of each strain twelve patient isolates from different culture materials were chosen, which had been identified and characterized by an antibiogram in the routine laboratory of the Hygiene Institute, University Hospital Heidelberg. In spite of their different origin and level of resistance to standard antibiotics, all isolates were susceptible to catnip and lemon balm oils with MIC values ranging from 0.25 % to 0.008 % (v/v). MIC values within the groups of isolates did not differ from those obtained with essential oil sensitive reference strains by more than one dilution step, indicating that natural resistance to these essential oils and cross resistance to standard antibiotics are unlikely to occur in these bacteria. Lemon balm oil, whose main components were geranial (23 %) and neral (17 %), exhibited the highest antibacterial activity, followed by lemon catnip oil, whose main components were the monoterpene alcohols nerol, citronellol, and geraniol (≈ 50 %), and catnip oil which contained mainly nepetalactones (77 %) and only small amounts of monoterpene aldehydes. **Reference**: 1. Harkenthal, M. *et al.* (1999), Pharmazie 54: 460 – 463.

P 028

Screening of medical plants from Mali for antitrypanosomal activity

Aderbauer B[1], Clausen PH[2], Melzig MF[1]

[1]Department of Pharmacy, Freie Universitaet Berlin, Koenigin-Luise-Str. 2 + 4, D-14195 Berlin, Germany; [2]Institute for Parasitology and Tropical Veterinary Medicine, Freie Universitaet Berlin, Koenigsweg 67, D-14163 Berlin, Germany

Dichlormethane extracts of 50 plant parts collected in Mali, traditionally used against trypanosomes were investigated for their *in vitro* and *in vivo* activity against *Trypanosoma brucei brucei*. [1]. Six extracts showed high efficacy *in vitro,* using the Long-term Viability Assay (LtVA) [2] with MIC-values of 50 μg/mL. Four extracts showed low cytotoxicity and good tolerance in mice and were tested *in vivo* using the standard mouse test by Eisler et al. (2001), [3]. The extracts of *Guiera senegalensis* J.F. leaves and of *Securidaca longependuncula*ta Fres. roots were able to reduce parasitaemia in mice treated at a dose of 150 mg/kg b.w. (*i.p.*, two times daily for three days) with a reduction in parasitaemia of 42 and 48.5 % compared to the untreated control group. These extracts are now phytochemicallly analysed for active principles. *Acknowledgements:* The authors thank Dr. Bizimana for providing the plant material and for technical support. **References**: 1. Bizimana, N. *et al.* (2006), J. Ethnopharmacol. 103: 350 – 356. 2. Kaminsky, R. *et al.* (1989), Exp. Parasitol. 69: 281 – 289. 3. Eisler, M.C. *et al.* (2001), Vet. Parasitol. 97: 171 – 182.

P 029

Methanolic extracts of two Betulaceae species from Spain, induced apoptosis in tumoral cell lines

Acero N, Muñoz-Mingarro D, Domínguez MT, Llinares F

Facultad de Farmacia Universidad San Pablo CEU. Urb. Montepríncipe. 28660 Boadilla del Monte MADRID. SPAIN

The citotoxic activity of methanolic extracts of leaves and barks from *Alnus glutinosa* L. and *Betula alba* L. were studied over three tumoral cell lines (PC-3, HeLa and HT-29). The LDH test allows the determination of the necrotic effect of the extracts. Apoptosis was microscopically visualized after staining [1]. The extracts capacity to regulate the Tumoral Necrosis Factor-α (TNF- α) and the Vascular Endothelial Growth Factor (VEGF) synthesis, was also analyzed by Sigma ELISA kits. The plants antioxidant capacity was estimated by the DPPH assay [2]. The method evaluates antioxidation as the ability of the extracts to scavenge DPPH radical. All the extracts show

citotoxic activity against all cells, especially, *B. alba* leaves extracts over PC-3 cells (IC_{50} < 30 μg/mL). The extracts citotoxicity was established via apoptosis. No necrosis was detected in treated cells. Leaves and barks extracts from both plants decreased ($p < 0.05$) the TNF- α synthesis induced by OK and TPA in HL-60 cell line. The VEGF expression was not altered by these plants. *B. alba* leaves and *A. glutinosa* barks have strongest DPPH radical scavenging activity than the ascorbic acid. These activities must be due to the phenolic compounds present in the extracts, as had been demonstrated in other Betulaceae species [3]. *Acknowledgements*: Universidad San Pablo CEU **References***:* 1. Chen, S.Y., Chen, C.H. (2002), J. Ch. Phar. Sci. 11: 48 – 51. 2. Koleva, I.I. (2000), Analytical Chem. 72: 2322 – 2328. 3. Ju, E.M. *et al.* (2004) Life Sci. 8: 1013 – 1026.

P 030

Hyraceum, the fossilised metabolic products of rock hyraces, shows affinity to the GABA-benzodiazepine receptor

Jäger AK[1], Olsen A[1], Prinsloo LC[2], Scott L[3]
[1]Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen O, Denmark; [2]Department of Physics, University of Pretoria 0002, South Africa; [3]Department of Plant Sciences, University of the Free State, Nelson Mandela Avenue, PO Box 339; Bloemfontein 9300, South Africa

Hyraceum, the fossilised urine and dung of rock hyraces (*Procavia capensis*), was traditionally used in South Africa by Hottentots and Afrikaner settlers for the treatment of epilepsy. 14 hyraceum samples were collected at different geographical locations in South Africa and tested for affinity to the GABA-benzodiazepine receptor using flumazenil Ro-15 1788 as ligand [1]. The clinically used benzodiazepines bind to the GABA-benzodiazepine site and exert their anti-epileptic effect via the GABAergic system. Ethanolic extracts of 4 of the hyraceum samples showed affinity to the GABA-benzodiazepine site, displacing over 50 % of the flumazenil at 0.45 mg/mL extract (in total assay volume); whilst aqueous extracts were inactive. One of the active samples was carbon-dated to be about 10.000 year old. A TLC analysis of the ethanolic extracts showed a complex pattern of compounds with no constituents present in all the 4 active samples, but absent in the inactive samples. Infrared spectra did not indicate similarity between the 4 active samples, and also showed that the heterogeneity of the samples can influence the concentration of the active ingredient. **Reference**: 1. Risa, J. *et al.* (2004), J. Ethnopharmacol. 93: 177 – 182.

P 031

Screening of plants used in Danish folk medicine to treat depression for MAO-A inhibition and affinity to the serotonin transporter

Jäger AK, Gauguin B, Gudiksen L, Adsersen A
Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen O, Denmark

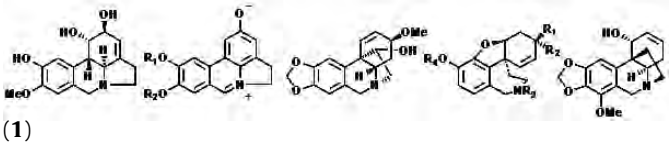
Plant species used in Danish folk medicine to treat depression were selected based on the ethnobotanical standard work Folk og Flora [1]. Water and ethanol extracts of different plant parts from 18 plant species, resulting in 43 extracts, were tested for inhibition of MAO-A and affinity to the serotonin transporter. MAO-inhibitors and selective serotonin reuptake-inhibitors (SSRI) are clinically used to treat depression. The MAO-A assay was performed in microtitre plates as a spectrophotometric peroxidase-linked assay measuring the production of a quinoneimine dye. The serotonin assay was performed as a binding assay using a rat brain homogenate with [^{3}H]citalopram as ligand [2]. 11 extracts had IC_{50} values below 0.025 mg/mL extract (in total assay volume). The most active plant extracts in the MAO-A assay were the water extract of *Hypericum perforatum* L. (IC_{50} 3.6 μg/mL); ethanol extract of *Trigonella foenum-graecum* L. (IC_{50} 3.6 μg/mL); ethanol extract of *Apium graveolens* L. (IC_{50} 4.8 μg/mL) and the water extract of *Calluna vulgaris* (L.) Hull (IC_{50} 8.5 μg/mL) In the serotonin transporter assay the most active extract was an ethanolic extract of aerial parts of B*orago officinalis*. The other extracts did not have affinity to the transporter. **References**: 1. Brøndegaard, V.J. (1978), Folk og Flora. Vol. 1 – 4. Rosenkilde og Bagger, Denmark. 2. Nielsen, N.D. *et al.* (2004), J. Ethnopharmacol. 94: 159 – 163.

P 032

Alkaloids from Phaedranassa dubia (Amaryllidaceae). In vitro antiprotozoal activity

Osorio E[1,2], Brun R[3], Viladomat F[2], Codina C[2], Cabezas F[4], Bastida J[2]
[1]Grupo de Investigación en Sustancias Bioactivas, Facultad de Farmacia, Universidad de Antioquia, A.A. 1226-Medellín, Colombia; [2]Departament de Productes Naturals, Facultat de Farmàcia, Universitat de Barcelona, Avda Diagonal 643, 08028-Barcelona, Spain; [3]Parasite Chemotherapy, Swiss Tropical Institute, Socinstraβe 57, CH-4002 Basel, Switzerland; [4]Departamento de Química, Grupo de Química de Compuestos Bioactivos, Universidad del Cauca, Popayán, Colombia

Continuing our work on Amaryllidaceae alkaloids, the bulbs of *Phaedranassa dubia* (H.B. & K.) were studied and found to contain eight alkaloids: pseudolycorine (**1**), ungeremine (**2**), zefbetaine (**3**), haemanthamine (**4**), sanguinine (**5**), galanthamine (**6**), epinorgalanthamine (**7**) and buphanamine (**8**). Their structures were established using physical and spectroscopic methods. Each alkaloid was tested against the following parasitic protozoa: *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. The results showed a good activity of the betaine ungeremine against *P. falciparum* (IC_{50} = 0.09 μg/mL). Additionally, haemanthamine presented noteworthy activity against *T. brucei rhodesiense* (IC_{50} = 0.49 μg/mL) and *P. falciparum* (IC_{50} = 0.69 μg/mL) and pseudolycorine was active against *P. falciparum* (IC_{50} = 0.24 μg/mL).



(**1**)
(**2**): $R_1 + R_2 = CH_2$
(**4**
(**5**): R_1 = OH, R_2 = R_4 = H, R_3 = Me
(**8**)
(**3**): R_1 = H, R_2 = Me
(**6**): R_1 = OH, R_2 = H, R_3 = R_4 = Me
(**7**): R_1 = R_3 = H, R_2 = OH, R_4 = Me

Acknowledgements: Edison Osorio thanks the Fundación Carolina for his doctoral fellowship.

P 033

Nematicidal compound from the seeds of Balanites aegyptiaca. Isolation and structure elucidation

Gnoula C[1,2], Guissou P[3], Dubois J[1], Frédérich M[4], Duez P[2]
[1]Laboratoire de Chimie Bioanalytique, de Toxicologie et de Chimie Physique Appliquée, Institut de Pharmacie; [2]Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine. Université libre de Bruxelles. Bd du triomphe, CP205/9. 1050 Bruxelles. Belgique; [3]Unité de Formation et Recherche en Sciences de la Santé, Université de Ouagadougou; [4]Laboratoire de Pharmacognosie. Université de Liège. CHU du Sart Tilman – B36, 4000 Liège – Belgique

Testing for anthelmintic activity using the target species in its normal host requires relatively large quantities of chemicals and animals breeding facilities, which can be quite expensive. To circumvent this problem, *Caenorhabditis elegans*, a free-living soil nematode, susceptible to all commercially available anthelmintics, has been proposed for the development of *in vitro* drug screening assays [1], a fluorescence-based microscopy method has been recently de-

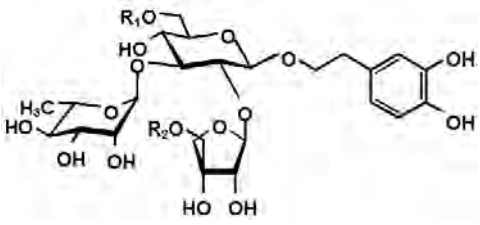
veloped and fully validated from the biological and analytical points of view [2]. *Balanites aegyptiaca* (L.) Delile (Zygophyllaceae) is a tropical plant largely used in Africa; every parts of the plant are medicinal and compose a number of remedies to treat various ailments. The seeds are notably used as anthelmintic against different nematode and cestode species. The crude aqueous extract of *Balanites aegyptiaca* seeds showed *in vitro* anthelmintic activity against *Caenorhabditis elegans*. Bioassay-directed fractionation based on this model led to the isolation of a known cytostatic steroidal saponin, balanitin 7 [3.] as the principal nematicidal agent. The structure elucidation was based on NMR spectroscopic analysis and chemical methods. Preliminary testing for the mechanism of action of balanitin 7 shows that this "new" anthelmintic agent does not inhibit acetyl cholinesterase and so differs from the well-known drugs pyrantel and piperazine. *Acknowledgement:* C. Gnoula is a PhD scholarship recipient from the Université Libre de Bruxelles (ULB). **References**: 1. O'Grady, J. *et al.* (2004), Exp. Parasitol.106: 164 – 72. 2. Gnoula, C. *et al.* (2006), Talanta (submitted). 3. Pettit, G.R. *et al.* (1991), J. Nat. Prod. 54: 1491 – 1502.

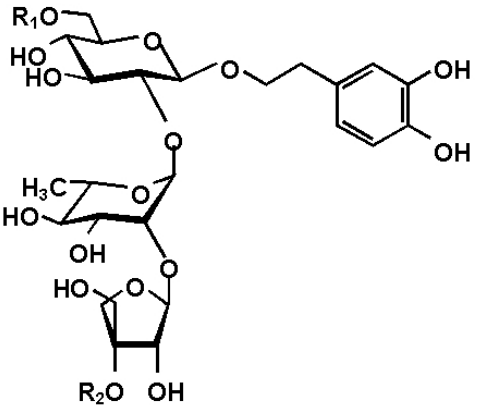
P 034

Newbouldiosides A-C, phenylethanoid triglycosides from Newbouldia laevis

Gormann R, Kaloga M, Kolodziej H
Freie Universität Berlin, Institute of Pharmacy, Pharmaceutical Biology, Berlin, Germany

Newbouldia laevis SEEM (Bignoniaceae) is a shrub or small tree distributed in the tropical rain forest and Savannah zones of Western Africa. The stem bark is traditionally used for the treatment of a variety of ailments including dysentery, rheumatoid arthritis, epilepsy and skin infections. Previous chemical studies have revealed the presence of furanonaphthoquinones, atraric acid and a benzofuran derivative [1]. Continued studies have led to the characterization of three new phenylethanoid glycosides, designated as newbouldioside A-C. In addition, a sodium salt of analogue B representing the first phenolate within this group was encountered. Newbouldioside C represents the first member possessing a linear glc-rha-api chain and a sinapoyl moiety. The structures of the newbouldiosides were elucidated by spectroscopic methods as ß-(3,4-dihydroxyphenyl)ethyl *O*-5-*O*-syringoyl-*ß*-*D*-apiofuranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-*ß*-*D*-gluco-pyranoside, ß-(3,4-dihydroxyphenyl)ethyl *O*-5-*O*-syringoyl-*ß*-*D*-apiofuranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→3)]-6-*O*-*E*-feruloyl-*ß*-*D*-glucopyranoside, and ß-(3,4-dihydroxyphenyl)ethyl *O*-3-*O*-*E*-feruloyl-*ß*-*D*-apiofuranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→2)-6-*O*-*E*-sinapoyl-*ß*-*D*-glucopyranoside, respectively.





Reference: 1. Gormann, R., Kaloga, M. *et al.* (2003), Phytochemistry 64: 583 – 587.

P 035

Novel flavonoids from leaf extracts of Markhamia acuminata and Spathodea campanulata

Gormann R, Kaloga M, Kolodziej H
Freie Universität Berlin, Institute of Pharmacy, Pharmaceutical Biology, Berlin, Germany

The medicinal uses of *Markhamia acuminata* (Klotzsch) Schum. (syn. *M. zanzibarica*;) and *Spathodea campanulata* P. Beauvois (Bignoniaceae) in traditional medical systems of Africa and the limited information regarding their chemical constituents prompted the present investigation. This report deals with the flavonoid patterns of the two species with a view of chemotaxonomical significance for Bignoniaceae. The series of naturally occurring flavonoids is extended by identification of 3',4',5,7-tetrahydroxy-5'-methoxyflavanone and apigenin 5-*O*-α-L-rhamnopyranosyl-7-O-ß-D-glucopyranoside from a methanol extract of *M. acuminata*, while dihydrokaempferol-7-O-(2''-O-formyl)-ß-D-glucopyranoside was obtained from *S. campanulata*. Formic acid as an acylating acid of a carbohydrate moiety in the flavonoid series is a new discovery. They are accompanied in the leaves of *M. acuminata* by a series of known flavones (apigenin, luteolin, luteolin-7-rutinosid) and flavanones (naringenin, naringenin-7-rutinosid, eriocitrin), while flavones (apigenin, luteolin, diosmetin) were associated with common flavonols (quercetin-glycosides) in *S. campanulata*. However, it should be noted that this is the second report [1] on the presence of flavanones within the Bignoniaceae, apparently confined to the tribe Tecomeae. The occurrence of flavones and flavonols meet the known flavonoid patterns encountered in members of the Bignoniaceae. The structures of these compounds were established from spectroscopic studies. **Reference**: 1. Mansoor, A., Neeru, J. *et al.* (1991), J. Chem. Res., Synopses 5: 109.

P 036

Binding of natural products and their derivatives to bovine β-lactoglobulin

Riihimäki L[1], Vuorela P[2,1]
[1]Drug Discovery and Development Technology Center, DDTC, and Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, P. O. Box 56 (Viikinkaari 5E), 00014 University of Helsinki, Finland; [2]Department of Biochemistry and Pharmacy, Åbo Akademi University, Biocity, Tykistökatu 6 A, 20520 Turku, Finland

β-lactoglobulin (βLG) is the main whey protein in bovine milk. In this study, the binding of a group of natural compounds and their derivatives (44 in total) to βLG was studied. βLG may act as a binder molecule for natural products. βLG binding studies were made using

our earlier miniaturised microplate screening assay based on fluorescence quenching [1], where the apparent dissociation constant (K_d) and the number of independent ligand binding sites (n) were determined. Of the compounds investigated the major of flavones, flavonols, flavanones and isoflavones were bound to βLG with high affinity, K_d between 0.203 – 0.633 μM. In addition some compound from the group of catechins and derivatives, coumarins and phenolic acids and derivatives were slightly bound to βLG, K_d > 0.7 μM. These studies showed that βLG could act as a binder for phenolic natural products and in this way enhance their health benefit characters when fortified in health promoting products. **Reference**: 1. Riihimäki, L. *et al.* (2006), J. Biochem. Biophys. Methods. Accepted.

P 037

Miniaturisation and automatisation of Caco-2 permeability studies for screening of natural and synthetic ligands

Galkin A[1], Pakkanen J[2], Vuorela P[3]
[1]Drug Discovery and Development Technology Center (DDTC) and Division of Pharmaceutical biology, Faculty of Pharmacy, P.O. Box 56, 00014, University of Helsinki, Finland; [2]Division of Pharmacology, Faculty of Pharmacy, P.O. Box 56, 00014, University of Helsinki, Finland; [3]Department of Biochemistry and Pharmacy, Åbo Akademi University, BioCity, Tykistökatu 6 A, 20520, Turku, Finland

Caco-2 cell monolayers have been widely accepted by pharmaceutical companies and by regulatory authorities as a standard *in vitro*–model system to predict permeability of compounds in human. To obtain these monolayers Caco-2 cells are traditionally grown on 12 and 24 wells for 21 to 28 days which is time consuming, laborious and expensive. To adapt the model to the needs of modern high-throughput screening, we replaced the 12- and 24-well plates with 96-well plates and reduced the growing time to 7 days. A set of standard compounds with different permeabilities, various permeability markers and confocal microscopy were used to assess the utility of our new method on Biomek FX automation. The permeability results obtained from standard compounds used were comparable to those obtained from traditionally performed experiments. These results indicate that we managed to build up a fast miniaturized and automated protocol to make a first evaluation of permeation of new compounds in the drug discovery process. *Acknowledgements*: The European Commission 6th framework program Pro-Kinase Research project no. 503467

P 038

Biological activity of Gunnera tinctoria, an invasive plant in the island of S. Miguel (Azores)

Barreto MC[1,2], Baptista J[1,2], Teixeira DM[2], Monteiro M[1,2]
[1]CIRN, Azores University, 9500 Ponta Delgada, Portugal
[2]DCTD, University of Azores, 9501 – 801 Ponta Delgada, Portugal

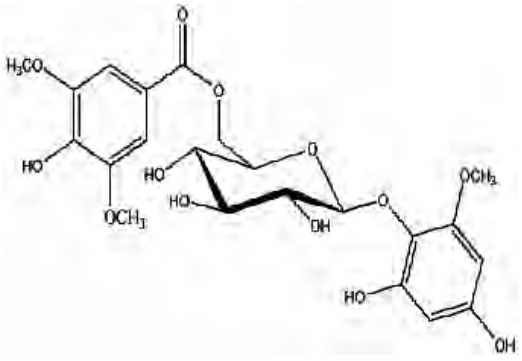
Gunnera tinctoria (Mol.) Mirb. is a Halogaracea originary from South America, which was introduced in the island of S. Miguel (Azores). An ornamental plant, it escaped from the Furnas botanical garden in the 1960 s and has been slowly invading the central area of the island [1]. This plant has been the object of preliminary work in our laboratory [2], and there are reports about biological activities detected in another species belonging to the same genus [3]. Dichloromethane and methanol leaf extracts of *G. tinctoria* were prepared either by soxhlet extraction (hot – $CHCl_2$ and hot – MeOH, respectively), or at room temperature (cold – $CHCl_2$ and cold – MeOH). The extracts were screened both for antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* and *Micrococcus luteus*, and for antitumour activity against HeLa cell line. The only antibacterial activity detected was from the hot – $CHCl_2$ extract against *Micrococcus luteus* (EC_{50} = 169.15 μg/mL). All extracts were active against HeLa tumour cell line, both in antiproliferative and in cytotoxycity assays. The extract which exhibited higher activity was hot – MeOH, with EC_{50} values of 25.3 and 49.5 μg/mL for antiproliferative and cytotoxicity assays, respectively. Soxhlet extraction yielded better results than cold extraction, suggesting that the active compound(s) involved are not particularly heat labile. The results obtained are promising and further work will be carried out to identify the molecules responsible for the effects detected. **References**: 1. Sjögren, E. (1984), Azores Flowers. Direcção Regional do Turismo, Horta. 2. Medeiros, J., Macedo, F.W. *et al.* (1999), Açoreana 9: 55 – 61. 3. Drewes, S.E., Khan, F., *et al.* (2005), Phytochemistry 66: 1812 – 1816.

P 039

Proanthocyanidins and phenolglycosides from Rumex acetosa L

Anke J[1], Petereit F[1], Hensel A[1]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

Rumex acetosa L. (Polygonaceae), also known as "sauerampfer" or "sorrel", is a perennial herb, which grows abundantly in most parts of Europe and North America. It is a traditional ingredient in salad or soup due to its vitamin C content. Additionally, preparations of *Rumex* species were used for constipation or for treatment of chronic skin diseases [1]. This claimed therapeutic use and the close relationship to rhubarb (*Rheum officinale*) indicate a high content of tannins, especially proanthocyanidins. In the literature, the amount of tannins found in different related *Rumex* species varies from about 2 to 15 % [2, 3]. However, only few investigations concerning tannin composition in *Rumex* have been carried out so far. In order to isolate and elucidate new proanthocyanidins from *Rumex acetosa* we purified an acetone/water extract via elution on Sephadex LH20, MLCCC, MCI-Gel and RP-18 material. Different proanthocyanidins, a polymer fraction and the new phenolglycoside 1-O-β-D – (2,4-Dihydroxy-6-methoxyphenyl)-6-O-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside were obtained. The isolated proanthocyanidin fractions contain mono-, di-, tri-, and tetramers consisting of catechin, epicatechin and epiafzelechin as flavan-3-ol components. A- and B-type interflavan-linkage was found as well as substitution with gallic acid. The polymer fraction was characterized by NMR and MS techniques.



References: 1. Williamson, E.M. (2003), Potter's Herbal Cyclopaedia. C.W. Daniel Company Limited. Essex. 2. McGuffin M. (1997) American Herbal Products Assosiation's Botanical Safety Handbook. CRC Press. Boca Raton. 3. Demirezer, L.Ö. *et al.* (1997), FABAD 22: 153 – 158.

P 040

New oxidative derivatives of atractyligenin and their cytotoxic activity

Bruno M[1], Rosselli S[1], Maggio A[1], Bellone G[1], Lee KH[2]
[1]Dipartimento di Chimica Organica, Università di Palermo, Viale delle Scienze, Parco d'Orleans II, 90128 Palermo; [2]Natural Products Laboratory, University of North Carolina at Chapel Hill, USA

ent-Kauranes are naturally occurring diterpenoids isolated from several families, such as Asteraceae and Lamiaceae. These compounds have attracted interest because of their structures and their biological activities as anti-tumorals, anti-HIV and anti-bacterials [1]. Extensive chemical work [2] was carried out on the structure of atractyligenin, the nor-diterpene aglycone of the glucoside atractyloside, occurring, together with its diterpene homologous carboxyatractyloside, in the root of *Atractylis gummifera* L. (Compositae). The interest for these compounds was stimulated by the high toxicity [3] of both glucosides, responsible of many deadly poisoning in past time. Due to the 15-hydroxyl group of atractyligenin, it was possible to design a series of chemical reactions in order to build an α,β-unsaturated ketone in the kaurane skeleton. In fact, it is well know that the main determining factor responsible for cytotoxicity is the presence of an α,β-unsaturated system that likely serves as an alkylating center and can be part of an ester, ketone, or lactone moiety [4]. The same oxidative reaction carried out on atractylitriol gave unexpected products in which the allylic alcohol moiety was preserved. The cytotoxic tests of the compounds having an insaturated moiety (15-oxo-atractyligenin methyl ester and 2,15-dioxo-atractyligenin methyl ester) and of several ester derivatives of 15-oxo-atractyligenin methyl ester were performed against KB and KB-VIN tumor cell lines. They showed a good activity between 2.9 – 1.1 μM comparable to mitomycin C (0.6 μM). **References**: 1. Hanson, J.R. (2005), Nat. Prod. Rep. 22: 594 – 602. 2. Piozzi, F. *et al.* (1965), Tetrahedron Lett. 1829 – 1836. 3. Santi, R. *et al.* (1978), Atractyloside: Chemistry, Biochemistry and Toxicology, Medical Books, Piccin, Padova, Italy. 4. Lee, K.H. *et al.* (1977), Science 196: 533 – 536.

P 041

Screening of Acetylcholinesterase Inhibitors from Fungal Extracts

Oinonen P[1,2,3], Mettälä A[3], Vuorela P[1,4], Hatakka A[3]
[1]Drug Discovery and Development Technology Center, Faculty of Pharmacy, University of Helsinki, Viikki Biocenter, P.O. Box 56, FIN-00014 University of Helsinki, Finland; [2]Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, Viikki Biocenter, P.O. Box 56, FIN-00014 University of Helsinki, Finland; [3]Department of Applied Chemistry and Microbiology, Faculty of Agriculture and Forestry, University of Helsinki, Viikki Biocenter, P.O. Box 56, FIN-00014 University of Helsinki, Finland; [4]Department of Biochemistry and Pharmacy, Åbo Akademi University, BioCity, Tykistönkatu 6 A, FIN-20520 Turku, Finland

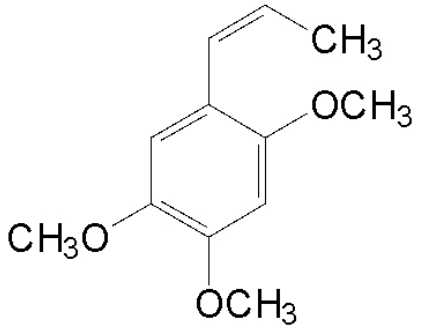
The purpose of this study was to screen fungal extracts for their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Most of the fungi studied were basidiomycetous wood-rotting fungi belonging to the genera *Trametes, Phellinus, Pycnoporus, Ganoderma* and *Piptoporus*. Altogether 125 strains of fungi were grown in liquid media for 8 weeks. The growth media were extracted with ethylacetate and the extracts were screened for the inhibitory effect. In addition some of the media were also extracted with water. We used TLC bioautograpical assay described by Marston et al. (2002) for screening. Physostigmine was used as positive control for both enzymes. We detected 146 bands inhibiting AChE selectively, 72 bands inhibiting BChE selectively and 56 bands inhibiting both enzymes. Selective AChE inhibitory effect was detected in 92 extracts whereas selective BChE inhibory effect was detected in 41 extracts. Non-selective effect was detected in 44 extracts. **Reference**: 1. Marston, A. *et al.* (2002), Phytochem. Anal. 13: 51 – 54.

P 042

Acetylcholinesterase inhibition of oil from Acorus calamus rhizome

Houghton PJ[1], Mukherjee PK[2], Kumar V[2], Mal M[2]
[1]Pharmacognosy Research, Pharmaceutical Sciences Research Division, KCL, 150 Stamford St, SE1 9NH London, UK; [2]School of Natural Product Studies,Department of Pharmaceutical Technology,Jadavpur University; Kolkata 700032, India

Acorus calamus L. has been used in traditional Indian prescriptions and its beneficial effects on memory disorder, learning performance and other aspects of ageing effect have been reported [1]. The hydro-alcoholic extract and essential oil of Indian *Acorus calamus* rhizomes were tested for *in vitro* AChE inhibitory activity based on Ellman's method in 96-well micro plates [2] using acetylcholinesterase (AChE) from bovine erythrocytes. The hydro-alcoholic extract gave an inhibition of AChE (IC_{50} value 182.31 ± 16.78 μg/mL) but the oil was stronger (IC_{50} value 106.75 ± 8.08 μg/mL). GC analysis of the oil was performed on a Varian 3400 programmable capillary GC, using D.B.5 'Wax' capillary column [30m x 0.32 mm i.d., film thickness 0.25 μ]. Oven temperature was programmed at 140 – 180^{0}C, at 3^{0}C/min and held isothermal at 180^{0}C for 7.67 minutes. Injector temperature was 210^{0}C; Detector temperature was 250^{0}C [FID]; carrier gas Helium. 2 μL samples were injected with the Frit-splitter at ratio 12:1. GC analysis of the oil revealed that β-asarone **1** was the major constituent (52.33 % w/w with respect to dried rhizomes) while α-asarone content was 1.026 % w/w. **1** and α-asarone (the *trans* isomer of **1**) were tested for AChE inhibition and found to have IC_{50} values of 3.33 ± 0.02 μM and 46.38 ± 2.69 μM respectively. Physostigmine was used as standard and showed inhibition of AChE with an IC_{50} value of 0.28 ± 0.015 μM.



1

The AChE-inhibitory activity of the oil can be ascribed to β-asarone. Since cognitive performance and memory are related to acetylcholine levels, the AChE inhibitory effect of the plant may account for its traditional use. *Acknowledgements*: Commonwealth Fellowship for financial support. for PKM. **References**: 1. Howes, M.R., Houghton, P.J. (2003), Pharmacol. Biochem. Behavior 75: 513 – 527. 2. Perry, N.S.L. *et al.* (2000), J. Pharm. Pharmacol. 52: 895 – 902.

P 043

Antimicrobial polysaccharide of durian-rinds and natural essential oils combination in an effective non-alcoholic antiseptic lotion for hands

Pongsamart S[1], Lipipun V[2], Jesadanont S[1], Pongwiwatana U[1]
[1]Department of Biochemistry, [2]Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

This study aimed to evaluate antimicrobial activity of natural essential oils, tea-tree oil (TTO) from *Malaleuca alterifolia* Cheel, and betel vine oil (BO) from *Piper betle* L.; and to prepare non-alcoholic antiseptic hand-lotion by using antimicrobial polysaccharide gel (PG) isolated from fruit-rind of durian, *Durio zibethinus* Murr., [1, 2] in

combination with essential oils. PG, TTO or BO each showed good inhibitory effect against *Staphylococcus aureus*, *S. epidermidis*, *icrococcus luteus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris* and *Propionibacterium acnes* by agar diffusion and broth macrodilution methods [3]. Minimum bactericidal concentrations (MBCs) of each single component, *i.e*. PG, TTO and BO against the tested bacteria were 2.5 %, 0.156 – 0.625 % and 0.020 – 0.156 % w/v, respectively. TTO or BO each by itself also killed other bacteria and fungi including *Streptococcus typhimurium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*. PG at 2.5 % w/w was used in combination with 1 % w/w TTO and 0.5 % w/w BO to prepare hand-lotion. Antimicrobial activity of the hand-lotion prepared was evaluated where clear inhibition zone against all tested microorganisms by using agar diffusion test was observed. For *in vitro* Time-kill analysis, the hand-lotion prepared killed all the tested bacteria, *S. aureus* and *E. coli*, within 15 min. Upon hand-washing test [4], the treated hands exposed to the antiseptic hand-lotion prepared showed significant reduction of hand normal flora compared to a positive control exposed to a commercially available alcoholic antiseptic hand gel. Thus, PG in combination with TTO together with BO is of good potential to be used for preparing a non-alcoholic antiseptic lotion used topically on hands or skin. *Acknowledgements*: This work was supported by Annual Research Budget of Faculty of Pharmaceutical Sci., Chulalongkorn University. **References**: 1. Pongsamart, S. *et al*. (2005), Acta Hortic. 678: 65 – 73. 2. Hokputsa, S. *et al*. (2004), Carbohydrate Polymers 56: 471 – 481. 3. Lorian, V. (1996), Antibiotics in laboratory medicine. 4th ed. Williams&Wilkins. Baltimore. 4. Messager, S. *et al*. (2004), J. Hosp. Infect. 59: 220 – 228.

P 044

Oral hypoglycemic activity of water extract from Ya-Tevada, Malvastrum Coromandelianum Garcke, equivalent to insulin injection

Jesadanont S, Sitthiwej C, Poungshompoo S, Pongsamart S
Chulalongkorn University, Bangkok 10330, THAILAND

Water extract from whole plant of Ya-Tevada, *Malvastrum coromandelianum* Garcke; Malvaceae, showed strong hypoglycemic activity when given orally. Feeding the spray-dried crude water extract as low as 50 and 100 mg/kg body weight (bw) to streptozotocin-induced male Wistar Rats, reduced significantly non-fasting blood glucose from approx. 350 – 450 mg/dL within one hour after administration to a level of blood glucose in normal non-diabetic rats which is about 150 mg/dL, equivalent to *i.p.* injection of Insulin (Humulin R®) 5U/kg bw. The hypoglycemic effect lasted for at least 5 hours. This strong hypoglycemic activity of this plant extract would fulfill the search for an oral hypoglycemic agent equivalent to insulin injection. Feeding the extract at repeated doses of 50, 100 and 500 mg/kg bw/day for 30 days suppressed only fasting blood glucose to a level equivalent to a level shown in the group of diabetic rats injected with insulin 5 U/kg bw/day and the group of normal rats fed water. Previous report showed no chronic toxic effect upon feeding this water extract at daily doses of 0.2, 2.0 and 20 g/kg bw for 6 months in male and female Wistar rats [1]. This largest dose is 400 times the effective hypoglycemic dose of 50 mg. This agent also showed antibacterial activity against *Staphylococcus aureus* both methicillin-sensitive and methicillin-resistant and good wound healing effect. Thus, water extract of this plant showed great potential as oral hypoglycemic agent to be used in diabetic condition. *Acknowledgements:* Fac Pharm Sci, and Fac Vet Sci, Chulalongkorn U., Bangkok 10900, Thailand. **Reference**: 1. Attawish, A, *et al*. (1998), Bull. Dept. Med. Sci. 40: 261 – 271.

P 045

Screening of Antibacterial, Antifungal and Antiviral Properties of the Selected Turkish Helichrysum Species

Aslana M, Özçelik B, Orhana I, Karaoglu T, Sezika E
[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey; [2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey; [3]Department of Virology, Faculty of Veterinary, University of Ankara, 06100 Ankara, Turkey

Various *Helichrysum* species have been widely used as folk remedy in Turkish folk medicine for diuretic, and anti-asthmatic properties as well as against kidney stones, stomache as decoction. Besides, the powder of capitulums have been also used like pomade, prepared by mixing with barley flour, for wound healing (1 – 3). In the present study, Fourteen extracts prepared with hot water and ethanol (80 %) from the capitulums obtained from seven *Helichrysum* species including *H. armenium* ssp. *araxinum* (Takht. ex Kirp.) Tahkt, *H. armenium* ssp. *armenium* DC, *H. arenarium* (L.) DC, *H. pallasii* (Spreng.) Ledeb., *H. stoechas* (L.) Moench, *H. sanguineum*, and *H. graveolens* (Bieb.) Sweet (Compositae) growing in Turkey were screened for their antibacterial, antifungal and antiviral activity against both standard and the isolated strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacer baumannii, Staphylococcus aureus, Enterococcus faecalis*, *Candida albicans* and *C. parapsilosis* by microdilution method. Both *Herpes simplex* (HSV) (DNA) and *Para-influenza*-3 (PI-3) viruses (RNA) were used for the determination of antiviral activity of the water and ethanol extracts of *Helichrysum* species by using MDBK and Vero cell line. Ampicilline, levofloxacin, ofloxocin, ketoconazole, fluconazole, acyclovir and oseltamivir were used as the control agents. The same degree of inhibitory effect has been observed on Gram (-) bacteria. All the extracts have shown more potent effect against Gram (+) bacteria than Gram (-) ones. Moreover, all the extracts screened have exerted a better inhibitory effect towards ATCC strains than the isolates. In particular, all of the ethanolic extracts were found to have a very significant inhibition against ATCC strain of *S.aureus* with MIC value of 8 µg/mL. Ethanolic extracts of *H. arenarium* and *H. armenium* ssp. *armenium* showed antiviral action against both HSV and PI-3, whereas the rest was completely inactive with the related activity. **References**: 1.Sezik, E., Tabata, M. *et al.* (1991), J. Ethnopharmacol. 35: 191 – 196. 2. Fujita, T., Sezik, E., *et al.* (1995), Econ. Bot. 49: 406 – 422. 3. Sezik, E., Yesilada, E. *et al.* (2001), J. Ethnopharmacol. 75: 95 – 115.

P 046

Antimutagenic effects of ethanolic extracts from three Palestinian medicinal plants

Khader M, Eckl PM, Bresgen N
Department of Cell Biology, University of Salzburg, Hellbrunnerstrasse 34, Salzburg, A-5020, Austria

Eryngium creticum L., *Nigella sativa* L., and *Teucrium polium* L. have been traditionally used for the treatment of inflammations, liver disorders, and arthritis. Several studies on these plants revealed antioxidant, anti-inflammatory, hepatoprotective, antimutagenic and antiulcerogenic activities. In this study the antimutagenic activity of these plant species was tested in rat hepatocyte primary cultures by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), a directly acting mutagen, which was shown to induce massive chromosomal damage in hepatocytes [1]. Since it cannot be excluded that the active constituents of the plant extracts require biotransformation or induce metabolic enzymes, causing antimutagenic or detoxifying effects, the present investigation was carried out with metabolically competent primary cultures of rat hepatocytes. Rat hepatocytes were isolated as described by Michalopoulos *et al.* [2]. Establishment of primary cultures and cytogenetic studies were performed according to Eckl *et al.* [1]. Plant extracts were prepared by Soxhlet continuous extraction method, 6 gm of ground plant materials were extracted in 50 mL of absolute ethanol at 85 °C

for 20 hours, ethanol was air dried and the remaining oily extracts were dissolved in 5 mL of dimethyl sulfoxide (DMSO). Antimutagenicity testing was done in three modes: pre-treatment, combined treatment and post-treatment of the primary cultures with plant extracts and MNNG. Therefore, both the induction of metabolizing enzymes, direct interaction of plant constituents with the mutagen and increased recovery, i. e. enhanced repair of induced DNA damage can be evaluated. Student's double sided t-test for independent samples was used to evaluate the levels of significance. The results of our investigation clearly indicate an inhibitory effect on MNNG mutagenicity by the three plant extracts, and this effect can be attributed to a direct antimutagenic activity and an increased recovery. *Acknowledgments*: This investigation was supported by a stipend of the Austrian Exchange Service (OEAD). **References**: 1. Eckl, P.M. *et al.* (1987), Carcenogenisis 8:1077 – 1083. 2. Michalopoulos, G. *et al.* (1982), Cancer Res. 42:4673 – 4682.

P 047

Screening for PDE inhibitory activity from Thai traditional plants used as rejuvenating and aphrodisiac agents

Temkitthawon P[1], Viyoch J[2], Ingkaninan K[1]
[1]Department of Pharmaceutical Chemistry and Pharmacognosy, Naresuan University, Thapol, Muang, 65000, Phitsanulok, Thailand; [2]Department of Pharmaceutical Technology, Naresuan University, Thapol, Muang, 65000, Phitsanulok, Thailand

Phosphodiesterase (PDEs) [1, 2], are a group of enzymes that have powerful effect on cellular signal because they regulate the second messengers, cAMP or cGMP. PDEs inhibitors have been used for treatment in many indications such as cardiovascular disease, chronic obstructive pulmonary disease and erectile dysfunction. In our studies, more than twenty of Thai traditional plants used as rejuvenating and aphrodisiac agents were collected. The plant ethanolic extracts were tested for PDE inhibitory activity using malachite green assay [3] in 96-welled microplates. The amount of phosphate which was liberated from the enzymatic reaction was from a complex with malachite green reagent and showed the absorbtion at 640 nm. The results showed that ethanolic extracts of *Ficus pubigera* Wall. (Ma-kra-thuep-rong) and *Piper spp.* (Sakhan-dang) at concentration 1 mg/mL inhibited more than 80% and 70% of PDE activity, respectively. **References**: 1. Essayan, D.M. (1999), Biochem. Pharmacol. 57: 965 – 973. 2. Corbin, J.D.*et al.* (2002), Urology 60(suppl. 2B): 4 – 11. 3. Roengsamran, S. *et al.* (2000), J. Sci. Res. Chula. Univ. 25: 169 – 176.

P 048

Identification of $GABA_A$-modulators obtained from Valeriana officinalis L

Trauner G[1], Khom S[2], Benedek B[1], Hering S[2], Kopp B[1]
[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; [2]Department of Pharmacology and Toxicology, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria

Valeriana officinalis L. is used in phytotherapy due to its sedative and sleep enhancing effects. Its application is principally focused on disorders of initiating sleep and problems in sleeping through, states of anxiety as well as depressive moods. Nevertheless, little is known about the mode of action and substances determining the efficacy. One of the jointly responsible mechanisms of action for sedative substances is stimulation of the $GABA_A$-receptor. The aim of our study was to identify substances from *Valeriana officinalis* L. which stimulate the $GABA_A$-receptor. Isolated frog-oocytes from the genus *Xenopus laevis* were employed. Centring on heterologously expressed $GABA_A$-channels, the Two-Electrode Voltage-Clamp (TEVC) mode was used for the measurements [1]. In a first screening of commercial extracts we observed coherence between different polarity and stimulation of the receptor. Apolar extracts revealed high activity, whereas polar extracts showed no effect. All extracts were characterised by HPLC analysis of the sesquiterpene acids according to Pharmacopoeia Europea. Fractionating a highly potent apolar extract confirmed our first results: Apolar fractions containing high amount of sesquiterpene acids showed strong stimulation. In order to verify correlation between content of sesquiterpene acids and receptor stimulation, valerenic acid and acetoxyvalerenic acid were tested on the $GABA_A$-receptor. Valerenic acid showed strong stimulation, whereas acetoxyvalerenic acid inhibited the receptor. In conclusion, one possibility for the mode of action of valerian roots is the stimulation of the $GABA_A$-receptor, and one of the major compounds, valerenic acid, is not only a marker for standardisation but also a potent activator of the $GABA_A$-receptor. **Reference**: 1. Hering, S. (1998), Pflugers Arch. 436: 303 – 307.

P 049

Effects of piceatannol derivatives in the antiproliferative activity of the anticancer-drug doxorubicine and on apoptosis induction in MDR cancer cell lines

Ferreira MJU[1], Duarte N[1], Gyémant N[2], Varga A[3], Molnár J[2]
[1]CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600 – 083 Lisbon, Portugal; [2]Department of Medical Microbiology, Univercity of Szeged, Szeged, Hungary; [3]Department of Molecular Parasitology, Humboldt University, Berlin, Germany

Historically, plants have provided a source of novel drug compounds and have shown great promise in the treatment of diseases, particularly cancers. A great number of phytochemicals have been demonstrated to have antitumor activity in various experimental systems. Their mechanism of action may affect many different targets of the signal transduction pathway that modulate gene expression, cell cycle progression, proliferation, cell mortality, metabolism and apoptosis [1]. In this study, piceatannol, was isolated from the methanolic extract of *Euphorbia lagascae* L. defatted seeds. This compound was methylated with diazomethane to afford three derivatives that were identified by their physical and spectroscopic data. Piceatannol and the three methylated derivatives were evaluated as multidrug resistance modulators, by using the rhodamine 123 exclusion test, and apoptosis inducers on multidrug resistant mouse lymphoma cells. Furthermore, the antiproliferative effects of the anticancer drug doxorubicine in combination with one of these resistance modifiers were studied on human MDR1 gene transfected mouse lymphoma and doxorubicine resistant human breast cancer cell lines. Verapamil and 12H-benzo(α)-phenothiazine were used as positive controls for the MDR and apoptosis assays, respectively. Piceatannol and its methylated derivatives can be considered as apoptosis inducers. On the other hand, one of the methylated compounds was found to be a powerful inhibitor of p-glycoprotein activity, and has shown in combination with doxorubicine, an additive effect on human MDR1 gene transfected mouse lymphoma cells. *Acknowledgements*: The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant. **Reference**: 1. HemaIswarya, S. *et al.* (2006), Phytotherapy Res. 20: 239 – 249.

P 050

Usnic acid: anti-proliferative, apoptotic and morphological effects on human malignant cell lines

Hardardottir G[1,2], Ogmundsdottir HM[1,2], Ingólfsdóttir K[3]
[1]Molecular and Cell Biology Research Laboratory, Icelandic Cancer Society, Skogarhlid 8, 105 Reykjavik, Iceland; [2]Faculty of Medicine, University of Iceland, Vatnsmyrarvegur 16, 101 Reykjavik, Iceland; [3]Faculty of Pharmacy, Hagi, Hofsvallagata, 107 Reykjavik, Iceland

The aim was to investigate the effects of two enantiomers of usnic acid on proliferation and survival of human malignant cell lines. R- and S- usnic acid were isolated in pure form from the lichens *Cladonia arbuscula* (Wallr.) Flot. and *Alectoria ochroleuca* (Hoffm.) Massal., respectively, and solubilized in DMSO. T47-D (breast cancer)

and Capan-2 (pancreatic cancer) were from ATCC and the myeloma cell lines RPMI-8226*, U266 – 84* and LP-1*. Anti-proliferative effects were tested by thymidine-uptake, results expressed as IC_{50}. To test for apoptosis cells were exposed to four times this concentration for 24 hours; a commercial TUNEL assay was used. The morphology of MG-G-stained cells was investigated after 2, 6 and 24 hour-exposure to usnic acid at the four times IC_{50} concentration. Usnic acid had anti-proliferative effects against T47-D (IC50 = 4.2 μg/mL) and Capan-2 (IC_{50} = 5.3 μg/mL). No difference was found between the enantiomers and only (+) usnic acid was used for further testing. None of the myeloma cell lines was significantly affected. Usnic acid did not induce apoptosis in any of the cell lines. T47-D cells, but not Capan-2 cells, showed morphological changes indicative of necrosis in a small proportion of cells after 6 hours exposure to usnic acid. In conclusion, both enantiomers of usnic acid have significant anti-proliferative effects against two human carcinoma cell lines but not against myeloma cell lines. Usnic acid did not induce apoptosis, which is in line with its non-genotoxic mode of action (1), but mild signs of necrosis were seen. *A kind gift from Kenneth Nilsson.

P 051

Diterpenic compounds as antineoplasic agents in classical and atypical multidrug resistant cancer cells

Duarte N[1], Lage H[2], Ferreira MJU[1]
[1]CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600 – 083 Lisbon, Portugal; [2]Charité Campus Mitte, Institute of Pathology,Schumannstr. 20/21, D-10117 Berlin, Germany

The successful chemotherapy of neoplasic diseases requires that the antitumor combination of drugs employed displays a sustained activity against malignant cells. Simultaneous resistance of cancer cells to multiple classes of structurally and mechanistically unrelated antitumor drugs can be defined as multidrug resistance (MDR), and it is one of the main causes of chemotherapy failure [1]. In addition with the classical multidrug phenotype mediated by increased activity of the ATP-binding cassette transporters P-gp / MDR1, that were responsible for the efflux of drugs out of the cells, there are other multidrug resistant tumors with resistance caused by different mechanisms. This is called atypical resistance and could be due to the decreased DNA topoisomerase (Topo II)-like activity. DNA topoisomerases are nuclear enzymes that are essential for DNA replication, transcription, chromosomal aggregation and DNA recombination. For these reasons, Topo II is one of the possible targets for the commonly used anticancer drugs [2]. The aim of this work is to study the antiproliferative effect of diterpenic compounds isolated from *Euphorbia* species in various human drug sensitive cancer cell lines (gastric, pancreatic and colon carcinomas) and in classical and atypical multidrug resistant sublines of these cell models. Etoposide was used as positive control. It could be demonstrated, that most of the drug resistant cell variants decreased the expression levels of both Topo II isoforms on mRNA level as well as on protein level. None of the tested compounds were as effective as etoposide, but some of them are much more effective in drug resistant cells that in drug sensitive cell lines. In conclusion, some of these compounds may be considered as potential new drugs for the treatment of drug-resistant human cancer cells. *Acknowledgements*: The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant. **References**: 1. Avendaòo, C. *et al.* (2002), Curr. Med. Chem. 9: 159 – 193. 2. Lage, H. *et al.* (2000), Lancet Oncol. 1: 169 – 175.

P 052

Search for P-glycoprotein modulators and apoptosis inducers on cancer cells among ergostane and stigmastane steroids

Duarte N[1], Ramalhete C[1], Gyémant N[2], Varga A[3], Molnár J[2], Ferreira MJU[1]
[1]CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600 – 083 Lisbon, Portugal; [2]Department of Medical Microbiology, University of Szeged, Szeged, Hungary; [3]Department of Molecular Parasitology, Humboldt University, Berlin, Germany

One of the most effective strategies in biological systems to demonstrate resistance to cytotoxic drugs is the efflux of these compounds out of the cell, via membrane transporter proteins. This phenomenon is called multidrug resistance (MDR) and is a mediator of drug resistance observed in tumor cells and in microorganisms (*in vivo* and *in vitro*) [1]. There are numerous resistance mechanisms and the MDR phenotype alone, can not completely explain this occurrence. In fact, several other related proteins are also overexpressed in resistant tumors. Furthermore apoptosis also play a vital role in resistance [2]. Because apoptosis is a major modality by which different tumor cell types can be eliminated, the identification of new drugs able to induce the programmed cell death is an important goal in cancer therapy, and may provide new useful tools for the treatment of patients with drug resistance malignancies. In this context, plants could be potential sources for the isolation of new metabolites that could be used as lead molecules on the treatment of neoplasic diseases. In our search for biologically active compounds from *Euphorbia* species, several ergostane and stigmastane steroids were isolated from the methanolic extract of *Euphorbia lagascae*, and its structures were deduced by the combination of physical and spectroscopic data (IR, MS, 1D and 2D NMR). The ability of some of the isolated steroids to modulate MDR reversion was investigated using mdr-1 (L5178) mouse lymphoma cell line. In addition, the apoptosis induction on the same cell line was also studied. 12H-benzophenothiazine was used as a positive control for apoptosis induction and verapamil for resistance reversal. The tested compounds were weak inducers of apoptosis in the studied mouse lymphoma cell line, but two of them were found effective on MDR reversion in a concentration dependent manner. *Acknowledgements*: The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant. **References**: 1. Borges-Walmsley, M. *et al.* (2003), Biochem. J. 376: 313 – 338. 2. Volm, M. (1998), Anticancer Res. 18: 2905 – 2917.

P 053

Antibacterial compounds from Vaccinium myrtillus (bilberry)

Bessadóttir M[1], Jónsdóttir Í[1], Omarsdottir S[1], Erlendsdóttir H[2], Ingólfsdóttir K[1]
[1]Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland; [2]Department of Bacteriology, Landspitali-University Hospital, IS-101 Reykjavik, Iceland

Bilberries have been used in herbal medicine against various diseases [1]. The aim of the study was to determine the antibacterial activity of three different (petroleum ether (PB), acetone (A) and methanol (M)) extracts of Icelandic bilberries, using broth microdilution assay. Bioguided fractionation was used to isolate active compounds. The PB extract exhibited potent antibacterial activity against antibiotic sensitive and resistant Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis* and *E. faecium*) where minimum inhibitory concentration (MIC) was shown to be from 8 to125 μg/mL. The extracts showed no significant activity against Gram-negative bacteria and *C. albicans*. A fraction composed of three pentacyclic triterpenoids was isolated from the PB extract using LC and HPLC. These triterpenoids were tested against seven resistant Gram-positive bacteria. The MIC value for the triterpenoids was determined to be: 8 μg/mL against coagulase-negative staphylococci and ampicillin resistant *E. faecium*; 16 μg/mL against ampi-

cillin-and vancomycin resistant *E. faecium* and 31 μg/mL against methicillin-resistant *S. aureus*, gentamicin resistant *E. faecalis* and vancomycin resistant *E.faecalis*, respectively. Aqueous extract of bilberries has previously been shown to exhibit some antibacterial activity (2). However, the MIC value was ranging from 15 – 31 mg/mL [2]. *Acknowledgements*: Icelandic Council of Science, University of Iceland Research Fund **References**: 1. Morazzoni, P., Bombardelli, E. (1996), Fitoterapia 66: 3 – 29. 2. Brantner, A., Grein, E. (1994), J. Ethnophamacol. 44: 35 – 40.

P 054

Cucurbitacin R reduces delayed-type hypersensitivity reaction induced by dinitrofluorobenzene and sheep red blood cells in mice

Ríos JL, Escandell JM, Cerdá-Nicolás M[1], Recio MC
Departament de Farmacologia, Facultat de Farmacia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain; Departament de Patologia, Facultat de Medicina, Universitat de València, Av. Blasco Ibáñez 15, 46010 Valencia, Spain

The roots of *Cayaponia tayuya* (Vell.) Cogn. (Cucurbitaceae) are used in folk medicine as an anti-inflammatory and anti-allergic crude drug [1]. We previously reported its anti-inflammatory effect [2] and the anti-arthritic properties [3] of two cucurbitacins isolated from the chloroform extract. Now we tested the effects of cucurbitacin R (CCR) on different experimental models of systemic delayed-type hypersensitivity (DTH) in mice [3, 4]. CCR showed anti-allergic effects in two of the three models assayed. In the dinitrofluorobenzene (DNFB) model inhibited the ear oedema with a DI_{50} of 0.56 mg/ear at 48 h. In the sheep red blood cells (SRBC) model, the inhibition reached 64 % (18 h), 58 % (24 h) and 62 % (48 h). In the oxazolone-induced DTH there was not significant effect. In the histological studies of the DNFB-induced contact dermatitis, the CCR-treated group inhibited the oedema formation and the inflammatory cell infiltration, along with a reduction of the tissue damage. In the DTH-induced by SRBC, when compared with the non-treated group, the paws of the CCR-treated group (12.5 mg/kg) present a mild inflammatory lesion and scarce mixture of inflammatory cells, and a reduction of tissue damage. In addition, CCR abolished the production of TNF-α and IL-1β in the paws of the CCR-treated group giving a 100 % (TNF-α) and 90 % (IL-1β) of inhibition. *Acknowledgements*: J.M.E. is recipient of a grant from the Generalitat Valenciana. This work was supported by the Spanish Government (SAF2002 – 00723) **References**: 1. Ríos, J.L. *et al.* (1990), Fitoterapia 61: 275 – 278. 2. Recio, M.C. *et al.* (2004), Planta Med. 70: 414 – 420. 3. Escandell, J. *et al.* (2006), Eur. J. Pharmacol. 532: 145 – 154. 4. Góngora, L. *et al.* (2000), Life Sci. 66: PL183.

P 055

Evaluating the effects of phenolics from Phagnalon rupestre (L.) DC on cellular nitration and oxidation

Giner RM, Olmos A, Máñez S
Departament de Farmacologia, Facultat de Farmacia, Universitat de València, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain

Not only do reactive nitrogen species modify proteins and nucleotides by means of oxidative or nitrating reactions, but they can also cause other structural alterations which have a physiopathological significance in a number of human diseases. Given that some naturally occurring phenolics, such as hydroxycinnamate and hydroxybenzoate derivatives, are particularly effective at preventing the degradation of biomolecules, such compounds should actually protect against these unfavourable conditions. The present communication describes our ongoing investigations of three phenolics isolated from *Phagnalon rupestre* (Asteraceae): 2-isoprenylhydroquinone-1-glucoside (IHG), 3,5-dicaffeoylquinic acid (DCA) and its methyl ester (DCE) [1], previously described as peroxynitrite-scavengers for free tyrosine [2], on nitrating and oxidative reactions in two different cellular systems. We examined the effect of these compounds on bovine seroalbumin nitration by human neutrophils [3] and on dihydrorhodamine 123 (DHR 123) oxidation in macrophages [4], both stimulated with tetradecanoylphorbol acetate. All the compounds tested significantly reduced protein-bound tyrosine nitration in neutrophils with IC_{50} values of 27.9, 10.5, 11.0 and 20.7 microM for IHG, DCA, DCE and the reference epigallocatechin gallate (EGCG), respectively. DCE was the most active compound in preventing DHR 123 oxidation with 46 and 61 % inhibition at 50 and 100 microM, respectively. Since caffeoylquinic derivatives have previously been described as human leukocyte myeloperoxidase (MPO) inhibitors [5], this may have an influence on their ability to impair the formation of the nitrating agent NO_2, which is generated by MPO in the neutrophils. Because of their phenolic nature, such compounds should manifest noteworthy antioxidant activity; however, they do not behave uniformly in preventing DHR 123 oxidation. *Acknowledgements*: A.O. is recipient of a grant from Generalitat Valenciana. This work was supported by the Spanish Ministry of Science and Technology (SAF 2002 – 00723). **References**: 1. Góngora, L. *et al.* (2002), Planta Med. 68: 561 – 564. 2. Olmos, A. *et al.* (2005), Nitric Oxide 12: 54 – 60. 3. Eiserich, J.P. *et al.* (1998), Nature 391: 393 – 397. 4. Walrand, S. *et al.* (2003), Clin. Chim. Acta 331: 103 – 110. 5. Góngora, L. *et al.* (2002), Life Sci. 71: 2995 – 3004.

P 056

Phytochemical investigation of the Mongolian medicinal plant Saussurea amara (L.) DC (Asteraceae)

Glasl S[1], Mayr K[1], Daariimaa K[2], Narantuya S[2], Kletter C[1]
[1]Department of Pharmacognosy, University of Vienna, PharmaCenter Vienna, Althanstrasse 14, A-1090 Vienna, Austria; [2]Health Sciences University of Mongolia, Choidog 3, Ulaanbaatar, Mongolia

In Mongolia, the genus *Saussurea* is represented by 42 different species [1]. Among them, some species such as *S. amara* (L.) DC, *S. involucrata* (TCSaul) Kar. & Kir. and *S. salicifolia* (L.) DC are frequently used as medicinal plants. In traditional Mongolian medicine the herbal parts of *Saussurea* species are considered to be useful to treat fever, infectious diseases, rheumatism, indigestion and haemorrhages [2, 3]. *S. amara* is added to medical preparations which are applied to treat hepato-biliary disorders. To determine the effect of this plant on liver functions, four different extracts (crude water – extract **1**, ethyl acetate – extract **2**, methanol – extract **3**, water – extract **4**) were investigated for their potential to stimulate bile secretion (choleresis). Extract **3** exerted a dose-dependent increase in bile flow (16 %, 37 %, 53 %, 61 %) in the applied isolated rat liver perfusion system in concentrations of 50 mg/L, 100 mg/L, 250 mg/L and 500 mg/L. Extracts **1** and **2** also showed a dose-dependent increase, but at the highest concentrations (1000 mg/L and 100 mg/L, respectively) a continuous decrease in bile flow could be observed. However, in order to trace the active, liver-affecting principles in *S. amara* the respective extracts were investigated phytochemically. We identified the flavonoids apigenin, luteolin, genkwanin, quercitrin and apigenin-7-O-glucoside besides the terpenoids taraxasterol, taraxasterol-acetate, cynaropicrin and desacylcynaropicrin. *Acknowledgements:* We thank Dr. Enebishiin Ganbold, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia, for his participation in the expeditions and for the identification of the plant species. We are greatly thankful to Peter Wyskovsky, Centre for Physiology and Pathophysiology, Medical University Vienna, for his technical assistance in the liver perfusion tests. **References**: 1. Grubov, V.I. (1982), Key to the vascular plants of Mongolia. Leningrad. 2. Ligaa, U. (1996), Medicinal plants of Mongolia used in Mongolian traditional medicine. KCA Press Korea. 3. Khaidav, T., Altanchimeg, B., Varlamova T.S. (1985), Medicinal plants in Mongolian medicine, State Printing House, Ulaanbaatar.

P 057

Antioxidant activity of water and ethanol extracts from roots of Cassine transvaalensis Burtt-Davy from Botswana

Motlhanka DMT[1], Miljkovic-Brake A[2], Houghton PJ[2], Habtemarium S[3], Hylands PJ[2]
[1]Medicinal Plant Research Laboratory, Basic Sciences Department, Botswana College of Agriculture, Bag 0027, Gaborone, Botswana; [2]Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College University of London, 150 Stamford Street, SE1 9NH, London, UK; [3]Pharmacognosy & Phytotherapy Research Laboratory, School of Chemical and Life Sciences, The University of Greenwich at Medway, Central Avenue, Chatham Maritime, Kent, ME4 4TB, KENT, UK

Several studies have described the antioxidant properties of medicinal plants rich in phenolic compounds. Botswana, a country with a strong history of traditional healing, hosts a variety of plant species with therapeutic reputation. There is however, very little or scanty information regarding the phytochemical composition of medicinal plants of Botswana. In this work, water and ethanol extracts of roots from *Cassine transvaalensis* Burtt-Davy (Celastraceae) were assessed for *in vitro* antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay. The ethanolic extract exhibited higher free radical scavenging effect than the water extract at all tested concentrations. Above 100 µg/mL, the ethanolic extract showed 80 % scavenging activity, similar to control antioxidant compounds quercetin, rutin and L-ascorbic acid. The water extract reached a similar level of activity (80 %) at 200 µg/mL. Between 25 – 50 µg/mL, 4'-O-methyl-epigallocatechin isolated by bioassay directed fractionation exhibited (65 %) scavenging activity greater than that of either the ethanolic or aqueous extract. However, at concentrations above 50 µg/mL, the scavenging activity of the ethanolic extract exceeded that of 4'-O-methyl-epigallocatechin. This shows that there are additional active compounds than the isolate. The results suggest that extracts from the roots of *Cassine transvaalensis* have strong antioxidant activity. These findings support the ethnomedical use of this plant to promote good health. *Acknowledgements*: Botswana College of Agriculture for funding. King's College, London Pharmacognosy Research Laboratory.

P 058

Antifungal and multidrug resistance modulatory effects of diterpenic and phenolic compounds

Ferreira MJU[1], Duarte N[1], Kołaczkowski M[2], Michalak K[2]
[1]CECF, Faculty of Pharmacy, University of Lisbon, Av. das Forças Armadas, 1600 – 083 Lisbon, Portugal; [2]Department of Biophysics, Wrocław Medical University, Chałubińskiego 10, PL-50 – 368 Wrocław, Poland

Azoles (fluconazole, itraconazole and ketoconazole) are among the few classes of antifungals available for the treatment of systemic yeast infections. Nowadays, due to the global AIDS pandemic and the use of immunosuppressive drugs in anticancer chemotherapy, the incidence of fungal infection as increased [1]. For these reasons, the resistance of yeasts to treatment is very common and the effectiveness of antifungal drugs is reduced by the activity of multidrug transporters from the ATP-binding cassette superfamily, such as Cdr1p and Cdr2p of the major human fungal pathogen *Candida albicans.* These proteins reduce intracellular drug concentration by actively extruding them out of cells. One of the strategies employed to overcome this type of resistance is the combination treatment with efflux pump inhibitors. The aim of this work is to study the antifungal and multidrug resistance modulatory effect of diterpenic and phenolic compounds isolated from *Euhorbia* species in the model eucaryote *Saccharomyces cerevisiae.* Yeast strains either deleted in major endogenous multidrug ATP-binding cassette transporters *PDR5*, *SNQ2* and *YOR1* or specifically overproducing each pump separately were used. The effect of analysed compounds on the inhibition of heterologously overproduced Cdr1p of *Candida albicans* was also verified. Trifluoperazine was used as positive control exerting both growth inhibitory and modulatory activity. The analysed compounds exert weak antifungal activity and modulate to a different extent azole antifungal resistance mediated by Pdr5p, Snq2p, and Cdr1p. *Acknowledgements*: The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant. **Reference**: 1. Kolaczkowski, M. *et al.* (2003), Int. J. Antimicrobial Agents 22: 279 – 283.

P 059

Vasorelaxant effect of ethanolic extract from Cecropia obtusifolia in Guinea pigs aortic rings

Cassani J[1], Luna HM[1], Magos GA[2], Tato P[3], Jiménez-Estrada M[4]
[1]Departamento de Sistemas Biológicos, UA M- Xochimilco, Calz. del Hueso 1100, Col. Villa Quietud, Coyoacán 04960 México, D.F; [2]Facultad de Medicina. Departamento de Farmacología, UNAM, Coyoacán 04510, México, D.F; [3]Departamento de Microbiología y Parasitología, Facultad de Medicina, UNAM, Coyoacán 04510, México, D.F; [4]Instituto de Química, UNAM, Coyoacán 04510, México, D.F

Cecropia obtusifolia (Moraceae) is a 20 – 25 m tall tree, which grows in tropical rain forest. It is commonly known as "guarumbo", "chancarro" or "trumpet". It is widespread in México, especially in Veracruz, Oaxaca, Hidalgo and from Sinaloa to Chiapas [1]. Several species of Cecropia had been described for its different biological activities like cardiovascular, anxiolytic, and mainly hypoglycemic effect [4, 5]. However, the vascular effect of *C. obtusifolia* has been poorly investigated. The aim of this work was to study the effects of *C. obtusifolia* on the *Guinea pigs* aortic model *in vitro*. The activity of the ethanolic extract was assayed, showing efficient relaxing activity; 3 mg/mL produced 50 % of relaxation in aortic rings pre-treated with norepinephrine (1 X 10^{-7} M). After the pigments removal, the residue was taken in water and dialyzed using molecular porous membrane with a cutoff of 3.500 Da. We then assayed again the activity *in vitro*, the effect was better than the crude ethanolic extract, because 3 mg/mL produced 98 % relaxation over pre-treated with norepinephrine (1 X 10^{-7} M). Further purification can be done by precipitation by salting out using ammonium sulfate and desalting by dialysis, conducing to a potential vasorelaxant agent. **References**: 1. Consolini, A.E., Migliori, G.N. (2005), J. Ethnopharmacol. 96: 417 – 422. 2. Rocha, F.F. *et al.* (2002), Pharmacol. Biochem. Behavior 71:183 – 190. 3. Andrade-Cetto, A., Wiedenfeld. H. (2001), J. Ethnopharmacol. 78:145 – 149. 4. Pérez, G. *et al.* (1984), J. Ethnopharmacol. 12: 253 – 262. 5. Pérez-Guerrero, C. *et al.* (2001), J. Ethnopharmacol. 76:279 – 284.

P 060

Some cardiovascular effects of the aqueous extract of the leaves of Starchytarpheta jamaicensis L. (Vahl)

Idu M[1], Omogbai EKI[2], Amaechina F[2], Ataman JE[3]
[1]Department of Botany, University of Benin, PMB 1154, Benin City, Nigeria; [2]Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria; [3]Department of Anatomy, University of Benin, PMB 1154, Benin City, Nigeria

Traditionally, many herbal doctors claim, some plants are known for their anti-hypertensive effects. The high patronage of sellers of such herbs may be an indication of the plants efficacy. However, their mechanisms of action as well as the active constituents may not have been documented. The efficacy of powdered *Stachytarpheta jamaicensis* (L.) Vahl. leaves, known for treating hypertension in some Nigerian communities, was investigated in anaesthetized normotensive male rabbits. The extract was administered intravenously at doses ranging from 2.5 – 80 mg/kg. The extract caused a dose-dependent fall in blood pressure and heart rate. 2.5 mg/kg of the extract reduced the mean arterial pressure (MAP) from the initial 102.8 ± 4.2 mm Hg to 96.6 ± 7.3 mm Hg and the heart rate (HR) from 398.3 ± 8.3 beats/min to 373.1 ± 9.7 beats/min. 80 mg/kg reduced MAP and HR to 38.9 ± 3.1 mm Hg and178.3 ± 83.7beats/min respectively. The results showed that the water extract has a significant

dose-dependent hypotensive effect. It has been shown in this study that the extract may not be acting through histamine release or through the stimulation of muscarinic receptors. Neither atropine nor promethazine inhibited the hypotensive effect of the extract. The acute hypotensive effect of the extract may be partly due to the negative chronotropic effect or to a direct effect on vascular smooth muscle.

P 061

Effect of masticadienonic acid on the induction of micronuclei in polychromatic erythrocytes in mouse peripheral blood

Hernández MS[1], Acevedo HR[1], Rojas MD[2], Barriga SD[2]
[1]Colegio de Postgraduados, Km 35 – carretera México-Texcoco, Montecillo, Texcoco, C.P. 56230, Estado de México, México; [2]Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México, Av. Primero de Mayo s/n, Cuautitlán, Izcalli, C.P. 54740, Estado de México, México

The bark of *Amphipterygium adstringens* Schiede ex Schltdl. is widely used in the traditional Mexican medicine for treating ailments such as gastric ulcers, gastritis and stomach cancer. The masticadienonic acid was isolated from the bark of this species. In previous papers have been informed that this compound possess anti-inflammatory and cytotoxic activities [1, 2]. Now we describe cytotoxic and genotoxic effects of this compound. The cytotoxic and genotoxic effects of masticadienonic acid on CD1 male mice were determined with micronucleus assay at 24, 48 and 72 h after oral administration of doses of 250, 500 and 1000 mg/kg. Peripheral blood samples were drawn from the caudal vein and analyzed by Giemsa-stained technique. The results showed that the ratios of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) in mice treated with 250, 500 and 1000 mg/kg were not statistically different compared with their negative control animals at 0, 24 and 72 h. PCE/NCE ratios were increased at 48 h at all doses. The masticadienonic acid showed cytotoxic activity at 48 h after administered at all doses. The test compound do not increased the frequency of micronucleated polychromatic erythrocytes; this compound may not lead to chromosome damage at the evaluated doses. **References**: 1. Oviedo-Chavez, I. *et al.* (2004), Phytomed. 11: 436 – 445. 2. Giner-Larza, E.M. *et al.* (2002), Planta Med. 68: 311 – 315.

P 062

Anti fungal activity of alkaloid extract of Erythrina coralloides A. DC. against five phyto pathogen fungi

Soto-Hernández M, San Miguel-Chávez R
Postgrado en Botánica. Campus Montecillo. Colegio de Postgraduados. Carr. México-Texcoco km. 36.5 CP 56230, Montecillo, México

The genus *Erythrina* (Leguminoseae) produces a high variety of secondary metabolites, such as flavonoids, isoflavonoids and alkaloids with high pharmacological, antimicrobial and anti-inflammatory activities [1]. The African species of Erythrina have been investigated in connection with the anti microbial properties of the isoflavonoids but in the American species there is not information about the antimicrobial activity of their constituents The aim of this work was to assess the antifungal activity of the alkaloid extract of *E. coralloides*. The alkaloid extract was obtained from one month age seedlings grew in a controlled environment chamber at 25°C, relative humidity of 80% and 12 h of light. The seedlings were lyophilised, and ground to a fine powder and then mixed with 0.1% trifluoroacetic acid. The mixture was filtered and the pH was adjusted to 8 with NH_4OH. The filtrate was extracted with dichloromethane (three times) and the solvent was evaporated under vacuum [3]. The antifungal activity evaluation of the crude extract showed that *Penicillium* sp. had a minimum inhibitory concentration (MIC) of 6000 µg/L, *Alternaria solani*, *Botrytis cinerea* and *Fusarium oxysporum* of 4000 µg/L and *Monilia fructicola* of 2000 µg/L. It is proposed that the interaction of the various alkaloids detected in the extract could cause the growth inhibition of the fungi *Acknowledgements*: To the mycology laboratory of the Phytopathology Department of the Colegio de Postgraduados by the donation of the fungi. **References**: 1. García-Mateos, R. *et al.* (2001), Econ. Bot. 55: 391 – 400. 2. Majinda, R.T. *et al.* (2001), Pure Appl. Chem. 73: 1191 – 1208. 3. San Miguel-Chávez, R. *et al.* (2003), Biotech. Lett. 25 (13): 1055 – 1059.

P 063

Acetylcholinesterase inhibitors from Huperzia selago

Borloz A, Marston A, Hostettmann K
Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland

At the present time, most comercially available drugs to slow down the progression of Alzheimer's disease (AD) are acetylcholinesterase (AChE) inhibitors. In an effort to find new active substances, different Lycopodiaceae species were screened by TLC bioautography [1]. The alkaloid extract of the whole plant of *Huperzia selago* (L.) Bernh. ex Schrank et Martius (Lycopodiaceae) was the only one found to contain huperzine A, in addition to other AChE inhibitors. Active constituents were isolated by bio-guided fractionation, using different chromatographic methods such as centrifugal partition chromatography, low-pressure chromatography and semi-preparative HPLC. Three active compounds were isolated with minimal inhibitory quantities (MIQ) as low as 0.01 ug (MIQ (±)-huperzine A = 0.2 ng). *Acknowledgements:* The Swiss National Science Foundation (grant no. 2153 – 066874.01/1 to K. Hostettmann) is gratefully acknowledged for financial support. **Reference**: 1. Marston, A. *et al.* (2002), Phytochem. Anal. 13: 51 – 54.

P 064

β-Secretase (BACE1) Inhibitors from Pomegranate (Punica granatum) L. Husk

Kwak HM[1], Choi SH[1], Yang EJ[1], Kwon SH[1], Jeong HH[1], Bae KH[2], Seong YH[3], Song KS[1]
[1]College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702 – 701, Korea; [2]College of Pharmacy, Chungnam National University, Yusung, Daejon305 – 764, Korea; [C]College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk361 – 763, Korea

Alzheimer's disease (AD) is a neurodegenerative disorder clinically characterized by progressive dementia that inevitably leads to incapacitation and death. Two characteristic brain lesions define AD at the microscopic level: (1) amyloid plaques, extracellular deposits primarily composed of 4 kDa, 40 – 42 amino acid Aβ peptide, a product of APP proteolysis, and (2) neurofibrillary tangles, and intracellular aggregates of the microtubule associated protein tau. The relationships between amyloid plaques, neurofibrillary tangles, and the pathogenic mechanisms of AD are controversial. Evidence, however, suggests that Aβ is critically involved at an early stage in AD pathology. Two proteolytic cleavage events are required to generate Aβ from its precursor, one at the *N*-terminus by an enzyme termed β-secretase and one at the *C*-terminus by an enzyme termed γ-secretase. Among the secretases, a novel transmembrane aspartic protease BACE1 (for β-site APP, cleaving enzyme 1), also known as Asp2 (for novel aspartic protease 2) and memapsin 2 (for membrane aspartic protease/pepsin 2), is at present the most attractive target for the inhibition of amyloid production. In the course of screening anti-dementia agents from natural products, two β-secretase (BACE1) inhibitors were isolated from the ethyl acetate soluble fraction of pomegranate husk. Chromatographic separation including silica gel, Sephadex LH-20, and RP-HPLC afforded two active principles. They were identified as ellagic acid (**1**) and punicalagin (**2**) and were shown to non-competitively inhibit β-secretase (BACE1) with the IC_{50} values of 3.9×10^{-6} M and 4.1×10^{-7} M, respectively. The *Ki* values of **1** and **2** were 2.4×10^{-5} M and 5.9×10^{-7} M. They were less

inhibitory to α-secretase (TACE) and other serine proteases such as chymotrypsin, trypsin, and elastase, suggesting that they were relatively specific inhibitors of BACE1.

P 065

Inhibitory Effects of the Constituents of Prunus mume on Bradykinin and Prostaglandin E_2 Production in Abdominal Cavities of Mice

Ina H[1], Yamada K[1], Matsumoto K[2], Miyazaki T[1]
[1]School of Pharmacy, Tokyo University of Pharmacy & Life Science, 1432 – 1 Horinouchi, Hachioji, Tokyo 192 – 0392, Japan; [2]Umekenkyuukai Foundation, 1 – 1-26 Gakuenchou, Settsu, Osaka 566 – 8566, Japan

The fruits of *Prunus mume* Sieb. *et* Zucc. (Rosaceae) have been traditionally used as medicinal food in Japan. In regard to the chemical constituents, we previously reported on isolation of benzyl β-D-glucopyranoside (BG) and chlorogenic acid (CA) [1]. In the course of our research on the pharmacologically active constituents of the fruits of *P. mume*, we recently found that BG and CA inhibited acetic acid-induced writhing behavior of mice. To elucidate the analgesic mechanisms of BG and CA, we compared the inhibitory effects of BG and CA on bradykinin and prostaglandin E_2 production [2, 3] in abdominal cavities of mice with those of aspirin (Asp). In this symposium we report the effects of BG, CA and ASP on acetic acid-induced writhing behavior of mice, and on bradykinin and prostaglandin E_2 production in abdominal cavities of mice. BG, CA and Asp equally inhibited bradykinin production. Though the inhibitory effects of BG and CA were smaller than that of Asp in the case of prostaglandin E_2 production, BG and CA meaningfully inhibited it. These results indicate that the inhibitory effects of BG and CA on acetic acid-induced writhing behavior were brought about by inhibiting both bradykinin and prostagrandin E_2 production in abdominal cavities of mice. **References**: 1. Ina, H. *et al.* (1999), Nat. Med. 53: 109 – 110. 2. Ando, T. *et al.* (1982), Recent Progress on Kinins, ed. by Fritz, H., Dietze, G. *et al.*, Birkhauser Verlag, Stuttgart, pp. 222 – 232. 3. Kawano, K. (1987), Enshou, 7: 511 – 515.

P 066

Anticancer properties of brassinosteroids

Swaczynová J[1], Malíková J[2], Hoffmannová L[1], Kohout L[3], Strnad M[1]
[1]Laboratory of Growth Regulators, Institute of Experimental Botany ASCR & Palacký University, Šlechtitelu 11, 78371 Olomouc, Czech Republic; [2]Laboratory of Molecular Pathology, Institute of Pathology, Faculty of Medicine, Palacký University, Hnìvotínská 3, 775 15 Olomouc, Czech Republic; [3]Institute of Organic Chemistry and Biochemistry ASCR, Flemingovo námìstí 2, 16610 Praha 6, Czech Republic

Brassinosteroids (BRs) represent a large group of plant steroids which include more than 70 congerners distributed from lower to higher plants. BRs have been detected and isolated from seeds, fruits, leaves, galls and pollen. Physiological functions proposed for BRs include plant cell elongation, cell division and modulation of stress responses when applied at very low concentrations [1]. Some medically oriented applications of BRs have also been already reported [2 – 4]. Wachsman et al.[2, 3] showed that some natural BRs (28-homocastasterone, 28-homobrassinolide) and their synthetic analogues have *in vitro* antiviral activity against several pathogen viruses, like herpes simplex virus type 1 (HSV-1), arenaviruses and measles virus (MV). The aim of our study was to determine whether natural types of BRs can affect the viability, proliferation, differentiation, apoptosis and expression of some cell cycle related proteins in cancer cell lines. Cytotoxic activity of BRs were tested *in vitro* by Calcein AM assay. IC_{50} values were estimated for human breast adenocarcinoma cell lines (MCF- 7 – estrogen-sensitive, MDA-MB-468 – estrogen-insensitive), human acute lymphoblastic leukemia cell line (CEM) and human myeloma cell line (RPMI 8226)[5]. TUNEL, DNA ladder assay, and immunoblotting were used for analysis of changes of cell viability, proliferation, differentiation and apoptosis. 28-Homocastasterone inhibited the viability of cancer cell lines and significantly reduced or induced the expression of *p21*, *p27*, *p53*, cyclins, proteins of Bcl-2 family, and ER-alpha. The antiproliferative properties can be usable for development of new brassinosteroid-derived generation of anticancer drugs. *Acknowledgements:* This work was supported by the grant MSM 6198959216 of the Ministry of Education of Czech Republic. **References**: 1. Clouse, S.D. (2002), Brassinosteroids. In: The Arabidopsis book. American Society of Plant Biologists, Washington D.C., pp. 1 – 23. 2. Wachsman, M.B. *et al.* (2002), Antivir. Chem. Chemother. 13: 61 – 66. 3. Wachsman, M.B. *et al.* (2000), Antivir. Chem. Chemother. 11: 71 – 77. 4. Michelini, F.M. *et al.* (2004), Steroids 69: 713 – 720. 5. Swaczynová, J. *et al.* (2006), Polish J. Chem. 80: 629 – 635.

P 067

Structure elucidation of bioactive pectins from Opilia celtidifolia (Guill. & Perr.) Endl. Ex Walp. (Opiliaceae)

Togola A[1], Diallo D[2], Michaelsen TE[3], Paulsen BS[1]
[1]Section of Pharmacognosy, Department of Pharmaceutical Chemist, University of Oslo PO box 1068 Binder, 0316 Oslo, Norway; [2]Department of Traditional Medicine Bamako Mali; [3]National Institute of Public Health Oslo, Norway

Many plants contain polysaccharides that exhibit biological activities of different kinds. Immunostimulatory, antitumor, antiviral, antibacterial and anti-inflammatory activities are among the numerous demonstrated biological proprieties [1]. Polysaccharides from the leaves of *Opilia celtidifolia* (*Oc*), a medicinal plant used in wound healing processes in traditional medicine in Mali (West Africa), possesses a potent activity in the complement system. The activity was tested using the inhibition of haemolysis of human sensitized erythrocytes [2]. A pure pectin fraction PMII, with Rhamnogalacturonan (RG) type I structure, from the leaves of *Plantago major* L. was used as positive control. *Oc* polysaccharides were more active than PMII. The ICH_{50} of the most active fraction was 0.6 µg/mL while that of PMII was 8.6 µg/mL. We elucidated the structure of these active polysaccharides. They were water soluble; they were purified by gel filtration and anion exchange chromatography. The structure was determined by enzymatic degradation followed by methylation using GC-MS. The monosaccharide composition determined by gas chromatography and a positive reaction with the Yariv-β-glucosyl reagent coupled with the GC-MS results indicated that the active polysaccharides are pectic type and contain some structural elements associated with arabinogalactans type II. **References**: 1. Paulsen, B.S., and Barset, H. (2005), Adv. Polym. Sci. 186: 69 – 101. 2. Michaelsen, T.E. *et al.* (2000), Scand. J. Imm. 52: 483 – 490.

P 068

Functionality of oligo- and polysaccharides against gastrointestinal epithelial membranes: bioadhesive and mucin-stimulating carbohydrates

Lengsfeld C[1], Schmidgall J[1], Hensel A[1]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

Within a screening on bioadhesive carbohydrates against gastrointestinal tissues rhamnogalacturonans with a low degree of esterification and linear oligogalacturonids derived from pectin as well as polygalacturonic acid showed significant bioadhesion against intestinal mucous membranes. Bioadhesion was based on the occurrence of linear, strongly acidic homogalacturonide parts in the polymers. Esterification, branching or non-linear backbone structures reduced the adhesive properties. The bioadhesive effects were concentration-dependent and due to exclusive localization of the polysaccharides on the apical membrane surface of the membranes. The bioadhesive effects were tissue specific: no adhesion occurred on porcine small intestine, while porcine stomach and porcine and human co-

lonic membranes strongly bound the carbohydrates. The bioadhesion was due to an interaction of the acidic polymers with the endogenous mucin via bivalent positive cations as shown by *in situ* investigations on tissue and by a rheological mucin-galacturonide synergism. The artificial mucin layers provided protective effects on colonic mucous membranes against exogenous toxic agents. Chitooligosaccharides (N-Acetyl-D-glucosamintetramer and -pentamer) were found to stimulate the endogenous mucin synthesis from intestinal membranes. This stimulating effect was deduced from colorimetric methods and Western blots of mucins with lectin staining. While colonic and stomach tissue were susceptible for this mucin stimulation, membranes from ileum did not show these effects.

P 069

Okra polysaccharides inhibit adhesion of Campylobacter jejuni to mucosa from poultry in situ but not in vivo within infection study

Hensel A[1], Lengsfeld C[1], Faller G[2]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany; [2]University of Erlangen-Nürnberg, Pathology, Krankenhausstr. 8 – 10, D-91054 Erlangen, Germany

With a potential use of prophylactic functional food additives to animal feed, the application of antiadhesive compounds against the gastrointestinal docking of pathogenic microorganisms is increasingly under discussion. High-molecular glycosylated compounds (polysaccharides and glycoproteins) isolated from the immature fruits of the okra plant, *Abelmoschus esculentus* (L.) MOENCH, were shown to have a strong antiadhesive activity against *Helicobacter pylori*, leading to an inhibition of binding to mucosal epithelia from human stomach *in situ*. In order to evaluate a further potential use these polymers were additionally tested concerning the adhesion of *Campylobacter jejuni* towards intestinal epithelia derived from chicken, the transducer of this germ to humans. Using an *in situ* adhesion model with fluorescent-labelled *Campylobacter* cells, the bacterial adhesion was shown to occur predominantly within jejunum and colon sections of the GUT of these animals, but only to a low extend on stomach, ileum and caecum tissue. Under these conditions, isolated okra polysaccharides strongly inhibited the microbial adhesion to colonic tissue. Within a controlled *in vivo infection* study over 42 days with chicken broilers infected with *Campylobacter* and fed with okra aqueous extract (5 and 10%) no significant reduction in *Campylobacter* excretion was observed, indicating that intestinal decontamination is not possible by the oral application of these compounds.

P 070

Antiplasmodial, $GABA_A$-benzodiazepine receptor binding and acetylcholinesterase inhibitory activities of plants used in traditional medicine in Mali, West Africa

Bah S[1,3], Jäger AK[2], Adsersen A[2], Diallo D[3], Paulsen BS[1]
[1]University of Oslo, School of Pharmacy, Department of Pharmaceutical Chemistry, PO Box 1068 Blindern, N-0316 Oslo, Norway; [2]The Danish University of Pharmaceutical Sciences, Department of Medicinal Chemistry, 2 Universitetsparken, 2100, Copenhagen O, Denmark; [3]Institut National de Recherche en Santé Publique, Département de Médecine Traditionnelle, BP 1746, Bamako, Mali

The recourse to traditional medicine and medicinal plants could be an alternative to expensive synthetic drugs for developing countries. Malaria is the leading cause of morbidity and mortality in Mali. Plants used in traditional medicine for treatment of epilepsy and convulsions are potential sources to look into in order to find substances that enhance GABA's affinity to the $GABA_A$-receptor. An important approach in the symptomatic treatment of Alzheimer's disease (AD) involves the inhibition of acetylcholinesterase. Five medicinal plants: *Boscia angustifolia* A. Rich, *Cissus quadrangularis* L., *Securidaca longepedunculata* Fers, *Stylosanthes erecta* P. Beauv. and *Trichilia emetica* Vahl., used traditionally in Mali to treat malaria, old age-related memory loss, epilepsy and convulsion have been evaluated for their antiplasmodial activities, their ability to bind to the $GABA_A$-benzodiazepine receptor [1] and acetylcholinesterase inhibitory activity on the TLC assay [2]. The strongest antiplasmodial activity was observed with dichloromethane extracts of leaf of *S. longepedunculata* with IC_{50} of 7 μg/mL (95% CI: 5 – 9) and leaf of *T. emetica* IC_{50}: 12 μg/mL (95% CI: 12 – 14). The strongest binding to $GABA_A$-receptor was obtained with the methanol extract of aerial part of *S. erecta*. No acetylcholinesterase inhibitory activity in the TLC assay was observed with any of the tested extracts. The $GABA_A$-benzodiazepine receptor assay results suggest that the active compounds are of apolar nature. The results of this study justify some of the traditional indications of the plants investigated. **References**: 1. Kahnberg, P., Howard M.H. *et al.*(2004), J. Mol. Graph. Model. 23: 253 – 261. 2. Rhee, I. K., van Rijn, R.M., Verpoorte R. (2003), Phytochem. Analysis 14: 145 – 149.

P 071

Antiviral and antimicrobial activities of three sesquiterpene lactones from Centaurea solstitialis L. ssp. solstitialis

Gürbüz I[1], Özçelik B[2], Karaaoglu T[3], Yesilada E[4]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara- Turkey; [2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, Etiler 06330, Ankara-Turkey; [3]Department of Virology, Faculty of Veterinary, Ankara University, Diskapi 06100, Ankara-Turkey; [4]Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Kayisdagi, Kadiköy 34755 -Istanbul, Turkey

Three sesquiterpene lactones (centaurepensin = chlorohyssopifolin A, chlorojanerin and 13-acetyl solstitialin A) were isolated from *Centaurea solstitialis* L. ssp. *solstitialis* (Asteraceae). Antimicrobial and antiviral properties of centaurepensin, chlorojanerin and 13-acetyl solstitialin A were screened against both standard and the isolated strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida albicans* and *C. parapsilosis* by microdilution method. *Herpes simplex* type-1, as representative of DNA viruses and *Parainfluenza* as representative of the RNA viruses were employed for the determination of antiviral activity of this three sesquiterpene lactones by using Vero cell line. Ampicilline, ofloxacine, ketoconazole, fluconazole, aciclovir and oseltamivir were used as the control agents. 13-acetyl solstitialin A displayed remarkable antibacterial activity against isolated strains of *E. faecalis* at 1 μg/mL concentration, which is close to the effective concentrations of the ampicillin. The data obtained from antiviral activity screening showed that 13-acetyl solstitialin A had significant activity against DNA virus same as the aciclovir at maximum and minimum concentration of 16 and 0.00006 μg/mL

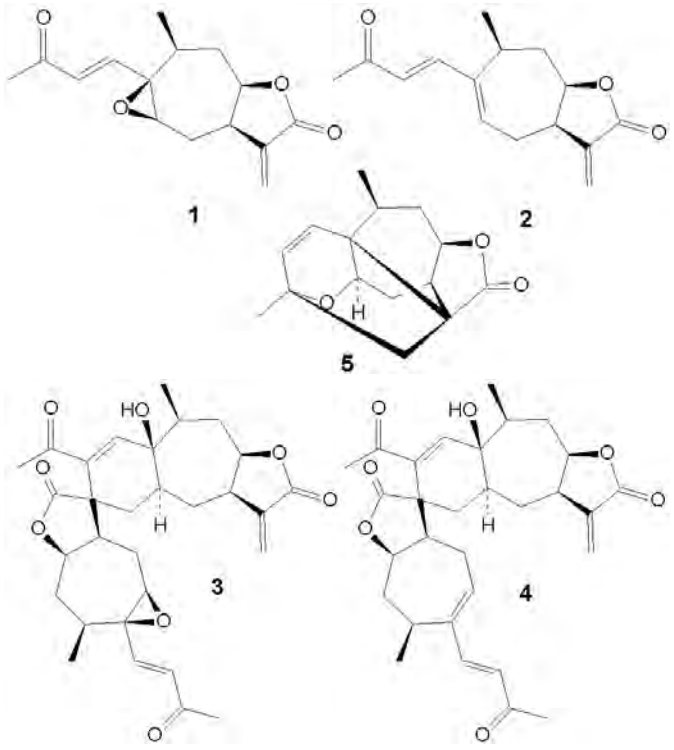
P 072

Antiprotozoal activity of Xanthium brasilicum and bioactivity-guided isolation of its active constituents

Nour AMM[1], Khalid SA[2], Abdallah WE[3], Kaiser M[4], Brun R[4], Schmidt TJ[1]
[1]Westfälische Wilhelms-Universität, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstraße 56, D-48149 Münster, Germany; [2]University of Khartoum, Department of Pharmacognosy, Faculty of Pharmacy, P.O. box 1996, Khartoum, Sudan; [3]Medicinal and Aromatic Plants Research Institute, P.O. box 2404-Khartoum, Sudan; [4]Swiss Tropical Institute, Socinstraße 57, CH-4002 Basel, Switzerland

In the course of a screening of Sudanese Asteraceae against protozoa causing tropical diseases, a crude DCM extract of *Xanthium brasilicum* Vell. was found highly active against *Trypanosoma brucei rhodesiense* (East African Sleeping Sickness, IC_{50} = 0.1 μg/mL). Bioactivity-guided fractionation yielded two monomeric and two dimeric xanthanolide sesquiterpene lactones (STL **1**-**4**), all highly active.

The unusual compound **5** was almost inactive as a pure compound. Compound **1** (8-epixanthatin) showed the lowest IC_{50} values against *T. brucei* and *Leishmania donovani* (visceral Leishmaniasis) of 0.34 μM and 0.60 μM, respectively. All compounds were reported as constituents of *X. pungens* [1] but no information on their bioactivity existed. Structure-activity studies with a variety of STL [2] are in progress.



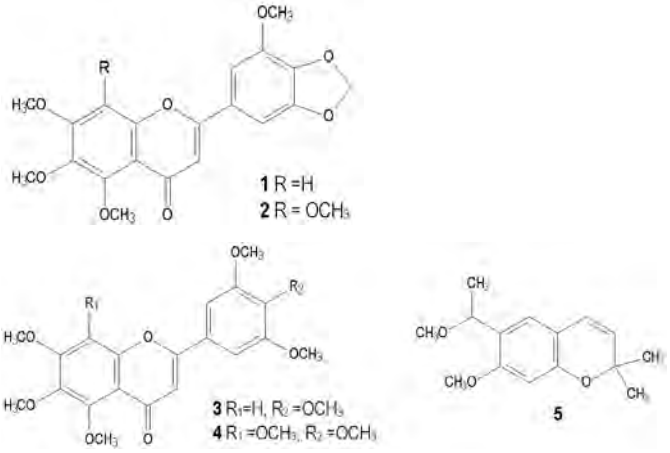
Acknowledgements: A. M. M. Nour acknowledges a scholarship from the German Academic Exchange Service (DAAD). **References**: 1. Ahmed, A. A. *et al.* (1990), Phytochemistry 29: 2211 – 2215. 2. Schmidt, T. J. *et al.* (2002), Planta Med. 68: 750 – 751.

P 073

Trypanocidal Flavonoids from Ageratum conyzoides

Nour AMM[1], AKhalid S[2], Abdallah WE[3], Kaiser M[4], Brun R[4], Schmidt TJ[1]
[1]Westfälische Wilhelms-Universität, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstraße 56, D-48149 Münster, Germany; [2]University of Khartoum, Department of Pharmacognosy, Faculty of Pharmacy, P.O. box 1996, Khartoum, Sudan; [3]Medicinal and Aromatic Plants Research Institute, P.O. box 2404-Khartoum, Sudan; [4]Swiss Tropical Institute, Socinstraße 57, CH-4002 Basel,Switzerland

The crude extract (MeOH sol. part of DCM extract) of *Ageratum conyzoides* L. (Asteraceae) was found to be active against the trypomastigote forms of *Trypanosoma brucei rhodesiense* (IC_{50}=0.78 μg/mL), the protozoan that causes East African sleeping sickness. Bioassay-guided fractionation of this extract so far afforded four polyoxygenated flavonoids (**1**-**4**) and a chromene (**5**). Compounds **1 – 4** were active with IC_{50} values of 16 μM, 18 μM, 21 μM and 11 μM, respectively, whereas compound **5** was almost inactive with an IC_{50} of 316 μM. The cytotoxicity level of all compounds against murine L6 cells was negligibly low. Although polyoxygenated flavonoids have previously been reported from *A. conyzoides* [1] no information was hitherto available on the trypanocidal activity of the crude extract or pure compounds. These results suggest that the polyoxygenated flavone skeleton deserves further investigation as a template for novel trypanocidal compounds. Structure-activity studies in relation with other flavonoids, see, e. g. [2], are under way. Search for further active constituents from this species is still in progress.



Acknowledgements: Amal Nour acknowledges a scholarship from the German Academic Exchange Service (DAAD). **References**: 1. Vyas, A.V. *et al.* (1986), Phytochemistry 25: 2625 – 27. 2. Tasdemir, D. *et al.* (2006), Antimicr. Agents Chemother. 50: 1352 – 64.

P 074

Analytical and functional aspects on Saffron from Crocus sativus L.: development of quality control methods, species assortment and affinity to sigma-1 and NMDA receptors

Hensel A[1], Niehues M[1], Lechtenberg M[1], Quandt B[1], Schepmann D[2], Wünsch B[2]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany; [2]University of Münster, Institute of Pharmaceutical and Medicinal Chemistry, Hittorfstr. 58 – 62, D-49149 Münster, Germany

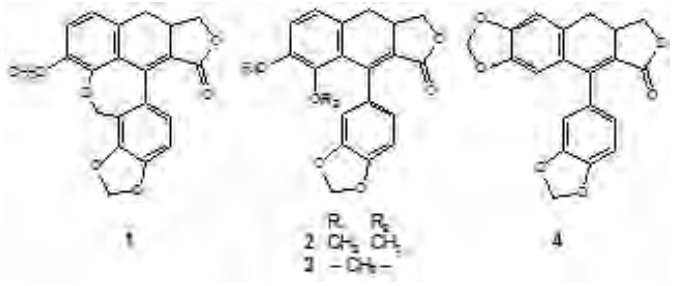
Saffron from *Crocus sativus* L. (Iridaceae) is getting into focus of medicinal development because of antidepressant and anticancer activities. Within the present investigation a potential influence on central receptor systems of ethanolic saffron extract and the crocins was investigated. While the extract showed no affinity to NMDA receptor binding of the crocin was found with IC_{50} 10 μM. Using sigma-1 receptors a IC_{50} of 30 μM was determined for both, the extract and crocins. No affinity was found against sigma-2 receptors. Using saffron for product development a strong need for validated control methods and use of reference standards is obvious. For that reason methods for isolation of reference material are described. Crocin-1,-2,-4, cis-crocin-1 and picrocrocin were isolated in good yields and high purity; identity was proven by NMR and MS. For determination of crocin distribution analytical RP-18 HPLC method with acetonitrile-TFA as mobile phase was developed. Using this method the different crocins can be quantified beside degradation products. Using an RP18 column picrocrocin was quantified from extracts effectively. Volatile compounds were analyzed by GC-MS, using isophoron, ketosiophoron and safranal for calibration. For investigation of drying conditions residual enzyme activity was investigated. All methods were validated. Short time stability was investigated using crocin-1, indicating sufficient stability of test solutions at RT; degradation was observed at higher temperatures. A broad investigation using 21 saffron samples indicated that products from certain proveniences (Spain, Iran, Greece, others) are not superior to others.

P 075

A novel Aryldihydronaphthalene Lignan from Linum perenne L

Schmidt TJ, Vößing S
Westfälische Wilhelms-Universität Münster, Institut für Pharmazeutische Biologie und Phyochemie, Hittorfstraße 56, D-48149 Münster, Germany

In the course of our study on the chemical diversity of lignans in the genus *Linum*, we investigated the aerial parts of *L. perenne* L. By HPLC-MS, the cytotoxic, antiviral and anti-inflammatory arylnaphthalene lignan justicidin B, previously reported by us as a constituent of *L. austriacum* [1] was identified. Further peaks corresponded to lignans hitherto unreported for *Linum* species. Compound **1** was found to be an aryldihydronaphthalene lignan with a hitherto unreported additional oxepine ring connecting the two aromatic moieties. Its structure was unambiguously proven by heteronuclear 2D-NMR (HSQC, HMBC). Compound **2** was previously obtained synthetically [2] but not reported as a natural product up to present. The structure of **3** has not been reported before, to the best of our knowledge. Compound **4** has previously been found as a natural product in *Cleistanthus collinus* (Euphorbiaceae) [3]. It is noteworthy that such presumable biogenetic intermediates between aryltetralin-type lignans (e. g. podophyllotoxin) and arylnaphthalenes (e. g. justicidin B), found in other members of the genus, were now found in a *Linum* species together with an arylnaphthalene for the first time. Compound **1**, moreover, deserves special mentioning since its novel ring system including a dibenzo[b,e]oxepine structure has not been reported as part of a lignan or natural product so far, to the best of our knowledge. Isolation of further lignans from *L. perenne* and establishment of the absolute stereochemistry of **1**-**4**, as well as evaluation of their biological activity, is in progress.



Acknowledgements: TJS acknowledges DFG grant Schm 1166/2 – 2. We thank Dr. H. Luftmann, Organic Chemistry, Münster, for HR MS spectra. **References**: 1. Mohagheghzadeh, A. *et al.* (2002), J. Nat. Prod. 65: 69 – 71. 2. Stevenson, R. *et al.* (1971), J. Org. Chem. 36: 3453 – 5. 3. Ajaneyulu, A. S. R. *et al.* (1981), Tetrahedron 37: 3641 – 52.

P 076

FabI, FabZ and FabG, Three Key Enzymes from the Type II Fatty Acid System of Plasmodium falciparum, as Possible Drug Targets of Polymethoxyflavones of Artemisia annua

Bilia AR[1], Vincieri FF[1], Perozzo R[2], Tasdemir D[3]
[1]Department of Pharmaceutical Sciences, University of Florence, 50019 Sesto Fiorentino, Florence, Italy; [2]School of Pharmaceutical Sciences, University of Geneva, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; [3]Centre for Pharmacognosy and Phytotherapy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, United Kingdom

The sesquiterpene lactone artemisinin is the antimalarial principle of *Artemisia annua* L. (Asteraceae). The antimalarial activity of artemisinin and its derivatives has been postulated to result from the inhibition of SERCA, a *P. falciparum* Ca^{2+}-ATPase [1]. The plant contains also some polymethoxyflavones, which were shown to potentiate the antimalarial activity of artemisinin [2]. However, no data concerning the mechanism related to their synergistic effect has been reported. Recent discoveries reveal that *Plasmodium* is able to synthesize its own fatty acids in the apicoplast [3]. *Plasmodium* fatty acid synthase (FAS) is a type II multienzyme complex, as found in plants and bacteria, and as such, differs markedly from human type I FAS. FabG, FabI and FabZ represent three key enzymes of the FAS-II system and are ideal targets for malaria drug discovery. After discovering a flavonoid as the first natural product inhibiting the plasmodial FabI enzyme [4], we suspected the *A. annua* polymethoxyflavones to have similar effects. Thus, three flavonoids, crysophenol D, crysyoplenitin and artemetin isolated from *A. annua* were tested against purified FabG, FabI and FabZ. Indeed, crysophenol D and artemetin inhibited all three enzymes (IC_{50}s 15 – 50 μg/mL). Crysoplenitin inhibits both FabI and FabZ (IC_{50}s 20 and 40 μg/mL), but is inactive against FabG. These findings identify the FAS-II enzymes as possible targets of *A. annua* flavonoids and provide logical explanations for their synergistic activity when combined with artemisinin. The inhibition of multiple enzymes from the same pathway is very useful, as it increases the efficacy of the drug and reduces the risk of resistance. Consequently, the combinations of polymethoxyflavonoids with artemisinin(s) might be a promising option for treating drug-resistant malaria. *Acknowledgements:* The financial support of MIUR (PRIN 2004) and Ente Cassa di Risparmio di Firenze are acknowledged. **References**: 1. Eckstein-Ludwig, U. *et al.* (2003), Nature 21: 957 – 961. 2. Elford, B.C. *et al.* (1987), Trans. R. Soc. Trop. Med. Hyg. 8: 434 – 436. 3. Waller, R.F. *et al.* (1998), Proc. Natl. Acad. Sci. USA 95: 12352 – 12357. 4. Kirmizibekmez, H. *et al.* (2004), Planta Med. 70: 711 – 717.

P 077

Essential oil of Turkish Origanum onites L. and its main components, carvacrol and thymol show potent antiprotozoal activity without cytotoxicity

Tasdemir D[1], Kaiser M[2], Demirci F[3], Baser KHC[3]
[1]Centre for Pharmacognosy and Phytotherapy, University of London, 29 – 39 Brunswick Square, London WC1N 1AX, United Kingdom; [2]Department of Medical Parasitology, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland; [3]Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, TR-26470, Eskisehir, Turkey

Origanum species (Lamiaceae) are natural floristic elements of Turkey and widely used as traditional medicines and flavor enhancers in foods [1]. Oregano herb is also an important commercial product of Turkey, where *Origanum onites* accounts for 80 % of all the oregano exports of the country. The plant is characterized by high yield of essential oil that contains very high amounts (up to 80 %) of carvacrol as major constituent [2]. In the continuation of our search for new antiparasitic agents from Turkish plants [3], we have investigated the *in vitro* activity of the essential oil of *Origanum onites* L. against several parasitic protozoa, namely *Trypanosoma brucei rhodesiense*, *Leishmania donovani* and *Plasmodium falciparum*. The essential oil was obtained via hydrodistillation from the dried herbal parts of *Origanum onites* and the analyses were performed on a GC and GC-MS system simultaneously. Carvacrol (70 %), linalool (9.7 %), *p*-cymene (7 %), gamma-terpinene (2 %), and thymol (1.7 %) were identified as main components. The oil showed very significant activity against *T. b. rhodesiense* (IC_{50} 186 ng/mL), and moderate antileishmanial and antiplasmodial effects (IC_{50} values 17.8 and 7.9 μg/mL, respectively). As a next step, the main constituent, carvacrol, but also its position isomer, thymol, were tested. Interestingly, both compounds have retained the same activities as observed for the oil. Furthermore, their trypanocidal activity was even stronger (IC_{50} value for thymol: 114 ng/mL, for carvacrol: 149 ng/mL). Since both the oil and its two components are devoid of cytotoxicity on mammalian L6 cells (IC_{50}s > 50 μg/mL), they are currently being evaluated on animal models for *in vivo* trypanocidal activity. **References**: 1. Baser, K.H.C. (2002), Oregano. Taylor & Francis. London. 2. Demirci, F. *et al.* (2004), J. Agric. Food. Chem. 52: 251 – 254. 3. Tasdemir, D. *et al.* (2005), Phytochemistry 66: 355 – 362.

P 078

Antinociceptive profile of ethyl acetate extract of Hyptis fruticosa Salzm. ex Benth. (Labiatae)

Marçal RM[1], Ptak DM[1], Krempser RR[1], Krempser MR[1], Floresta SV[1], Moreno MPN[2], Cavalcanti SCH[1], Fernandes JB[2]
[1]Physiology Department, Federal University of Sergipe, Av. Marechal Rondon, s/n; CEP 49.100 – 000, São Cristóvão, SE, Brazil; [2]Chemistry Department; Federal University of São Carlos, Rodovia Washington Luis, KM 235, CEP 13.0565 – 905, São Carlos, SP, Brazil

Hyptis fruticosa, an aromatic medicinal shrub, is commonly used in Brazilian folk medicine to soothe pain. In this work, the antinociceptive profile of the ethyl acetate extract of *H. fruticosa* (EA) was investigated on three different pain models: the acetic acid-induced writhing reaction [1], the hot-plate test [2], and the formalin test [3]. *H. fruticosa* leaves, collected in São Cristóvão (Brazil), were dried, pulverized and macerated (r.t.) in ethyl acetate (17.8 % yield). The exploratory HPLC show compounds at A (236; 276 nm), B (242nm; 275 nm), C (196; 202; 236; 276 nm), D (198; 214; 229; 262; 273 nm) and E (198; 214; 229; 262; 273 nm). Indomethacine (7.5 mg/kg; $p < 0.001$) and morphine (2.5 mg/kg; $p < 0.001$) were used as positive control in the writhing and the hot-plate tests, respectively. The EA (25 – 400 mg/kg) dose-dependently reduced writhing induced by acetic acid ($p > 0.05$ – $p < 0.001$) and increased the latency time in the hot-plate test ($p > 0.05$ – $p < 0.001$). The analgesic effect of EA was reversed by naloxone (3 mg/kg) in the hot-plate model. Naloxone (3.0 mg/kg) was ineffective in the hot-plate method. Morphine (7.5 mg/kg) reduced the pain reaction in both the early ($p < 0.001$) and the late ($p < 0.001$) phases of the formalin model. Ethyl acetate extract (100 mg/kg, $p < 0.05$ and 200 mg/kg; $p < 0.05$), like morphine, showed analgesic effects in both phases of the formalin test. In conclusion, the ethyl acetate extract of *Hyptis fruticosa* showed an opioid-like antinociceptive effect. *Acknowledgments*: CNPq. **References**: 1. Koster, R. et al (1959), Fed. Proc. 18: 412 – 418. 2. Ankier, S.I. (1974), Eur. J. Pharmacol. 27:1 – 4. 3. Hunskaar, S., Hole, K. (1987), Pain 30:103 – 114.

P 079

Antinociceptive profile of (+)-citronellol in experimental animals

Marçal RM[1], Salinas RAM[1], Paula SPS[1], Santos AB[1], Souza DP[2]
[1]Physiology Department, Federal University of Sergipe, Av. Marechal Rondon, s/n; CEP 49.100 – 000, São Cristóvão, SE, Brazil; [2]Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, Cidade Universitária, Campus I, CEP 58 059 – 900, João Pessoa, PB, Brazil

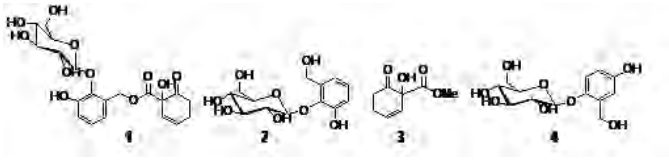
(+)-Citronellol, the natural occurring enantiomer monoterpene compound, is commonly found as a component of aromatic plants essential oil. Considering that several monotepenes have showed analgesic properties [1, 2], the aim of this study was to investigate the possible antinociceptive effect of (+)-citronellol on three different pain models, namely the acetic acid-induced writhing reaction [3], the hot-plate test [4], and the tail-flick model [5]. Indomethacine (7.5 mg/kg; *i.p.*; $p < 0.001$) and morphine (2.5 mg/kg; *i.p.*; $p < 0.001$) were used as positive control in the writhing and the hot-plate tests, respectively. (+)-Citronellol, at doses of 10, 25, and 50 mg/kg (s.c.), dose-dependently reduced the writhing induced by acetic acid ($p > 0.05$, $p < 0.01$ and $p < 0.001$, respectively) and increased the latency in the hot-plate test ($p > 0.05$, $p < 0.05$ and $p < 0.01$, respectively). The (+)-citronellol-induced antinociception (50 mg/kg; s.c.) in the hot-plate model was reversed by the opiate antagonist naloxone (3 mg/kg; *i.p.*). Naloxone (3.0 mg/kg; *i.p.*) was ineffective in the hot-plate method. Morphine (12 mg/kg; *i.p.*; $p < 0.001$) increased the reaction time in the tail-flick test. However, unlike morphine, (+)-citronellol did not manifest a significant effect in the tail-flick model (10 – 75 mg/kg; s.c.). In conclusion, (+)-citronellol showed analgesic properties, involving the opioid system activation in a supra-spinal, but not a spinal, site of action. Since it was effective in the acetic acid-induced writhing model, a peripheral antinociceptive effect can not be disregarded. *Acknowledgments*: CNPq. **References**: 1. Peana, A.T. *et al.*(2003), Eur. J. Pharmacol. 460: 37 – 41. 2. Santos, F.A., Rao, V.S.N. (2000), Phytother, Res, 14: 240 – 244. 3. Koster, R. *et al.* (1959), Fed. Proc. 18: 412 – 418. 4. Ankier, S.I. (1974) Eur. J. Pharmacol. 27: 1 – 4. 5. Langerman, L. *et al.* (1995), Pharmacol. Toxicol. 34: 23 – 27.

P 080

Effects of constituents from the fruits of Idesia polycarpa on lipopolysaccharide-induced nitric oxide production in BV2 microglial cells

Kim SH, Jeong EJ, Ha NR, Yang H, Hyun S, Young S, Kim C
College of Pharmacy, Seoul National University (a), San 56 – 1, Shillim-Dong, Kwanak-Gu, 151 – 742, Seoul, Korea

In our search for anti-inflammatory substances from natural products, we found that the total methanol extract of the fruits of *Idesia polycarpa* effectively inhibited nitric oxide (NO) production induced by lipopolysaccharide (LPS) in BV2 microglial cells [1]. Through the bioactivity-guided fractionation of this extract, four compounds (**1**~**4**), idescorpin (**1**), idesin (**2**), 1-hydroxy-6-oxocyclohex-2-enoic acid methyl ester (**3**) and salirepin (**4**), were isolated from the ethylacetate fraction. These compounds significantly inhibited LPS-induced NO production in BV2 microglial cells despite of their weak NO radical scavenging activity. Moreover, they showed significant inhibitory effect on inducible NO synthase (iNOS) activity without affecting iNOS expression as demonstrated by Western blot analysis.



Acknowledgements: Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, the Republic of Korea **Reference**: 1. Kim, S.H. *et al.* (2005), Org. Lett. 7: 3275 – 3277.

P 081

Investigation of anti-inflammatory activity of complex herbal oil extract in vitro and in vivo

Tikhonov VP[2], Rydlovskaya A[1], Makarov VG[1], Makarova MN[1], Pozharitskaya ON[1]
[1]Interregional Center "Adaptogen" Pyskarevsky pr.47/5, 195067, St.-Petersburg, Russia adaptogen@sp.ru; [2]Open joint-stock company "Diod", 11-A, ul. Derbenevaskaya, 115114, Moscow, Russia

Known from ancient China and India by their therapeutic qualities *Boswellia serrata* Roxb. and *Curcuma longa* L. now is an object of study of many scientists. Turmeric extract shows strong antioxidant activity and can inhibit COX enzymatic activity [1]. In one's turn, boswellic components inhibit 5-LOX activity [2] and NF-*k*B transcription factor [3]. As a result two perspective therapeutic nature substances work by different mechanisms of action and in sum can show synergistic effect. So we pooled dry extract of *B. serrata* resign (contented 85 % of boswelic acids in sum) with oil extract of *C. longa* roots in ratio 1/9. This product was named as BsCl. Using of mononuclear cells of healthy donor's blood we showed that BsCl (125 mkg/mL) had an ability to inhibit LPS-inducible production of pro-inflammatory cytokines (TNFα, IL-1β) on 40 – 50 %. BsCl showed some antiradical activity: it had 0.15 ± 0.004 of trolox equivalent antioxidant capacity in relation to HO. More effective BsCl was in relation to LOO°: 2.6 mkg/mL of BsCl decreased MDA concentration in blood plasma in 2.5 times. Using model of adjuvant arthritis on Wistar rats we showed high anti-inflammatory action of BsCl *in vivo*. 300 mg/kg of BsCl effectively and comparable by force with voltaren (8 mg/kg) and prednisolon (10 mg/kg) decreased edema,

temperature and ulcer formation on infected rat paws, normalized biochemical indexes (ESR, level of fibrinogen, sialic acids and leukocytes in blood) and decreased TNFα level in blood plasma to zero. Thus we determined high anti-inflammatory action of BsCl not only *in vitro* but *in vivo* too. **References**: 1. Strasser, E.M., Wessner, B. (2005), Biochem, Pharmacol. 70: 552 – 559. 2. Sailer, E., Schweizer, S. (1998), Eur. J. Biochem. 256: 364 – 368. 3. Syrovets, T., Buchele, B. (2005), J. Immunol. 174: 498 – 506.

P 082

Cytotoxic and apoptosis–inducing activity of ramentaceone – a naphthoquinone from Drosera sp

Kawiak A[1], Wasilewska A[1], Stasilojc G[2], Stobiecki M[3], Bigda J[2], Lojkowska E[1]
[1]Department of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Kladki 24, 80 – 822, Gdansk, Poland; [2]Department of Cell Biology, Intercollegiate Faculty of Biotechnology, University of Gdansk & Medical University of Gdansk, Debinki 1, 80 – 211, Gdansk, Poland; [3]Institute of Bioorganic Chemistry PAS, Noskowskiego 12/14, 61 – 704, Poznan, Poland

Naphthoquinones represent a group of compounds, which exhibit various biological activities including anticancer properties. The objective of this research was to evaluate the cytotoxic activity of ramentaceone (7-methyljugone) isolated from *Drosera* sp. and determine whether cell death induced by this compound is mediated through the induction of apoptosis. Ramentaceone exhibited high cytotoxic activity against various human tumor cell lines, with the highest activity observed against leukemic lines HL-60 and U937 (IC_{50} 1.5 μg/mL).The mode of cell death induced by ramentaceone was evaluated using the HL-60 cell line. Typical morphological features of cells undergoing apoptosis were examined, such as cell shrinkage, nuclear condensation and DNA fragmentation. The treatment of HL-60 cells with ramentaceone induced an increase in the sub-diploid DNA content. A loss in membrane phospholipids asymmetry determined by the externalization of phosphatidylserine as well as a loss in mitochondrial membrane potential ($\Delta\psi m$) were observed upon the treatment of cells with ramentaceone. Naphthoquinones are known redox cycling agents, therefore the generation of reactive oxygen species by ramentaceone was evaluated in HL-60 cells. To determine whether the induction of cell death by ramentaceone is mediated through the generation of ROS, cells were pretreated with a free radical scavenger N-acetylcysteine (NAC). NAC reversed the toxicity of ramentaceone as well as prevented the induction of DNA fragmentation in ramentaceone-treated cells pointing out to the involvement of ROS generation in the mechanism of cell death induced by ramentaceone. *Acknowledgements*: Funding from Grant No. BW/B051 – 5-00620 – 6, The Foundation for the Development of Gdansk University and The Integrated Regional Operational Programme (IROP).

P 083

Antibacterial activity of Nigella sativa seed essential oil and effect of different extraction methods on content of its active principle, thymoquinone

Kokoška L[1], Havlik J[1], Valterova I[2], Sovova H[3], Sajfrtova M[3], Marsik P[2]
[1]Institute of Tropics and Subtropics, Czech University of Agriculture Prague, Kamycka 129, 165 21 Prague 6, Czech Republic; [2]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 166 10, Prague 6, Czech Republic; [3]Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, Rozvojova 2, 165 02 Prague 6-Suchdol, Czech Republic

Nigella sativa L. is used in folk medicine all over the world for the treatment of a number of diseases. Its seed essential oil (EO) has previously demonstrated a wide range of biological activities, including antimicrobial effect [1]. In our study, we aimed to determine the influence of four different extraction methods on the chemical composition and antimicrobial activity of *N. sativa* seed EO, as well as on the content of its active principle, thymoquinone. EOs extracted by hydrodistillation (HD), dry steam distillation (SD), steam distillation of crude oils obtained by solvent extraction (SE/SD), and supercritical fluid extraction (SFE/SD) were tested for their antibacterial activities using broth microdilution method [2] against *Bacillus cereus*, *B. subtilis*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, and *Streptococcus pyogenes*. All samples were subsequently analyzed by gas chromatography and gas chromatography-mass spectrometry. The results showed that the EOs obtained by HD and SD were dominated by *p*-cymene, whereas the major constituent identified in both volatile fractions obtained by SD of extracted oils was thymoquinone (ranging between 0.36 and 0.38 mg/mL, whereas in oils obtained by HD and SD it constituted only 0.03 and 0.05 mg/mL). Both oils distilled directly from seeds showed lower antimicrobial activity (MICs ≥ 512 and 256 μg/mL for HD and SD, respectively) than those obtained by SE/SD and SFE/SD (MICs ≥ 4 μg/mL). Thymoquinone exhibited potent growth-inhibiting activity against Gram-positive bacteria with MICs ranging from 8 to 512 μg/mL. *Acknowledgements:* This research was supported by projects GACR 104/06/1174 and GA AV Z4 055 0506. **References**: 1. Ali, B.H., Blunden G. (2003), Phytother. Res. 17: 299 – 305. 2. Jorgensen, J.H. *et al.* (1999), Manual of Clinical Microbiology, Murray, P.R. ed., ASM Press, Washington, DC.

P 084

Pharmacophore modelling on the apoptosis regulating target XIAP-Bir3

Bliem CB[1], Schyschka L[2], Rollinger JM[1], Langer T[1], Vollmar AM[2], Stuppner H[1]
[1]Institute of Pharmacy / Pharmacognosy / CAMD; CMBI, Leopold-Franzens University, 6020 Innsbruck, Austria; [2]Department of Pharmacy, Center of drug research pharmaceutical biology, Ludwig-Maximilians University, 81377 Munich, Germany

XIAP (X-linked inhibitor of apoptosis protein) has been identified to be an endogenous protein that regulates the activity of both initiator (caspase-9) and effector caspases (caspase-3 and-7) and has therefore emerged as promising therapeutic target in cancer therapy [1]. The aim of our study was to generate a reliable pharmacophore model for small drug-like molecules binding at the Bir3 domain of XIAP at the same groove where endogenous Smac (second mitochondria-derived activator of caspases) and caspase-9 are binding. A GRID based pharmacophore model was recently published by Ortuso *et. al.* [2]. Since this model showed only poor selectivity it was further optimized using the ligand target interactions of the natural ligand Smac and its analogue (PDB entries 1G3F, 1TFQ, resp.; [3]). The refined model was validated by means of a small database containing 30 compounds with known inhibitory effects on the Bir3 domain; 29 out of 30 structures could be found by our hypothesis. Subsequently a virtual screening filtering experiment of commercial databases was performed revealing hit rates from 0.17 to 2.07 % depending on the used libraries. Fit values and docking experiments aided in the final selection of promising test candidates which were successfully evaluated for their ability to enhance apoptosis of Jurkat and XIAP overexpressing Jurkat cells affording the proof of concept. Evidence is given that natural products can be found by the pharmacophore model because embelin, a known natural product inhibitor of XIAP [4], could be retrieved from commercial databases. Our intention is to apply this validated *in silico* tool for the virtual screening of our in house natural product database. Thus, we hope to identify new lead structures from natural sources in a target-oriented way able to interact with XIAP in the apoptotic pathway. **References**: 1. Liston, P. *et al.* (2003), Oncogene 22: 8568 – 8580. 2. Ortuso, F. *et al.* (2006), Bioinformatics, doi: 10.1093/bioinformatics/btl115. 3. Berman, H. *et al.* (2000), Nucleic Acids Res. 28: 235 – 242. 4. Nikolovska-Coleska, Z. *et al.* (2004), J. Med. Chem. 47: 2430 – 2440.

P 085

Antimutagenic effects of ethanolic extracts from three Palestinian medicinal plants

Khader M, Eckl PM, Bresgen N
Department of Cell Biology, University of Salzburg, Hellbrunnerstrasse 34, Salzburg, A-5020, Austria

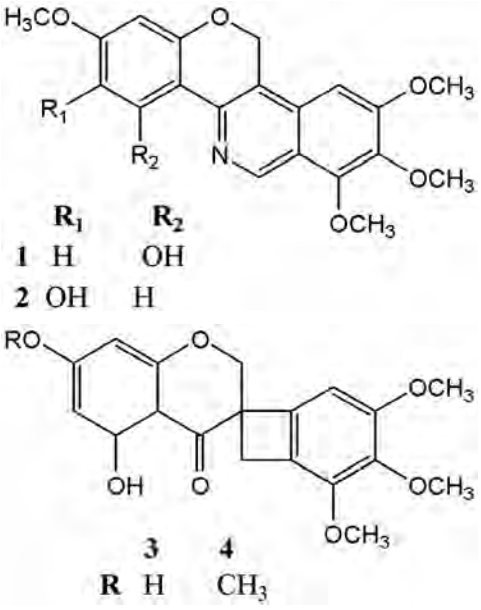
Eryngium creticum L., *Nigella sativa* L., and *Teucrium polium* L. have been traditionally used for the treatment of inflammations, liver disorders, and arthritis. Several studies on *N. sativa* revealed antioxidant, anti-inflammatory, hepatoprotective and antimutagenic activities. *T. polium* is reported to have antioxidant, anti-inflammatory, and antiulcerogenic activities, while *Eryngium* species are considered to have antioxidant and anti-inflammatory properties. In this study the antimutagenic activity of these plant species was tested in rat hepatocyte primary cultures by treatment with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), a directly acting mutagen, which methylates DNA and was shown to induce massive chromosomal damage in hepatocytes [1]. Since it cannot be excluded that the active constituents of the plant extracts require biotransformation or induce metabolic enzymes, causing antimutagenic or detoxifying effects, the present investigation was carried out with metabolically competent primary cultures of rat hepatocytes. Rat hepatocytes were isolated as described by Michalopoulos *et al.* [2]. Establishment of primary cultures and cytogenetic studies were performed according to Eckl *et al.* [1]. Plant ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO). Antimutagenicity testing was done in three modes: pre-treatment, combined treatment and post-treatment of the primary cultures with plant extracts and MNNG. Therefore, both the induction of metabolizing enzymes, direct interaction of plant constituents with the mutagen and increased recovery, *i. e.* enhanced repair of induced DNA damage can be evaluated. Student's double sided t-test for independent samples was used to evaluate the levels of significance. The results of our investigation clearly indicate an inhibitory effect on MNNG mutagenicity by the three plant extracts, and this effect can be attributed to a direct antimutagenic activity and an increased recovery. *Acknowledgments:* This investigation was supported by a stipend of the Austrian Exchange Service (OEAD) **References**: 1. Eckl, P.M. *et al.* (1987), Carcenogenisis 8 (8):1077 – 1083. 2. Michalopoulos, G. *et al.* (1982), Cancer Res. 42:4673 – 4682.

P 086

Secondary metabolites from Drimiopsis baterrii

Ngamga D[1], Tane P[1], Bezabih M[2], Abegaz BM[2]
[1]Chemistry Department, University of Dschang, Box 67, Dschang, Cameroon; [2]Department of Chemistry, Faculty of Science, University of Botswana, P.O. Box UB00704, Gaborone, Botswana

The genus *Drimiopsis* Lindl. (Hyacinthaceae) is endemic to sub-Saharan Africa, where it is represented by approximately 20 species [1]. *Drimiopsis barterii* Bak is the only specie of the genus which occurs in Cameroon. The plant is used by the Bamileke people of the Western province of Cameroon to treat fever. In a continuation of our search for bioactive compounds from natural source [2], a methylene chloride-methanol (1:1) extract of *Drimiopsis barterii* (whole plant) was investigated. Two new alkaloids (Drimiopsine A (**1**) and B(**2**)) and nine homoisoflavonids with two new structures (**3**, **4**) were isolated.The structure of the compounds were established by MS, 1D and 2D spectroscopy including DEPT, COSY, HMQC and HMBC experiments.



Acknowledgements: The authors gratefully acknowledge financial support from the African Network of Scientific and Technological Institutions (ANSTI) and the international program in the chemical Sciences (IPICS) through the Network for Analytical and Bioassay Services in Africa (NABSA). **Reference**: 1. Ngamga, D. *et al.* (2005), Zeitschrift für Naturforschung B 60b: 973 – 977.

P 087

In vitro and in vivo immunomodulatory activity of aqueous extract of Clerodendrum serratum L. roots

Juvekar AR, Nachankar RS, Hole RC, Wakade AS, Kulkarni MP, Ambaye RY
Department of Pharmaceutical Sciences and Technology, Mumbai University Institute of Chemical Technology, Nathalal Parikh Marg, Matunga, Mumbai-400 019, India

The aqueous extract of *Clerodendrum serratum* Linn (CSAQ) roots has been investigated for its immunomodulatory activity. The phytochemical screening revealed presence of D-mannitol, stigma sterols, three triterpenoids/ oleionolic acid, queretaric acid and cerratagenic acid. The CSAQ was examined for the ability to induce secretory and cellular responses in murine peritoneal macrophages. Macrophages treated with extract exhibited increased acid phosphatase and myeloperoxidase activity as well as significant increase in the production of nitric oxide (NO), hydrogen peroxide (H_2O_2), and O_2. Hence, *in vivo* studies were carried to confirm the immunomodulatory activity of CSAQ in mice. Administration of CSAQ at doses of 100 and 200 mg/kg *p. o.* significantly increased in total leukocyte count and in weight of spleen indicating an uplift of innate immunity. It has significantly increased carbon clearance index and ovalbumin induced delayed type hypersensitivity (DTH) reactions. It also produced a significant increase serum globulin content and specific antibody titer against ovalbumin. Treatment with CSAQ increased the number of bone marrow cells positive for nonspecific esterase and peroxidase activity In conclusion CSAQ has shown to stimulate both innate and adaptive immune response either through stimulation of macrophages or through stimulating the release of factors that are involved in proliferation of bone marrow cells. **References**: 1. Choi, E.M., Hwang, J.K. (2002), Fitoterapia 73: 629 – 637. 2. Kim, K.I., Shin K.S. *et al.* (2001), Biosci. Biotechnol. Biochem. 65: 2369 – 2377.

P 088

Carvacrol as the inhibitor of cyclooxygenase-1 and -2, the key enzymes of prostaglandin biosynthesis: in vitro assays

Marsik P[1], Landa P[1], Pribylova M[1], Vanek T[1], Kokoška L[2]
[1]Department of Plant Tissue Cultures, Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo n. 2, 166 10 Prague 6, Czech Republic; [2]Department of Crop Sciences and Agroforestry, Institute of Tropics and Subtropics, Czech University of Agriculture Prague, Prague, Czech Republic

Carvacrol is a phenolic monoterpene which is one of the most abundant constituents in essential oil of many aromatic plants such as oregano (*Origanum vulgare* L.), savory (*Satureja thymbra* L.), thyme (*Thymus vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), fennel (*Foeniculum vulgare* L.) and black cumin (*Nigella sativa* L.). These species are widely used in traditional medicine against various microbial diseases and gastrointestinal and inflammatory disorders. Antimicrobial, antiangiogenic, antioxidative and analgesic activity of carvacrol was also confirmed by recent studies [1, 2]. Carvacrol is closely related to other phenolic monoterpenes as thymol or eugenol, which anti-inflammatory effects have been published [3]. The inhibition of cyclooxygenase-1 (COX-1) and – 2 (COX-2) enzymatic activities by carvacrol is reported here. The anti-inflammatory assay was based on inhibition of activity of COX-1 and – 2, which catalyzes prostaglandin biosynthesis from [^{14}C] radioactive arachidonic acid. The inhibition was monitored as concentration of prostaglandin E_2 and D_2, the biosynthetic products of the COX reaction. The identification and quantification of the metabolites were performed by HPLC on C18 reversed phase column with an on-line radioactivity flow detector. IC_{50} values and percentage inhibition of different carvacrol concentrations were compared with COX inhibitors indomethacin and NS-398 as control samples. Student's two tailed t-test was employed for calculation of statistical significance and IC_{50} values were determined by regression analysis. Carvacrol as well as other inhibitors showed similar inhibition activity against COX-1 and -2. IC_{50} were almost identical for all tested substances. Inhibition effect of carvacrol (IC_{50} = 0.7) as well as indomethacin (IC_{50} = 0.6) on COX-1 was stronger in comparison with COX-2 (IC_{50} = 0.9 for both inhibitors). These results probably indicate non-specific but relatively strong inhibition of COX activity by carvacrol. *Acknowledgements:* This work was supported by 1P04OC926.001 research project and Z4055 0506 project **References**: 1. Faleiro, L. *et al.* (2005), J. Agr. Food Chem. 53: 8162 – 8168. 2. Aydin, S. *et al.* (1996), Phytoter. Res. 10: 342 – 344. 3. Marsik, *et al.* (2005) Planta Med. 71: 739 – 742.

P 089

COX-1 and COX-2 inhibitory activity of extracts produced from organic waste materials

Wenzig E[1], Erler J[2], Bauer R[1]
[1]Institute of Pharmaceutical Sciences, Dept. Pharmacognosy, University of Graz, Universitaetsplatz 4, 8010 Graz, Austria; [2]Bionorica AG, Kerschensteinerstrasse 11 – 15, 92318 Neumarkt, Germany

The aim of the European project SAFEWASTES is the processing of organic waste materials from the food, feed and pharmaceutical industry to high added value products of preventive or therapeutic potential for livestock or humans. As a part of the *in vitro* testings performed within this project, we screened aqueous, hydroethanolic and lipophilic extracts from 12 different waste materials for their inhibitory activity against the enzymes COX-1 and COX-2, which catalyze the first two steps of prostaglandin formation in the arachidonic acid cascade. From the 30 extracts tested up to now, a lipophilic extract from preextracted willow bark (*Salix sp.)* showed the highest inhibitory activity against the two isoenzymes, with IC_{50} values of 4.72 µg/mL against COX-1 and 1.86 µg/mL against COX-2. The aqueous extract of this material was inactive, and the ethanolic extract only showed moderate activity at the screening concentration of 20 µg/mL. In order to find out, whether the high activity of the extract is caused by the presence of high amounts of free fatty acids, which are known to possess a certain COX-1- and a quite strong COX-2 inhibitory activity *in vitro*, the free fatty acids in the extract were quantified by GC-FID. 3.02 % linoleic acid, 0.95 % oleic acid, 2.08 % palmitic acid and 0.12 % stearic acid were found in the extract. Comparison of the fatty acid concentration present in the assay mixture at the extract's IC_{50} with the IC_{50} values of the pure fatty acids [1] led to the conclusion that the free fatty acids do not significantly contribute to the COX-1 and COX-2 inhibitory activity of this extract. The bioassay guided fractionation of the extract in order to isolate the active principle is under progress. *Acknowledgements:* This project is supported by funding under the Sixth Research Framework Programme of the European Union. **Reference**: 1. Reininger, E.A., Bauer, R. (2006), Phytomedicine 13: 164 – 169.

P 090

Evaluation of the antiherpetical activities of Sideritis perfoliata L. subsp. perfoliata (Lamiaceae)

Lazari DM, Sylignaki GI, Matta MK, Panagiotidis CA
Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, GR 54 124 Thessaloniki Greece

Herpes simplex viruses (HSV) are ubiquitous pathogens which cause a variety of diseases ranging in severity from mild to severe, and in certain cases, they can even become life threatening, especially in immunocompromized patients. HSV becomes latent mainly in trigeminal ganglia, after primary infection, and persists for the lifetime of the host with periodic reactivations. Nucleoside analogues, such as aciclovir (ACV), are the only approved drugs for the treatment of HSV infections. However, the widespread use of nucleoside-based drugs has led to the emergence of resistance in HSV. Medicinal plants have been traditionally used for different kinds of ailments including infectious diseases. Continuing our chemotaxonomic examinations of the Greek flora belonging to Lamiaceae and our search for new compounds of pharmacological interest, we evaluate the aerial parts of *Sideritis perfoliata* subsp. *perfoliata* (a plant widely used in folk medicine in Greece since antiquity because of its antibacterial, anti-inflammatory, antirheumatic, anti-ulcer, digestive, and vaso-protective properties), for their virucidal activity or their abilities to inhibit HSV-1 propagation. Air-dried and powdered aerial parts of the above mentioned plant were extracted at room temperature with a series of solvents of increasing polarity, petroleum ether, CH_2Cl_2, MeOH, mixture of MeOH-H_2O 1:1 and H_2O. The dried extracts were dissolved in DMSO and tested for their ability to inhibit infection or delay the virus lytic cycle. Anti-HSV activities were found in dichloromethane extract. Bioguided fractionation of this extract led to the isolation of three active substances, which the mechanism of action is being evaluated.

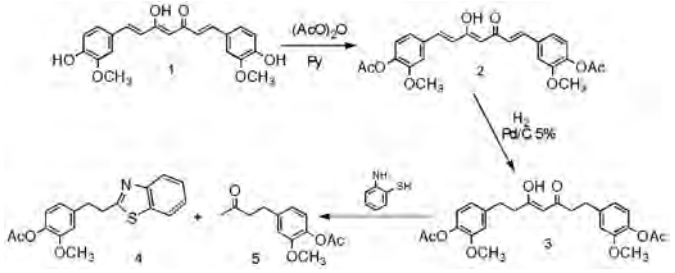
P 091

Synthesis and biological activity of a new benzothiazol derivative of curcumin

Lozada MC[1], Avila EV[2], Montiel JL[3], Villarreal ML[3], Enríquez RG[4], Gnecco D[5]
[1]Departamento de Sistemas Biológicos, UAM-Xochimilco, Calzada del Hueso 1100, Villa Quietud, Coyoacán, 04960, D. F, México; [2]Departamento de Ciencias de la Salud, UAM-Iztapalapa, Av. San Rafael Atlixco 186, Vicentina, Iztapalapa, 09340, D. F, México; [3]Universidad Autónoma del Estado de Morelos Av. Universidad 1001, 62210, Morelos, México; [4]Instituto de Química, UNAM, Cd. Universitaria, Coyoacán, 04510, D. F, México; [5]Centro de Química, Instituto de Ciencias, BUAP, 72000, Puebla, México

Recently much attention has been focused in the research of curcumin, a secondary metabolite isolated from *Curcuma longa* L. and from other species of Curcuma. Current investigation of curcumin is fastly increasing due to its biological activities (anti-inflammatory, [1] antioxidant, anti-HIV, [2] including cytotoxic effects on several cancer line cells [3, 4] and upon cystic fibrosis [5]). A benzothiazol derivative of curcumin was obtained by systematic structural modification of curcumin (Scheme)**.** We have described pre-

viously the synthesis of new heterocyclic derivatives of curcumin including the compound **4** [6].



The benzothiazol **4** was prepared by the reaction of **3** and 2-aminobenzenethiol. During the ring formation a part of the molecule of curcumin suffered fragmentation. The structure of **4** was assessed by spectroscopic methods (IR, 1D and 2D NMR, mass spectrometry), also the crystal structure was analyzed by X-ray crystallography. In our preliminary biological studies, compound **4** has shown important cytotoxic effect toward a nasopharyngeal carcinoma cell line KB (ED_{50} = 3.38 μg/mL) as well as modification of the percentage of cells on cell cycle phases of the monocytic human cell line TPH1. *Acknowledgements*: CONACYT of México (37821-N and 40959-Q); DGAPA of UNAM (IN232202). **References**: 1. Ali, M., *et al.* (1995), Ind. J. Chem. 34B: 884. 2. Artico, M., *et al.* (1998), J. Med. Chem., 41: 3948. 3. Huang, M.T., *et al.* (1992), ACS Symposium Series 507: 338. 4. Ishida, J. *et al.* (2002), Biorg. Med. Chem. 10: 3481. 5. Egan, M. F. *et al.* (2004), Science, 304: 600. 6. M. Concepción Lozada, *et al.*, (2004), Heterocycles 65: 49.

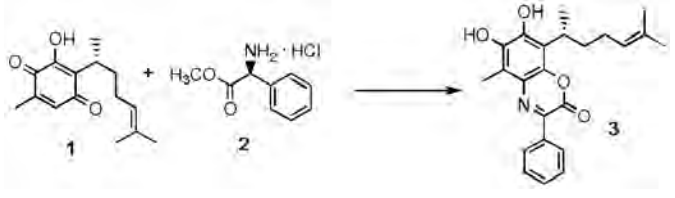
P 092

A potent cytotoxic semisynthetic derivative of perezone with phenylglycine

Enríquez RG[1], Alonso-Cortés D[2], Lozada MC[3], Avila EV[4], Montiel JL[5], Reynolds WF[6], Villarreal ML[5]

[1]Instituto de Química, UNAM, Cd. Universitaria, Coyoacán, 04510, México, D. F; [2]Centro de Investigación Biomédica del Sur IMSS, Argentina No 1, Xochitepec, Morelos México; [3]Departamento de Sistemas Biológicos, UAM-Xochimilco, Calzada del Hueso 1100, Villa Quietud, Coyoacán, 04960, México, D.F; [4]Departamento de Ciencias Biológicas, UAM-Iztapalapa, Av. San Rafael Atlixco 186, Vicentina, Iztapalapa, 09340, D. F, México; [5]Universidad Autónoma del Estado de Morelos Av. Universidad 1001, 62210, Morelos, México; [6] Deparment of Chemistry, University of Toronto, Ontario Canada

Perezone, is a naturally occurring quinone, first isolated in México (1852) from *Perezia* species and used in traditional medicine. We have isolated perezone from *Perezia cuernavacana*. This compound was reacted with methyl esthers of various amino acids. In particular, the phenylglycine esther derivative showed important cytotoxic activities against ovarian (ED_{50}= 4.0 μg/mL), renal (ED_{50}= 0.86 μg/mL), colon (ED_{50}= 0.82 μg/mL) and nasopharyngeal (ED_{50}= 4.0 μg/mL) cancer cell lines [1]. The chemical structure of this derivative was assessed by ^{13}C an ^{1}H NMR spectroscopy and X-ray crystallography [2]. The reaction scheme is given below



A biological screening has shown modification of the percentage of cells on cell cycle phases of the monocytic human cell line TPH1. *Acknowledgements*: to: CONACYT of México (37821-N and 40959-Q); DGAPA of UNAM (IN232202). **References**: 1. Alonso, D. (2003), M.Sc. Thesis. Cytotoxic effect of perezone derivatives. UAEM, Cuernavaca, Morelos. 2. Alducin, E. (1997), M.Sc. thesis. Analytic study of the reactivity of perezone. New heterocyclic derivatives of sulfur and nitrogen. UNAM, México.

P 093

Histopathologic effects of Stachytarpheta jamaicensis (L.)Vahl. on Wistar rats

Ataman JE[1], Idu M[2], Odia EA[2], Omogbai EKI[3], Amaechina F[3], Akhigbe AO[4], Ebite LE[5]

[1]Department of Anatomy, University of Benin, PMB 1154, Benin City, Nigeria; [2]Department of Botany, University of Benin, 1154, Benin City, Nigeria; [3]Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria; [4]Department of Radiology, University of Benin, PMB 1154, Benin City, Nigeria; [5]Department of Anatomy, Delta State University, Abraka, Delta State. Nigeria

The toxicity of powdered *Stachytarpheta jamaicensis* (L.)Vahl. leaves, known for treating different ailments such as diabetes, hypertension and bacterial infections [1] in some Nigerian communities, was investigated in rats to helps in determining the upper limits of administration [2]. Twenty Wister rats (male and female) were fed with different graded mixtures of Pfizer feed mash and the leaf powder. The animals were weighed and divided into four groups of three treatment groups and one control group with each group consisting of five rats. The rats were administered different concentrations of powdered *S. jamaicensis* leaves mixed with different amount of feed mash *i.e.* 75, 50 and 25 g of *S. jamaicensis* was mixed with 25, 50, and 75 g of normal feed mash. The control was fed only with feed mash all through the period of experiment. The results obtained showed slight variation on the physical signs/body appearance of the animals and mild histopathlogic 1esions such as congestion, fatty changes and necrosis in selective tissues such as the liver, blood vessels, kidney, lung and testis but the brain, eyes, intestines (small and large) and heart tissues were essentially normal. *S. jamaicensis* seem to cause mild non-dose dependent systemic toxicity in some specific tissues. **References**: 1.. Bonati, A., (1993), J. Ethnopharmacol. 2:167 – 171. 2. Sofowora, A., (1993), Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, pp 58 – 196.

P 094

Prediction of bioavailability of phenolic acids by potentiometric titration method and chromatographic techniques

Mornar A, Jasprica I, Medić-Šarić M

Department of Medicinal Chemistry, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

Early screening of physico-chemical and ADME (Absorption, Distribution, Metabolism and Elimination) properties has become the key interest in drug discovery. Molecular lipophilicity is a major physico-chemical property, which describes oral absorption, cell uptake, protein binding, blood-brain penetration and metabolism of the bioactive substances. Phenolic acids (a group of molecules present in majority of plants) exhibit protective effects against many diseases, but whether they can reach their sites of action, particularly in humans, is largely unknown. The aim of our work was to investigate several ADME parameters of 8 phenolic acids by chromatographic techniques (RP-TLC and RP-HPLC) and potentiometric titration method. Nowadays chromatography has a tendency to replace tedious „shake-flask" method for measuring lipophilicity. The TLC measurements were performed on 10 x 20 cm glass plates precoated with RP-$18F_{254s}$. The HPLC measurements were performed using Agilent 1100 LC System, with ZORBAX SB-C18, 4.6 x 150 mm, 3.5 μm particle size column. The binary solvent system, methanol–phosphate buffer (pH = 2.5), was used as a mobile phase in both techniques with a varying content of organic modifier (80 – 5 %). The potentiometric titration method was chosen to get detailed informa-

tion on the partitioning characteristics of target compounds at all pH values. pK_a values and lipophilic pH-profiles were determined using Sirius instrument GLpKa. Linear regression has shown good correlation between $R_{M(TLC)}$ and log$k_{w(HPLC)}$ values (r = 0.96). The correlation between experimental data and ADME parameters predicted by different computer programs was considered in order to evaluate the predictive power of the theoretical approaches applied to predict the lipophilicity of phenolic acids. Moderate correlations (r < 0.90) between experimental and theoretical values demand constant evaluation of calculated data validity.

P 095

Evaluation of anti-nociceptive effect of methanolic extract of Sambucus nigra leaves using Formalin test and Tail-Flick test models

Faizi M[1], Shafaghi B[1], Kamalinejad M[2]
[1]Department of Pharmacology and Toxicology, School of Pharmacy, SBMU, P.O.BOX: 14155 – 6153, Tehran, Iran; [2]Department of Pharmacognosy, School of Pharmacy, SBMU, P.O.BOX:14155 – 6153, Tehran, Iran

The interest in plant sources has increased during the last decades in order to obtain new pharmaceutically active compounds. There are many reports on analgesic and anti-inflammatory effects of some plants in traditional medicine. Considering the fact that there are some ambiguities and many difficulties to control pain in todays medicine, traditional medicine could be considered as a valuable source to find new analgesic and anti-inflammatory agents. In Iranian traditional medicine, Elder berry (*Sambucus nigra* L.) has been reported to have analgesic and anti-inflammatory effects. In the present study the anti-nociceptive effect of the above mentioned plant has been studied. For evaluating the anti-nociceptive or analgesic effect of this plant, Formalin test and Tail-Flick test have been employed in order to evaluate the effect of Elder berry extract on chronic and acute pain in male Sprague-dawley rats and male NMRI mice respectively. In this study the methanolic extract of the leaf with HRT (Herbal to Extract Ratio) of 10 % has been used. Anti-nociceptive effect of this extract has been compared with anti-nociceptive effect of a standard non-steroidal anti-inflamatory and analgesic drug (Sodium salicylate) by using one-way ANOVA and Tukey HSD. Extracts of Elder leaves at 200 mg/kg and 400 mg/kg doses has significant effect on acute and chronic pain. The induced analgesia by extract of this plant is not mediated by the opioid system since naloxon can not prevent the anti-nociceptive effect. The anti-nociceptive effect of this extract at 200 mg/kg dose is estimated to bioequivalent with 300 mg/kg of sodium salicylate. It has been suggested that this effect of the extract probably is produced by interaction of active components on prostaglandins.

P 096

Antioxidant activity of Galinsoga parviflora and Galinsoga quadriradiata

Derwińska M, Bazylko A, Kowalski J
Departament of Pharmacognosy,Medical University,1 Banacha St, 02 – 097 Warsaw, Poland

Galinsoga sp. is native to Mexico and South America. It has become naturalized and occurs as widespread weed in different areas whole over the world. It grows in gardens, crop fields and along roadsides. Galinsoga sp. is used in traditional medicine in Poland for treatment dermatitis. The aim of our study was to determine antioxidant activity of different fractions of Galinsoga parviflora and Galinsoga quadriradiata. Water-methanolic extracts (50:50) were fractionated successively by SPE-columns with water, 20 % methanol, 50 % methanol, 70 % methanol and pure methanol at the end. Antioxidant properties of each fraction were tested using three assays: DPPH photometric assay, xanthine oxidase assay and linoleic acid peroxidation assay. Mentioned above assays demonstrated the 50 % methanolic fractions and 20 % methanolic fractions of both species were the most active. Phytochemical determinations using TLC and HPLC showed that those fractions were rich of flavonoids.

P 097

Neuroprotective effect of methanol extract of Smilacis chinae rhizome on NMDA-induced neurotoxicity and cerebral ischemia in rats

Seonga YH, Bana JY, Songb KS
[1]College of Veterinary Medicine and Research Institute of Herbal Medicine, Chungbuk National University, Cheongju, Chungbuk 361 – 763,South Korea; [2]College of Agriculture and Life-Sciences, Kyungpook National University, Daegu, 702 – 701, South Korea

Smilax has various pharmacological effects including antiiflammatory, anticancer and antioxidant activity. We previously reported that *Smilacis chinae* rhizome from *Smilax china* L. (Liliaceae) inhibits amyloid β protein (25 – 35)-induced neurotoxicity in cultured rat cortical neurons [1]. The present study aims to investigate the effect of the methanol extract of *Smilacis chinae* rhizome (SCR) on N-methyl-D-aspartate (NMDA)-induced neurotoxicity in cultured rat cortical neurons. CSR, over a concentration range of 5 to 50 μg/mL, inhibited NMDA (1 mM)-induced neuronal cell death, which was measured by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. Pretreatment of CSR (50 μg/mL) inhibited NMDA (1 mM)-induced elevation of cytosolic calcium concentration ($[Ca^{2+}]_c$), which was measured by a fluorescent dye, Fluo 4-AM, and generation of reactive oxygen species (ROS). Furthermore, in middle cerebral artery occlusion model in male SD rats, SCR (30 and 50 mg/kg) potentially reduced the transient ischemia-induced cerebral infarct volume. These neuroprotective effects of SCR were mimicked by MK-801, an NMDA receptor antagonist. These findings suggest that SCR has a possible therapeutic role in neurodegenerative diseases such as Alzheimer's disease and stroke. *Acknowledgements*: This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea. **Reference**: 1. Ban, J.Y., Cho, S.O. *et al.* (2006), J. Ethnopharmacol. 106: 230 – 237.

P 098

Isolation, characterization and anti-inflammatory activity of pectin from common pondweed Potamogeton natans L

Popov SV, Popova GY, Ovodova RG, Ovodov YS
Institute of Physiology, Komi Science Centre, The Urals Branch of the Russian Academy of Sciences, 50, Pervomaiskaya str, 167982 Syktyvkar, Russia

The pectic polysaccharide named potamogetonan PN was obtained using extraction of the floating leaf of the aquatic plant *P. natans* L. (Potamogetanaceae) by 0.7 % aqueous ammonium oxalate and subsequent precipitation with ethanol. The polysaccharide obtained (yield 4 %) proved to compose mainly of D-galacturonic acid (82 %) mainly altogether with the minor residues of galactose (1.7 %), rhamnose (0.8 %), arabinose (1.4 %) and glucose (1.1 %). Potamogetonan PN was shown to contain sugar chains of molecular weights more than 300 kDa (78 %) as proved by membrane ultrafiltration. Anti-inflammatory capacity of potamogetonan was assessed in the carrageenan paw edema test in mice. Oral administration of PN 24 h prior to induction of inflammation was found to reduce the edema formation in a dose-related manner. The maximal effect of PN (50 mg/kg) was observed at 1 h after carrageenan injection (80 % reduction of footpad swelling) and was comparable with that of indomethacin (50 mg/kg, p. o.) The delayed edema (5 h) was less affected by the preadministration of PN (33 % reduction). Potamogetonan was shown to inhibit spontaneous and phorbol-12-myristate-13-acetate-activated adhesion of peritoneal leukocytes *in vitro*. Thus, pectin (potamogetonan PN) was isolated from *P.natans* and was found to possess strong preventive anti-inflammatory activity especially in relation to initial phase of inflammation development.

P 099

Formation of supramolecular structures of alkylamides from Echinacea – implications for cannabinoid type-2 receptor (CB_2) interactions in vitro

Raduner S[1], Bisson W[2], Altmann KH[1], Gertsch J[1]
[1]Institute of Pharmaceutical Sciences, ETH Zurich, 8093-Zürich, Switzerland; [2]The Scripps Research Institute, Molecular Biology, La Jolla, CA92037, USA

Various *N*-alkyl amides (alkylamides) from the medicinal plant *Echinacea* are cannabinoid type-2 receptor (CB_2)-specific cannabinomimetics. Based on biphasic effects observed in radioligand-based receptor binding assays it was postulated that *Echinacea* alkylamides may form aggregates. In this study we show that dodeca-2*E*,4*E*,8*Z*,10*Z*-tetraenoic acid isobutylamide (A1) and dodeca-2*E*,4*E*-dienoic acid isobutylamide (A2) assemble into micelles, whereas no micelle formation occurs for undeca-2*E*-ene,8,10-diynoic acid isobutylamide (A3) or the structurally related endogenous cannabinoids arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG). Light scattering spectroscopy was used to determine the size of premicelle aggregates, micelles and supermicelles. The critical micelle forming concentrations (CMC) of A1 (7.4 nmol/L) and A2 (150 pmol/mL) were determined by fluorescence spectroscopy. The experimental data were complemented by molecular dynamics (MD) simulations of the aggregation phenomenon based on Montecarlo calculations. The results of these studies suggest that both A1 and A2 readily aggregate into premicelles, whereas A2 forms more compact aggregates due to a better alignment of hydrophobic chains and higher curvature. This is in line with microscopic analyses, which show that A2 spontaneously forms both globular and rod-like surfactant micellar superstructures. The data on the self-assembly of A1 and A2 may provide a rationale for the concentration-dependent effects of alkylamides in the radioligand binding assay and suggest a partition between the receptor-bound, monomeric, premicellar, and micellar states.

P 100

Analysis of the Physiological Activity from Kalopanax septemlobus Koidz. Extracts in Korea

Kim SH, Chung HG, Han J, Kim SC
Korea Forest Research Institute, 44 – 3 Omokcheon, 441 – 350, Suwon, Republic of Korea

Aiming to find new uses and to select clones that contain high physiological active materials, stem and root barks of *Kalopanax septemlobus* Koidz., were analyzed to measure cell cytotoxicity(NR assay, MTT assay), anti-lipid peroxidation(TBA method), anti-free radical activity(DPPH test, NBT test) and oxidative stress(DCFH-DA method). NR50 and MTT50 values were 0.002~0.33 mg/mL, 0.003~0.80 mg/mL, respectively, and MeOH treatment of stem barks showed lowest toxicity. TBA analysis showed many differences by extraction methods and sampling parts. MeOH treatment of stem barks showed the best result, 98 % and that was better than control, vitamin C. Extracts of *Kalopanax septemlobus* showed superior anti-free radical activity on the DPPH and NBT tests. MeOH 0.1 mg/mL treatment of stem bark showed the best, 96 % on the DPPH test and that was better than control, vitamin E. Also, hot-water extracts soluble treatment of stem barks showed the best, 95 %. Hot-water extracts insoluble treatment of root barks showed the best in DCFH-DA analysis. The stem bark of *Kalopanax septemlobus* has been used in traditional Korean medicine for anti-inflammatory, expectorant, tranquilizer and effective on anti-rheumatic. Thirteen kinds of chemical components were isolated from the extracts of the dried stem bark of *K. septemlobus*. On the basis of physico-chemical, spectroscopic data and in comparison with those of authentic samples or values reported in the literatures, they were identified as β-sitosterol, oleanolic acid, caffeic acid, kalopanaxsaponin A, chlorogenic acid, protocatechuic acid, 3, 3'-bis(3,4-dihydro-4-hydroxy-6-methoxy-2H-1-benzopyran), (-)-balanophonin, liriodendrin, syringin, kalopanaxsaponin B, kalopanaxsaponin I and kalopanxsaponin H, respectively.

P 101

Development of antifungal agents from essential oil compounds in Ostericum koreanum

Shin S, Sim Y, Lim S, Byun Y, Choi S
College of Pharmacy, Duksung Women's University, Seoul, 132 – 714, Korea

Essential oils from plants are a promising source for novel natural antifungal drugs, though their activity against human pathogenic fungi is generally milder than commercial synthetic antifungal drugs. *Ostericum koreanum* (Max.) Kitagawa (Umbelliferae) is a perennial herb used in traditional Korean medicines for treatment of the common cold and for relief of rheumatic pains or headaches; the herb imparts a pungent and warm sensation. A rich source of essential oil, *Ostericum koreanum* is widely distributed in the wild and cultivated in Korea. In this study we analyzed the essential oil from *O. koreanum* and evaluated its antifungal activity by the broth dilution method and disk diffusion test against various pathogenic fungal species. On the basis of these results, checkerboard micro titer tests were performed and isobolograms were constructed to determine the combined effect of the essential oils and ketoconazole in order to develop more effective and safer anti-catharsis therapy. As the results, the essential oil of *O. koreanum* and its main components showed high susceptibility against the tested fungi. The antifungal activities were dose dependent. It exhibited significant synergism in combination with ketoconazole. **Reference**: Shin, S., Lim, S. (2004), J. Appl. Microbiol. 97: 1289 – 1296.

P 102

Antifertility activities of Acanthus montanus and its new sulphate ester on female rats with possible mechanism(s) of action

Asongalem EA[1], Nana P[2], Kamtchouing P[2]
[1]Pharmacology and Toxicology Unit, Department of Physiological Sciences, Faculty of Medicine & Biomedical Sciences, University of Yaounde 1, Yaounde, Cameroon, P.O. Box 8283, Yaounde Email: cpehw@yahoo.com, Fax: 237 2221873; [2]Department of Animal Biology & Physiology, Faculty of Science, University of Yaounde 1, Yaounde, Cameroon

This study centred on assessing the effects of aqueous extract (AE) of *Acanthus montanus* (Acanthaceae) and its new compound – Acanthus sulphate ester (ASE) on oestrous cycle, implantations and possible mode(s) of action. Oestrous cycles of Wistar rats (150 – 212 g) were monitored before, during and after oral administration of distilled water (control), AE (250, 500, 1000 mg/kg/day) and ASE (0.25, 0.5, 1.0 mg/kg/day; intravenous) for 6 consecutive days. Concerning implantations, pregnant rats received above doses of AE and ASE from days 1 – 6 (pre-implantation) or 6 – 15 (post-implantation) of gestation and sacrificed on day 8 or 20 of pregnancy(1). AE (1000 mg/kg/day) and ASE (2 mg/kg/day) were given to overiectomised rats in the presence and absence of exogenously administered oestrogen and or progesterone with uterine weight and deciduoma count assessed. $PGF_{2\alpha}$ was evaluated on pre-implantation in the presence and absence of AE and ASE. One-way ANOVA at $P < 0.05$ was used. AE and ASE dose independently prolonged metoestrous and dioestrous stages of the oestrous cycle which reversed at least 10 days post-dosing. On pre-implantation, the AE (1000 mg/kg/day) and ASE (0.5 mg/kg/day) caused appreciable pre-implantation losses of 36.8 ± 6.5 %, $P < 0.05$ and 42.5 ± 11.5 %, $P < 0.01$ respectively whereas AE (1000 mg/kg/day) and ASE (1.0 mg/kg/day) insignificantly caused post-implantation losses. AE and ASE did not alter the uterine weights or deciduoma counts (in the presence of progesterone) but reduced ($P < 0.05$) the number of implants of $PGF_{2\alpha}$-administered rats. AE and ASE caused infertility by prolonging oestrous cycle and promoting pre-implantation loss; abolished deciduoma formation and prostaglandin inhibition was implicated

but not sex hormones. *Acknowledgement:* This research was supported by the International Foundation for Science, Stockholm, Sweden and United Nations University (UNU), Tokyo, Japan, through a grant to Dr Emmanuel Acha ASONGALEM. **Reference**: 1. Asongalem, E.A., Akintonwa, A., (1997), Bull. Environ. Contam. Toxicol. 58: 184 – 189.

P 103

Antiinflammatory activity of the aqueous leaf extract of Manihot esculenta Crantz

Yemitan OK[2], Afolabi L[1], Adeyemi OO[1]
[1]Department of Pharmacology, College of Medicine of the University of Lagos, Idi-Araba, P.M.B. 12003 Lagos, Lagos, Nigeria; [2]Department of Pharmacology, Lagos State University College of Medicine, P.M.B. 21266, Ikeja, Lagos, Nigeria

The aqueous leaf extract of *Manihot esculenta* Crantz (MELE) has been used in traditional African medicine for the treatment of inflammation. The anti-inflammatory effects of MELE given through oral and topical routes, were tested in rodents. MELE (100 – 400 mg/kg, p.o) was given to rats and 30 min. later, 0.9 % carrageenan was injected into the right hind paw [1]. In another set, MELE (1 – 4 % w/w in petroleum jelly) was applied topically to either the paws or to shaved back portion of rats before carrageenan. Paw diameter was measured between 0 – 24 h post- carrageenan injection. In another experiment, MELE (100 – 400 mg/kg, p.o.) or (1 – 4 % applied to mouse abdomen) was administered and 30 min. later, 0.03 mL of xylene was applied to the right ear of mice; then sections of ear removed and weighed for oedema [2]. MELE (100 – 400 mg/kg, p.o) produced significant ($P < 0.001$) inhibition of carrageenan and xylene-induced oedema in rats and mice respectively. The percentage inhibition at 4 % w/w in petroleum jelly (52.3 ± 2.0 %) is comparable to those produced by acetylsalicylic acid (50.0 ± 2.6 %). At 1 – 4 % w/w, topically, MELE produced significant ($P < 0.01$) inhibition of carrageenan- induced rat paw oedema (68.0 ± 2.1 %) and xylene-induced ear swelling in mice (76.6 ± 2.2 %). Effects are significantly higher than those produced by indomethacin (74.0 ± 3.1 %, 47.0 ± 2.1 %, respectively). Based on the results, the extract may contain orally safe, anti-inflammatory principles, justifying its use in folklore medicine. **References**: 1. Winter, C.A. *et al.* (1962), Proc. Soc. Exp. Biol. 11: 533 – 547. 2. Tang, Xi Can, *et al.* (1984), Acta Pharmacol. Sinica 5: 85 – 89.

P 104

Analgesic activity of aqueous leaf extract of Manihot esculenta Crantz

Yemitan OK[2], Afolabi L[1], Adeyemi OO[1]
[1]Department of Pharmacology, College of Medicine of the University of Lagos, Idi-Araba, P.M.B. 12003 Lagos, Lagos, Nigeria; [2]Department of Pharmacology, Lagos State University College of Medicine, P.M.B. 21266, Ikeja, Lagos, Nigeria

The aqueous leaf extract of *Manihot esculenta* Crantz (MELE) has been used in traditional African medicine for the treatment of acute and chronic pain [1] and is claimed to be safe. The analgesic and acute toxicity effects of the extract, given through oral and topical routes, were tested in rodents. MELE (100 – 400 mg/kg, orally) was administered to mice 30 min before injection of 10mL/kg, acetic acid (0.6 % v/v in normal saline) intraperitoneally [2] or acetylcholine (8.3 mg/kg) [3]. In another set, 0.5mL MELE was rubbed onto the depilated abdomen of mice, and at 4 h and 8 h post-treatment, each mouse was challenged with 0.6 %, 10mL/kg, acetic acid or acetylcholine (8.3 mg/kg) intraperitoneally. The number of writhes was counted for 15 and 30 min., respectively in both experiments. MELE (100 – 400 mg/kg, orally) and (1 – 4 % w/w, topically), like aspirin exhibited significant ($P < 0.05$) inhibition of acetic acid (Orally: MELE: 61.3 ± 3.5 %, Aspirin: 70.4 ± 4.8 %; Topically: MELE: 47.4 ± 4.5 %; Aspirin: 54.9 ± 5.5 %) and acetylcholine (Orally: MELE: 54.7 ± 7.5 %; Aspirin: 70.1 ± 4.5 %; Topically: MELE: 68.0 ± 3.8 %; Aspirin: 57.8 ± 4.5 %) – induced mouse writhing tests, compared to untreated control. Acute oral administration up to 10 g/kg did not cause death within 14 days, but produced mortalities in i.p. administered extract with LD50 of 2.5 g/kg. Based on these, the extract may contain orally safe, analgesic principles, justifying its use in traditional African medicine. **References**: 1. Anderson, G.J., Coee, F.G. (1996), Econ. Bot. 50, 1:71 – 107. 2. Koster, R. et al. (1959), Fed. Proc. 18: 418 – 420. 3. Sancilio L.F. *et al.* (1977), Agents and Actions 7: 133 – 144.

P 105

Ultra low concentrations of sophoraflavanone G from Sophora pachycarpa C. Meyer enhanced the antibacterial activity of gentamycin aginst Staphylococcus aureus

Shahverdi AR[1], Iranshahi M[2]
[1]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; [2]Department of Pharmacognosy, Mashhad University of Medical Sciences, Mashhad, Iran

In this study the enhancement effect of *Sophora pachycarpa* roots' acetone extract on the antibacterial activity of gentamycin was evaluated against *Staphylococcus aureus*. Disk diffusion and broth dilution methods were used to determine the antibacterial activity of gentamycin in the absence and presence of plant extract and its various fractions separated by TLC. Clinical isolate of *S. aureus* was used as test strain. The active component of plant extract involved in enhancement of gentamycin's activity had $R_f = 0.72$ on TLC. The spectral data (^{1}H NMR, ^{13}C NMR) of this compound revealed that this compound was 5,7,2',4'-tetrahydroxy-8-lavandulylflavanone (sophoraflavanone G), previously isolated from *Sophora exigua*. In the presence of 0.03 mcg/mL of sophoraflavanone G the MIC of gentamycin for *S. aureus* decreased from 32 to 8 mcg/mL (a fourfold decrease). These results signify that the ultra low concentration of sophoraflavanone G potentiates the antimicrobial action of gentamycin suggestion a possible utilization of this compound in combination therapy against *S. aureus*. **Reference**: Tsuchiya, H., Iinuma M. (2000), Phytomedicine 7:161 – 165.

P 106

Alkaloids from the club moss Lycopodium annotinum L. – acetylcholinesterase inhibitory activity in vitro

Halldorsdottir ES[1], Olafsdottir ES[1]
[1]University of Iceland, Faculty of Pharmacy, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland

Plant species belonging to the genera *Lycopodium* are known to produce so-called Lycopodium alkaloids. The club moss *Huperzia serrata* (Thumb.) Trev., which has long been used traditionally against Alzheimer's disease in China, has been shown to contain Lycopodium alkaloids which are acetylcholinesterase inhibitors [1]. Five species of club mosses are found in Iceland. *Huperzia selago* L. Bernh. ex Schrank & C. Martius has previously been studied and shown to contain a new alkaloid, selagoline, in addition to the known huperzine A and serratidine. The inhibitory activity on acetylcholinesterase was not investigated [2]. The aim of this study was to examine the alkaloid content of the Icelandic *Lycopodium annotinum* and to determine their ability to inhibit the enzyme acetylcholinesterase *in vitro*. The plant extract was fractionated and the alkaloids purified using liquid chromatography methods (VLC, SPE, HPLC) and ^{1}H- and ^{13}C-NMR spectroscopy was used for the structure determination. The inhibitory activity of the alkaloids towards acetylcholinesterase was determined by an *in vitro* TLC acetylcholinesterase assay [3]. Three Lycopodium alkaloids from *L. annotinum* were found to inhibit acetylcholinesterase activity *in vitro* including the known annotine. Another known alkaloid from this plant, annotinine, did not show inhibition. Annotine and annotinine have only been found in *L. annotinum* and their effect on acetylcholinesterase

has not been described before. **References**: 1. Ma, X.Q. *et al.* (2004), Nat. Prod. Rep. 21: 752 – 772. 2. Staerk, D. *et al.* (2004), Nat. Prod. Res. 18: 197 – 203. 3. Rhee I.K. *et al.* (2001), J. Chromatogr. A 915: 217 – 223.

P 107

Anticonvulsant activities of the methanol extracts (leaf, root), saponins and n-butanol-insoluble fraction of Calliandria portoricensis Jacq (Benth) (Family: Mimosaceae)

Agunu A[1], Abdurahman EM[1], Musa KY[1], Zezi UA[2]
[1]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria; [2]Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria

Calliandra portoricensis Jacq (Benth) (family: Mimosaceae) is a plant used in Nigeria as an analgesic, anticonvulsant, antihelminthic, antidepressant and for treatment of skin rashes [1]. The methanol extracts (leaf, root), saponins and n-butanol-insoluble fraction [2, 3] were used in the investigation. The LD_{50} of the extracts (root, leaf) carried out in mice were 1292 mg/kg and 7041 mg/kg respectively. The anticonvulsant evaluation was carried out in mice using pentelyenetetrazole (PTZ) and electroshock (ES) by determining both antagonistic and potentiation properties [4] of the methanol extracts at 100 mg/kg, 200 mg/kg and 300 mg/kg, saponins and n-butanol-insoluble fraction at 50 mg/kg and 100 mg/kg. There were no antagonistic action to PTZ induced clonic convulsion by extracts, saponins and n-butanol-insoluble fraction. The root extract showed significant ($P < 0.05$) protection when subtreshold of PTZ was administered compared to leaf extract. The root extract, saponins and n-butanol-insoluble fraction showed higher protection against ES induced tonic seizures compared to the leaf. The root extract and n-butanol-insoluble fraction also increased pentobarbital-induced hypnosis compared with the leaf. This investigation provides scientific explanation for the anticonvulsant activities in the roots and leaves of *Calliandra portoricensis*. **References**: 1. Dalziel, J.M. (1937), Useful plants of West Tropical Africa pp. 303 – 304. 2. Cannell, R.J.P (1998), Natural product isolation pp. 343 – 348, Humana Press, U.S.A.. 3. Williamson, M.E; Okpako, T.D, *et al.* (1996), Selection, preparation and pharmacological evaluation of plant material pp 5 John Wiley and Sons Ltd, West Sussex. 4. Amagaya, S; Lizuka, A, *et al.* (2001), Phytomedicine 8: 338 – 347.

P 108

Reversible antifertility activity of methanol extract of Momordica dioica Roxb. in male albino rats

Kachhawa JBS, Sharma A, Gupta RS
Center for Advanced Studies, Reproduction Physiology Section, Department of Zoology, University of Rajasthan, Jaipur-302004, India

In the present era the major problem for developing countries is the increasing population. To fight this, various hormonal, chemical and surgical methods are developed for male but they have undesirable side effects and irreversibility. The present research work is going on the development of fertility regulating drug from plant organs. Therefore the present work was done to evaluate the antifertility activity of *Momordica dioica* Roxb. (Cucurbitaceae) in male albino rats. For this adult proven fertile male rats were gavaged 100% methanol extract of *Momordica dioica* root at the dose level of 5 mg/rat/day for 60 days. *Momordica dioica* reduced the fertility of male rats by 100%. A reduction was seen in the cauda epididymal sperm motility and density. Marked decline were also found in the testicular germ cell population, Leydig cell nuclear area and the number of mature Leydig cells, However no morphological changes were observed in Sertoli cells as well as in their counts. Serum testosterone level was also reduced after *Momordica dioica* treatment. The protein glycogen, sialic acid, acid phosphatase and alkaline phosphatase content of testes, protein and sialic acid in cauda epididymis and fructose in seminal vesicle was decreased significantly, whereas cholesterol content of testes increased significantly. *Momordica dioica* extract did not alter the blood & serum parameters, which shows the non-toxic nature. All the parameters were reversible after withdrawal of the drug. In conclusion methanol extract of *Momordica dioica* root have antifertility activity with their reversible nature. *Acknowledgment:* Authors are thankful to the Head, Department of Zoology, Prof. N.K. Lohiya Coordinator CAS, Department of Zoology for providing the necessary facilities and UGC, Regional Office, Bhopal, INDIA for financial support.

P 109

Evaluation of Narcissus tazetta L. under different habitats

Hassan NM, Habib AA, Abdel-Azim NS, Shams KA, Hammouda FM
Phytochemistry Dept, National Research Centre, 12311 Dokki, Cairo, Egypt

Narcissus tazetta belongs to the family Amaryllidaceae. In Egypt, it is commercially grown outdoors for cut flowers and essential oil extraction that used in perfume industry. In this work, the bulbs were cultivated in three different localities under different habitats representing the loamy soil, the new reclaimed sandy soil and the sandy soil; which were differentiated by their soil and water analysis. Evaluation of the cultivated plant samples from each locality; including essential oil content and the total alkaloids; was done. The loamy soil showed to be the most suitable habitat for the cultivation of the plant. Investigation of the essential oil revealed that the highest oil content was recorded in the plants grown in the loamy soil (0.13%). The plants cultivated at sandy soil gave the lowest yield of the oil (0.10%). GC/MS of the essential oil for all samples showed that the main constituents of the oil are the same, but only differ in their percentages. The maximum terpenoid percentage (65.1%) was determined in the plants cultivated in the loamy soil followed by sandy soil samples (52%), and then new reclaimed sandy soil samples (47.65%). The main constituents were α-pinene, limonine, linelool, methyl and ethyl cinnamate in all samples.The total alkaloids were extracted from the collected leaves and bulbs of the cultivated samples. The highest alkaloid percent was found in the loamy soil leave samples (0.28%) while the lowest one was found at the sandy soil samples (0.12). Also, the average of the total alkaloidal content of the collected bulbs showed the same tendency as the leaves. The highest percent was found in the loamy soil samples (0.13%) followed by the new reclaimed sandy soil samples (0.11%) and the lowest percent was found in the bulb samples collected from sandy soil (0.07). TLC-densitometric analysis indicated that the main spot of alkaloids might be identified as narcessine. Also, in vitro propagation by tissue culture technique was successful from callus formation from meristematic tips. **References**: 1. Guenther, E. (1952), The essential oils. Vol.5. Individual essential oils of the plant families, D. Van Nostrand Company, Inc. New York, pp. 343 – 351. 2. Furusawa, E., Furusawa, S. (1985), J. Ethnopharmacol. 16: 299. 3. El-Moghazy, A.M., Gomaa, G.S. *et al.* (1978), Egyptian J. Pharm. Sci. 17: 273 – 281.

P 110

Modification of the polarity of an anthocyanin pigment. Structure determination and antioxidant activity

Palé E[1], Duez P[2], Luhmer M[3], Nacro M[1]
[1]Laboratoire de Chimie Organique Appliquée, Département de Chimie, UFR-SEA, Université de Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso; [2]Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, Institut de Pharmacie, Université Libre de Bruxelles, Campus de la Plaine CP 205 – 4, Bld Triomphe, B-1050 Bruxelles, Belgique; [C]Laboratoire de RMN haute résolution, Département de Chimie Université Libre de Bruxelles, CP 160 – 08, 50 avenue Franklin Roosevelt, B-1050 Bruxelles, Belgique

In the aim to develop antioxidant compounds with cellular membrane anchoring potential, Cyanidin (3-O-(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl -5- O-β-D-glucopyranoside), an anthocyanin extracted from *Ipomoea asarifolia* (convolvulaceae), was acy-

lated with a palmityl group to yield two new acylated anthocyanins. Their structures were elucidated using chemical, mass and NMR spectroscopy methods (^{1}H and ^{13}C, TOCSY-1D, DQF-COSY and HMBC). Theses new pigments were found to consist of cyanidin 3-O-(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl-5-O-β-D-glucopyranosyl-7-O-palmitoyl and cyanidine 3-O-(2-O-β-D-glucopyranosyl)-β-D-glucopyranosyl -5- O-β-D-glucopyranosyl-7,3'-O-dipalmitoyl. Using MTT and DPPH assays for measuring the cytotoxic and antioxidant properties of these compounds, it was found that the acylated anthocyanins retain the non-toxicity and antioxidant properties of the parent cyanidin. Work is in progress to further characterize the antioxidant profile of these interesting compounds.

P 111

Pyridine Alkaloids of Senna multijulga (Cesalpiniaceae) as Acetylcholinesterase Inhibitors

da S. Bolzani V[1], Serrano MAR[1], Lopes MN[1], Young MCM[2], Torres LB[2], Cardoso EM[2]

[1]Núcleo de Bioensaios, Biossíntese e Ecofisiologia de Produtos Naturais – NuBBE, Universidade Estadual Paulista – UNESP – Instituto de Química, Departamento de Química Orgânica, Rua Prof. Francisco Degni s/n – 14.800 – 900 – Araraquara – São Paulo – Brazil; [2]Instituto de Botânica, Avenida Miguel Stéfano 3687 – 04301 – 902 – Água Funda – São Paulo – Brazil

As part of a search for naturally occurring acetylcholinesterase inhibitors, two new 2-methyl-3-hydroxyl-6-*n*-alquil-pyridine alkaloids 1 – 2 and the known flavonoid quercetin-3-*O*-glicopyranosyl-(1,6)-rhamnopyranoside, were isolated by bioassay-guided fractionation from the leaves of *Senna multijulga* (Rich.) (Cesalpiniaceae), an ornamental plant species, popularly named "canafistula", collected in Atlantic Forest, São Paulo State. Compounds 1 and 2 were submitted to preliminary TLC screening for selecting potential AchE inhibitors, in which both alkaloids 1 and 2 inhibited the enzyme at 0.1 and 0.5 mM concentrations. These compounds were also evaluated for their efficacy in an in vitro rat brain assay, to measure its AchE inhibitory potential, and additional results will be presented. (Supported by Program project Biota-FAPESP grant no 03/02176 – 7). *Acknowledgements*: To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), CAPES and CNPq for research funding.

P 112

Phytochemical and Antimicrobial investigation of Taleghan plants species

Mehrabadi Yari L, Hosein M, Surmaghi S, Amin G, Badami N, Emami M, Asgari T

Faculty of Pharmacy, Tehran University of Medical Sciences, Pursina Ave, 14155 – 3451; and faculty of pharmacy, Azad university, Tehran, Iran

Taleghan a highly beautiful area located in 80 km.Nw of Tehran, has a suitable ecology for growth of diverse plants. There are many species that are used in folk medicine by inhabitants for treatment of infection disease. In this study 158 species were collected, identified and deposited in the herbarium of faculty of pharmacy, Tehran University of medical sciences. Prephytochemical, antibacterial and anti fungal effects of plant extracts considered and for this proposes hydroalchoholic extracts were prepared using suxhelt apparatus, then dried over vacuum system. Qualitative tests for assessment of four components: alkaloids, flavonoides, saponins, and tannins were done via laboratory tests. Each extract was used due to find antifungal and antimicrobial properties against:Candida, Aspergillus, Nocardia, Microspom, Trychophyton, Streptococcus, Proteus, Escherchia, Salmonella, Staphylococcus and Pseudomonas. Blood agar and muller hinton were used as cultures media. Results of pre-phytochemical tests were as fallows: 84 % had saponin, 42 % tannin, 45 % flavonoids, 13 % alkaloids.69 species had attractive antimicrobial effect and three species including *Epilobium hirsutum* L., *Centaurea brugeriana* and *Centaurea virgata* were the best. Antifungal and antibacterial tests showed that the effective species were *Achillea micrantha* Wild., *Matricaria disciformis* C.A. Meyers, *Mentha longifolia*L., *Hypericum hyssopifolium* Chaix. and *Ducrosia anethifolia*(DC) Boiss. in conclusion the species of containing saponin were more effective.

P 113

Chemical composition, antiviral and antimicrobial activities of the essential oils of Ferula hormonis, Plectranthus coleoides and Magnolia grandiflora

Mohamed SM[1], Ibrahim NA[2], Ali MA[3], Faraid MA[4]

[1]Medicinal and Aromatic Plants; [2]Pharmacognosy; [3]Water Pollution; [4]Natural Products Department, National Research Center, Tahrir St. Dokki, 12311. Fax 3370931, Cairo, Egypt

Essential oils were obtained by hydrodistillation from the fresh leaves of *Magnolia grandiflora* L., aerial part of *Plectranthus coleoides* Benth. and dried roots of *Ferula hormonis* and were analyzed by GC/MS. The main constituents of the oil of Ferula were levomenol (23.42 %), alpha-humulene (13.80 %), cycloisolongifolene (8.35 %), spathulenol (7.89 %) and beta–oplopenone (7.76 %) while the oil of Plectranthus was characterized by thymol (57.57 %), gamma-terpinene (15.37 %), p-cymene (9.07 %) and trans-caryophyllene (5.81 %). The major component of Magnolia essential oil were trans-caryophyllene (9.70 %), caryophyllene oxide (9.55 %), dendrolasin (9.48 %), butanoic acid 2-methyl-1-methylpropyl ester (7.38 %) and spathulenol (7.23 %). Anti herpes simplex virus (HSV-1) was performed using Plaque inhibition assay [1]. The activity was calculated by percentage of viral plaque inhibition at a non-cytotoxic dilution of the oil (1:320 v/v). The most potent oil was obtained from ferula essential oil (81.4 %) against HSV-1. Antimicrobial screening was conducted using the disc diffusion assay against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger*, and the human pathogenic yeast, *Candida albicans*. The minimum inhibitory concentrations (MIC) were determined [2]. The essential oil of Plectranthus was the most active as antimicrobial. At dilution of 1:100 v/v essential oil of Plectranthus inhibited the growth of *S. aureus*, *B. subtilis*, *A. niger* and *C. albicans* while the dilution of 1:75 v/v inhibited the growth of *E. coli*. This chemical and biological investigation have not studied previously. **References**: 1. Bermejo, P. *et al.* (2002), Planta Med. 68: 106 – 110. 2. Gabraith, H. *et al.* (1971), J. Appl. Bact. 34: 803 – 813.

P 114

Investigation of free radical scavenging activity by ESR for coumarins isolated from Tecoma radicans

Hashem FA

Pharmacognosy depart. National Research Centre, Tahrir Street, Dokki, Cairo, Egypt

Tecoma radicans (*Campsis radicans* (L.) Seem.), *F. Bignoniaceae* is a species that belongs to a tropical family but has been introduced in many countries as ornamental. Phytochemical investigation of the aerial parts of *Tecoma radicans* (L.) DC, indicates the presence of four coumarins, 2', 3'-epoxide alloimperatorin (I), pabulenone (II), pereflorin B (III) and 17 –methylbothrioclinin (IV). One chromone was also isolated peucenin– 7-methyl ether (V), and showed the violet colour with $FeCl_3$ directly while the four coumarins showed this violet after alkalinization with ammonia solution. They were isolated, purified and identified from their spectroscopic analysis and comparing with the published data [1]. When successive extracts of *T. radicans* and coumarin fraction were screened for cytotoxic activity in concentration of 100 μg/0.1 mL DMSO, *in vitro* using a single tumour (Ehrlich ascites carcinoma cells) [2], they showed no cytotoxic activity. When the coumarin fraction and isolated compounds were examined for free radical scavenging activity, using the stable DPPH free radical [3] and recorded by ESR, using vitamin C as control, it was found that the whole coumarin fraction was the most

active, Fig.1, (89. 06 % inhibition of DPPH free radical), and the isolated components are 0, 25.9, 8.45, 13.85 and 50 % respectively, Table 1.
Fig.1. ESR of control (DPPH) and coumarin extract of *T.radicans*

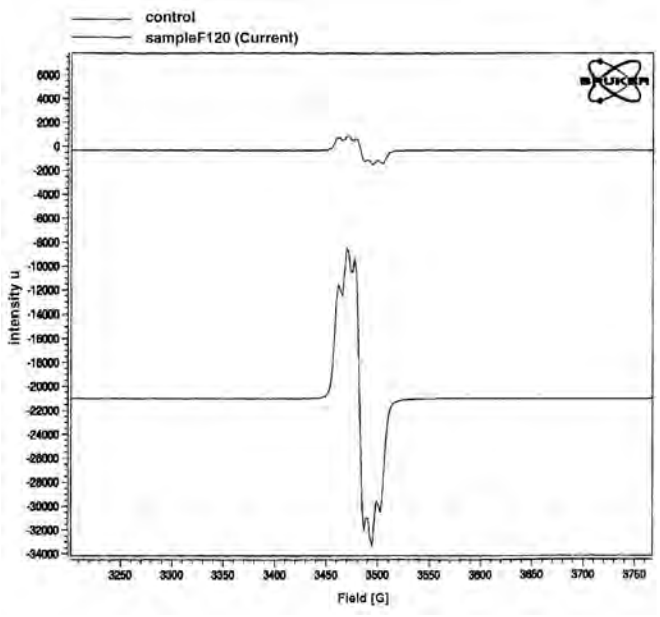


Table 1: Inhibition of DPPH radical by coumarins isolated from *T. radicans*.

Compound	Double integrationarea	Percentage inhibition
DPPH	638	
Vitamin C	29.0	95.45
Coumarin ext.	69.0	89.2
Compound I	638	-
Compound II	472.7	25.9
Compound III	584	8.45
Compound IV	549.6	13.85
Compound V	319.0	50.0

References: 1. Murray, R.D.H., Mendez, J., Brown, S.A.. (1982), The Natural Coumarins, John Wiley and Sons Ltd., New York. 2. El-Hossary, G.A., Fathy, M.M. (2000), Bull. Fac. Pharm. Cairo Univ. 38: 87 – 97. 3. Acqua, S.D., Innocenti, G. (2004), Fitoterapia, 75, 592 – 595.

P 115

Establishment of a cell-based screening system for NK-1 antagonists using SP-conjugated fluorescence; effects of plant extracts on the NK-1 receptor binding in U-373MG cultures

Koo KA[3], You JM[1], Xu P[3], Lee EJ[4], Youn HJ[2], Lee BJ[1]
[1]Biohealth Products Research Center and Department of Chemistry; [2]Biohealth Products Research Center and School of Biotechnology & Biomedical Science; [3]Biohealth Products Research Center, Inje University, Gimhae 621 – 749, Gyungnam, Republic of Korea; [4]Life Science R&D Center, SK Chemicals, Suwon 440 – 745, Kyungki-do, Republic of Korea

Substance P (SP) is a peptide neurotransmitter binding to neurokinin-1 (NK-1) receptors which are common in the central nervous systems. The interaction between SP and NK-1 receptor has been associated with a number of diseases, including inflammatory bowel disease, liver diseases, asthma, diabetes, migraine, emesis, depression and pain [2]. There is no such study for the discovery of a new agent from plant products even though their action could present value as a target in the treatment of many diseases. In this study, we have established a cell-based system for screening NK-1 receptor antagonists from plant extracts using a fluorescence probe, SP conjugated Oregon Green®488 (Molecular Probes, Eugene, OR), and U-373MG human malignant glioma cells, which dominantly expresses NK receptors [3 – 5]. The treatment of 10 nM SP-Oregon Green®488 to the -373MG culture resulted in the significant increase of the fluorescence intensity, which was selectively inhibited by L-733,060, a selective synthetic NK-1 antagonist having high affinity on human NK-1 receptor (6). L-733,060 blocked the binding of the SP fluorescence probe in a dose-dependent manner at the concentrations ranging from 0.1 to 100 nM (IC_{50}= 1.85 nM). We were able to investigate some prospective plant extracts with the NK-1 receptor antagonist activity using the assay system. *Acknowledgement:* This study was supported by a grant from the Ministry of Commerce, Industry and Energy (MOCIE) and the Korea Institute of Industrial Technology Evaluation & Planning (ITEP) through the Biohealth Products Research Center of Inje University. **References**: 1. Harrison, S. *et al.* (2001), Int. J. Biochem. Cell. Biol. 33: 555 – 576. 2. Duffy, R.A. (2004), Expert Opinion on Emerging Drugs 9: 9 – 21. 3. Bennett, V.J. *et al.* (2001), BMC Chem. Biol. 1: 1. 4. Rasley, A. *et al.* (2002), Glia 37: 258 – 267. 5. Walpole, C.S.J. *et al.* (1998), Br. J. Pharmacol. 124: 83 – 92. 6. Seabrook, G.R. *et al.* (1996), Eur. J. Pharmcol. 317: 129 – 135.

P 116

Anti-inflammatory effects of water-soluble fractions from Artemisia species using the LPS-induced inflammatory response in primary rat astrocyte cultures

Koo KA[3], Lee BJ[2], Youn HJ[1]
[1]Biohealth Products Research Center and School of Biotechnology & Biomedical Science; [2]Biohealth Products Research Center and Department of Chemistry; [3]Biohealth Products Research Cente, Inje University, Gimhae 621 – 749, Gyungnam, Republic of Korea

Water-soluble fractions have been purified from the crude aqueous extracts of *Artemisia* species, especially from *Artemisia folum* (AVF3) and *Artemisia iwayomogi* Kitam. (AIP1). The herbs are traditionally used as the medicinal plant to prevent or treat a number of liver diseases in Asia [1]. The AIP1 fraction has been shown to have diverse immuno-modulating activities including, anti-apoptosis and anti-cancer effects in our previous works [2, 3]. In this study, we have investigated the anti-inflammatory activity of the fractions using the LPS-induced inflammation model with primary rat astrocyte cultures. The treatment of the astrocyte culture either with AVF3 or AIP1 resulted in the suppression of the NO production by the LPS treatment, which was comparable to that of the dexamethasone treatment. Quantitative real-time PCR analysis revealed that the expression of iNOS gene was significantly suppressed by the samples, indicating that the inhibition of NO production could result from an inhibitory effect on iNOS gene transcription. The treatment also suppressed the up-regulation of the pro-inflammatory IL-6 and MIP-1β genes as the dexamethasone treatment. These results clearly demonstrate that the water-soluble fractions from *Artemisia* species might modulate the inflammatory response in brain astrocytes. Since NO production in brain astrocytes is important in the pathogenesis of a number of brain inflammatory diseases such as multiple sclerosis and Alzheimer's disease, the anti-inflammatory effect of the fraction could have considerable value for the protection or the treatment of the neurodegenerative diseases. *Acknowledgement:* This study was supported by a grant from the Ministry of Commerce, Industry and Energy (MOCIE) and the Korea Institute of Industrial Technology Evaluation & Planning (ITEP) through the Biohealth Products Research Center of Inje University. **References**: 1. Bensky, D. *et al.* (2004), Chinese Herbal Medicine; Materia Medica. Eastland Press Inc. Seattle, USA. 2. Koo, K.A. *et al.* (1994), Arch. Pharm. Res. 17: 371 – 374. 3. Hwang, J.S. *et al.* (2005), Biol. Pharm. Bull. 28: 921 – 924.

P 117

Aryltetralin lignans from Linum pamphylicum (Boiss.) Pod. sub sp. olympicum

Konuklugil B, Salık Ç, Yücel Z
University of Ankara, Faculty of Pharmacy 06100 Tandogan – Ankara, Turkey

Lignans are phenolic compounds that are very wide-spread in the plant kingdom. Lignans have been found in a large number of species belonging to more than sixty families of vascular plants and have been isolated from different parts of plants; roots and rhizomes, stems, leaves, fruits, seeds and resins [2 – 4] and show a wide variety of biological activities: antitumour, anti-HIV, immunosuppressive, hipolipidemic, antifungal, phytoestrogenic and antiasthmatic activities [1 – 3]. From a medical point of view, the most important compounds today are etoposide, teniposide and etopos, semisynthetic derivatives of podophyllotoxin which are used in cancer chemotherapy. Generally, aryltetralin types of lignans have been reported in the section *Syllinum* [5 – 8]. In Turkey, genus *Linum* is represented by 39 species. *L. pamphyllicum* is member of section *Syllinum* in a part of our ongoing study on the *Linum* species we identified podophyllotoxin and 6- methoxypodophyllotoxin from this species. *Acknowledgement*: This research was supported by The University of Ankara-Biotechnology Institute **References**: 1. Massanet, G.M., Pando, E., Rodriguez-Luis, F., Zubia, E. (1989), Fitoterapia 60: 3 – 35. 2. Row, R. (1978), Chemistry of Lignans, Andhra University Pres, Waltair, India. 3. Castro, M.A. *et al.* (1996), Phytochemistry, 41: 995 – 1011. 4. Ward, R.S. (1999), Nat. Prod.Rep. 16: 75 – 96. 5. Smolly, T., Wichers, H. *et al.* (1998), Phytochemistry 48: 975 – 979. 6. Konuklugil, B., (1996), Fitoterapia, 67: 379 – 381. 7. Konuklugil, B., (1997), Fitoterapia, 68: 183 – 184. 8. Konuklugil, B., (1997), Biochem. Syst. Ecol. 25: 75 – 79. 9. Konuklugil, B. (1998), Biochem. Syst. Ecol. *26*: 795 – 796.

P 118

A Maillard reaction product enhances eNOS enzymatic activity in human endothelial cells

Schmitt CA[1], Heiss E[1], Severin T[2], Dirsch VM[1]
[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, 1090 Vienna, Austria; [2]Department of Pharmacy, Center of Drug Research, University of Munich, Munich, Germany

Nitric oxide (NO) produced by the endothelial nitric oxide synthase (eNOS) is an essential signaling molecule in the cardiovascular system. Reduced eNOS activity is associated with the development of atherosclerosis [1]. Maillard reaction products (MRP), formed by the ubiquitious reaction between sugars and amins, possess antioxidant activity and other pharmacological effects [2]. We investigated a MRP, which is formed by the reaction between starch and a primary amine, and examined its effects on eNOS in the human endothelial cell line EA.hy926 [3]. We used EA.hy926 cells stably transfected with a plasmid containing 3600bp of the human eNOS promotor driving a luciferase reporter gene for measuring human eNOS promotor activity and western blot to quantify protein levels. ENOS enyzyme activity was investigated by an [^{14}C]L-arginine/L-citrulline conversion assay. NO was quantified by the reaction with the fluorescent probe DAF-2 [4]. After 18 hours of incubation (30 µM – 300 µM) we observed a significant and concentration-dependent increase of eNOS activity. NO production peaked at a concentration of 100 µM. Surprisingly we found a tendency towards a slight decrease of human eNOS promotor activity and protein levels. A time course with incubation times ranging from 30 minutes to 24 hours showed that eNOS enzyme activity was slightly attenuated during the first eight hours, but increased significantly afterwards. We therefore hypothesize that the de novo synthesis of another protein is needed to mediate this effect. This is the first time that positive effects of MRPs on eNOS activity and NO production are demonstrated *in-vitro*. Given the regular nutritional uptake of MRPs due to their great abundance in food, these results could be of physiologic importance. *Acknowledgments*: The authors would like to thank Dr. C.-J.S. Edgell (University of North Carolina) for EA.hy926 cells and Daniel Schachner for excellent technical assistance. **References**: 1. Naseem, K.M. (2005), Mol. Aspects Med. 26:33 – 65. 2. Somoza, V. (2005), Mol. Nutr. Food. Res. 49: 663 – 672. 3. Edgell, C.-J.S. *et al.* (1983), Proc. Natl. Acad. Sci. USA 80: 3734 – 3737. 4. Leikert, J.F. *et al.* (2001), FEBS Lett. 506: 131 – 134.

P 119

Antiplatelet activity of Ruta chalepensis L. (Rutaceae) grown in Jordan

Afifi-Yazar FU[1], Shahadeh M[1]
[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Queen Rania Street, 11942 Amman-Jordan

From the aerial parts of *Ruta chalapensis* L., grown in Jordan, two furanocoumarins (bergapten, chalepensin) one flavonoid glycoside (rutin) and several minor compounds have been isolated. The structural elucidation of these compounds was established based on spectral data. In Jordan, *R. chalepensis* is recommended for the treatment of rheumatism, mental and menstrual problems. Fresh and dried leaves are used as flavoring agent in food and beverages. Antiplatelet activities of the crude methanolic and ethylacetate extracts in addition to the three isolated major compounds were measured by aggrometric method [1] in venous blood taken from volunteers. Antiplatelet activity results of extracts and pure compounds on the aggregation of human PRP induced by ADP (ADP-IA) and collagen (C-IA) are shown in table 1.

Table 1

Compound/ Extract	Conc mg/mL	% Inhibition of ADP–IA*	% Inhibition of C-IA
Rc Ethyl acetate extract	5	100	50
Rc Methanolic extract	7	100	0
Rutin	0.06	98	Not tested
Bergapten	0.10.050.02	10097.596.3	7500
Chalpensin	0.10.050.02	97.898.394.9	75500

*Rc=*Ruta chalepensis*; 100 % platelet aggregation inhibition was calculated using aspirin13.9 mg/mL as a standard, 100 % aggregating induced by aggregating reagents: ADP 10 µM, Collagen 1 µg/mL

References: 1. Beretz, A., Cazenave, J.P. (1991), Methods in Plant Biochemistry, Hostettmann, K., Ed., Academic Press, New York, p.235

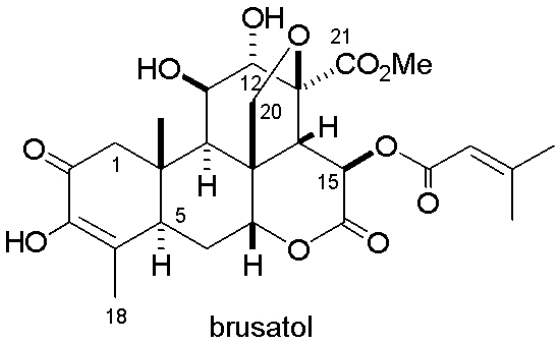
P 120

Antitumour Quassinoids from Brucea javanica and SAR of Brusatol

Takeya K, Kim IH, Hitotsuyanagi Y, Hasuda T
Tokyo University of Pharmacy and Life Sciences, School of Pharmacy, Horinouchi 1432 – 1, Hachioji, Tokyo 192 – 0392, Japan

Brucea javanica (L.) Merr. (Simaroubaceae) is a shrub which is distributed from Southeast to northern Australia. Its seeds, having been used for the treatment of dysentery, malaria and cancer, are known also as a rich source of quassinoids. We report the isolation, structure determination, and cytotoxic activity of new quassinoids and the related glycosides from the seeds of *B. javanica*. Their structures were elucidated by analysis of spectroscopic data and chemical evidence. Further we present preparation of analogues of the major constituent, brusatol, with a modified ring A or ring C structures, or different C-21 alkoxy chain lengths and some observations on the

chemical reactivity of the ring C moiety, and on the effects of chemical structures on the cytotoxic activity.



brusatol

References: 1. Kim, I.-H., *et al.* (2003), Tetrahedron 59: 9985 – 9989. 2. Kim, I.-H. *et al.* (2004), J. Nat. Prod. 67: 863 – 868. 3. Kim, I.-H. *et al.* (2004), Phytochemistry 65: 3167 – 3173. 4. Hitotsuyanagi, Y. *et al.* (2006), Tetrahedron 62: 4262 – 4271.

P 121

Phenolic compounds of plant origin and cell death

Lantto T[1], Raasmaja A[2], Hiltunen R[1]
Division of Pharmaceutical Biology[1] and Division of Pharmacology and Toxicology[2], Faculty of Pharmacy, University of Helsinki, P.O. Box 56 (Viikinkaari 5 E), 00014 University of Helsinki

Phenolic compounds of natural origin have been shown to possess many pharmacologically and nutritionally interesting properties. These compounds are abundant in fruits, berries and vegetables, and included also in normal human diet sometimes even in high concentrations. The aim of this work was to develop a cell model to study the effects of polyphenolic compounds in the modulation of apoptosis, a programmed cell death. Defects in apoptosis can result in pathological conditions, *e.g.* cancer and neurodegenerative diseases. The apoptotic inducing properties of two polyphenols, *i.e.* curcumin and resveratrol, and some natural extracts rich in phenolics, i.e. basil, ginger, laurel and parsley, in SH-SY5Y neuroblastoma cell line were studied. The cells were treated with different compounds for 12 h and the expression of p53 and β-actin was examined using Western blot technique. Arabinoside cytosine (AraC) was used as a positive control to induce apoptosis. Our results show that the addition of curcumin (25 and 50 μM) and resveratrol (100 μM) leads to an increase in p53 levels and the treatment with high concentrations of laurel and basil extracts leads to an increased cell death in SH-SY5Y cells. Further examinations of cell viability are in progress using MTT and LDH assays.

P 122

Lichens as a source of antibiotics against resistant bacteria

Elo H[1], Matikainen J[2], Pelttari E[1]
[1]Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, P. O. Box 56, 00014 University of Helsinki, Finland; [2]Department of Chemistry, Faculty of Science, University of Helsinki, P. O. Box 55, 00014 University of Helsinki, Finland

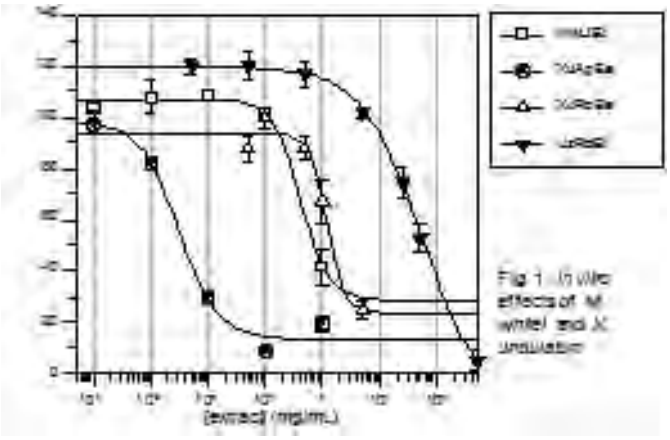
Resistant bacteria such as vancomycin-resistant enterococci (VRE) and methicillin-resistant staphylococci, especially methicillin-resistant *Staphylococcus aureus* (MRSA), are at present great clinical problems. The antibiotic arsenal available against them is limited and the situation is worsening because new resistant mutations are emerging. Therefore, an intensive search for new active agents is ongoing. We report that the lichen-derived old drug (+)-usnic acid and, especially, its sodium salt (sodium usniate) have potent antibacterial activity against VRE and MRSA. All MRSA strains tested were sensitive to sodium usniate (inhibitory zones of 21 mm with 10 μl of a 40 mg/mL solution in DMSO on a paper disk of 6 mm diameter). The activity of the free acid was somewhat lower, obviously because of lower solubility. In the case of VRE, sodium usniate gave inhibitory zone diameters of 32 mm, indicating very high activity of great clinical interest, while usnic acid itself gave diameters of 18 mm. Considering clinical applications, it must borne in mind that usnic acid has in some cases caused severe toxic manifestations such as fulminant hepatitis, and can also cause allergic reactions. In spite of this, it may possibly constitute a last rescue in life-threatening cases where other therapies have failed.

P 123

Pharmacological Studies on Xysmalobium undulatum and Mondia whitei – Two South African plants with in vitro SSRI activity

Pedersen ME[1,2], Weng A[1,2], Sert A[1], Stafford GI[2], van Staden J[2], Nielsen M[1], Jäger AK[1]
[1]Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark; [2]Research Centre for Plant Growth and Development, School of Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

Currently available therapy for depression is often associated with undesirable side effects. Therefore, the identification of alternative therapeutics for treatment of depression is still needed. This study focused on two South African plants that are used in traditional medicine. The root of *Xysmalobium undulatum* (L.) R. Br. (Asclepiadaceae) is traditionally used for numerous purposes, e. g. treatment of hysteria in young women and headache relief, whereas in Germany an alcoholic-aqueous root extract (Uzara®) is marketed for treatment of diarrhoea. *Mondia whitei* (Asclepiadaceae) has traditionally been used as aphrodisiac, arrow poison or headache relief. Both plants showed affinity to the serotonin transport protein in rat brain in a screening of plants used for anxiety and depression [1]. In the present study (Figure 1) root extracts and aerial part extracts of *X. undulatum* (XuRoEa and XuApEa respectively) and the Uzara® commercial product (UzRoEt) as well as leaf extracts from *M. whitei* (MwLfEt) showed *in vitro* affinity for the SSRI binding site in rat brain in a radioligand assay. The IC_{50} values were estimated to 3.0 μg/mL; 1.2 mg/mL; 0.4 mg/mL and 24 mg/mL for XuApEa, XuRoEa, MwLfEt and UzRoEt respectively based on dry extracts. However, a bioassay guided isolation of the Uzara® commercial product showed no fraction with specific activity.



Reference: 1. Nielsen, N.D. *et al.* (2003), J. Ethnopharmacol. 94: 159 – 163.

P 124

Two new triterpene saponins from Nylandtia spinosa

Lacaille-Dubois MA[1], Mitaine-Offer AC[1], Diome C[1], Miyamoto T[2], Delaude C[3]

[1]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique, UMIB, UPRES EA-3660, Faculté de Pharmacie, Université de Bourgogne 7, Bd. Jeanne D'Arc, BP 87900, 21079 Dijon Cedex, France; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan; [3]Centre de Recherche Phytochimique, Université de Liège, Institut de Chimie-B6, Sart Tilman, B-4000-Liège, Belgique

In a continuation of our study on saponin constituents of medicinal plants of the Polygalaceae family [1 – 3], we have examined the saponin fraction of the roots of *Nylandtia spinosa* (L.) Dum.. Two new triterpene saponins were isolated by successive MPLC over silica gel. Their structures were established mainly by 600 MHz 2D NMR techniques (COSY, TOCSY, NOESY, HSQC, HMBC) and mass spectrometry as 3-*O*-ß-D-glucopyranosyl-presenegenin-28-*O*-ß-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-ß-D-xylopyranosyl-(1→4)-[ß-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-ß-D-fucopyranosyl ester and 3-*O*-ß-D-glucopyranosyl-presenegenin-28-*O*-ß-D-galactopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→3)]-ß-D-xylopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)-ß-D-fucopyranosyl ester. **References**: 1. Mitaine-Offer, A.-C. *et al.* (2005), Helv. Chim. Acta 88: 2986 – 2995. 2. Mitaine-Offer, A.-C. *et al.* (2003), Helv. Chim. Acta 86: 2404 – 2413. 3. Mitaine-Offer, A.-C. *et al.* (2002), J. Nat. Prod. 65: 553 – 557.

P 125

New steroidal saponins from Asparagus acutifolius

Lacaille-Dubois MA[1], Sautour M[1], Miyamoto T[2]

[1]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique, UMIB, UPRES-EA 3660, Faculté de Pharmacie, Université de Bourgogne, 7, Bd. Jeanne d'Arc, BP 87900, 21079 Dijon Cedex, France; [2]Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

Asparagus acutifolius L. belongs to the Liliaceae family in which the steroidal saponins are fairly widespread. A survey of the literature showed that several *Asparagus* species have already been chemically studied and found to contain bioactive steroidal saponins. As part of our ongoing search for biologically active steroid saponins as potent antifungal agents [1 – 3] a phytochemical investigation of the roots of *A. acutifolius* has led to the isolation by several chromatographic steps on normal and reversed phase silica gel of three new steroidal glycosides. Their structures were determined by spectroscopic methods including 1D- and 2D-NMR (COSY, TOCSY, HSQC and HMBC) and FAB-MS as (25*S*)-3β,5β,22α-22-methoxyfurostan-3,26-diol 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranosyl 26-*O*-β-D-glucopyranoside [1], (25*S*)-5β-spirostan-3β-ol 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside [2] and (25*S*)-5β-spirostan-3β-17α-diol 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→4)]-β-D-glucopyranoside [3]. In addition, the antifungal activity of these compounds was tested against three human pathogenic yeasts (*Candida albicans, C. glabrata and C. tropicalis*). Spirostanol saponins presented antifungal activity with MICs values between 12.5 and 50 μg/mL whereas furostanol compounds were inactive. **References**: 1. Sautour, M. *et al.* (2004), Planta Med. 70: 90 – 92. 2. Sautour, M. *et al.* (2004), Chem. Pharm. Bull. 52: 1353 – 1355. 3. Sautour, M. *et al.* (2005), J. Nat. Prod. 68: 1489 – 1493.

P 126

Hypolipidemic and antioxidant effect of Ajuga iva in rats fed a high-cholesterol diet

Lacaille-Dubois MA[3], Chenni A[1], Yahia DA[1], Boukortt FO[1], Prost J[2], Bouchenak M[1]

[1]Laboratoire de Nutrition Clinique et Métabolique, Faculté des Sciences, Université d'Oran Es Sénia, 31000, Algérie; [2]UPRES Lipides- Nutrition EA 2422. 6, Bd Gabriel, Université de Bourgogne, 21000 Dijon, France; [3]Laboratoire de Pharmacognosie, Unité de Molécules d'Intérêt Biologique, UMIB UPRES EA 3660, Faculté de Pharmacie, Université de Bourgogne, BP 87900, 21079 Dijon Cedex, France

Ajuga iva (L.) Schreb. has been reported to have a variety of biological effects including hypoglycemic, vasorelaxant, hypolipidemic, antiinflammatory, antifungal, antimicrobial and anthelmintic activity [1, 2]. The present study explores the possible antioxidant and hypolipidemic effects of the aqueous extract of *Ajuga iva* (*Ai* 0.5 % in the diet) in rats fed a high-cholesterol (1 %) diet (HCD). The results indicated that the HCD-Ai *vs.* HCD treatment led to many changes in biochemical parameters. They showed a decrease by 18 % of plasma total cholesterol (TC) and by 29 % VLDL-cholesterol but an increase by about 35 % of HDL_2-cholesterol. The triacylglycerol contents were reduced by 31 % in plasma and 74 % in VLDL. The lipid peroxidation determined by TBARS was decreased by 75 % in plasma. TBARS in liver, heart and kidneys were highly reduced excepted in the adipose tissue. *A.i.* treatment enhanced superoxide dismutase activity in liver and kidney. Glutathione reductase activity was lowered in adipose tissue but increased in liver and in kidney. A significant increase was noted in glutathione peroxidase activity in liver, heart and kidney but a low value in adipose tissue was observed. In conclusion, this study demonstrates that in addition to its potent TG and TC-lowering effects, *Ai* is effective in improving the antioxidant status by reducing lipid peroxidation in plasma and tissues and enhancing the antioxidant enzymes in HCD fed rats. A phytochemical screening indicated the presence of flavonoids and terpenoids. Their isolation and characterization are currently in progress. **References**: 1. El Hilaly, J. *et al.* (2006), J. Ethnopharmacol. 105: 441 – 448. 2. Bondi, M.L. *et al.* (2000) Biochem. Syst. Ecol. 28: 1023 – 1025.

P 127

ChemGPS-NP – tuned for navigation in biologically relevant chemical space

Larsson J[1], Gottfries J[2], Muresan S[3], Bohlin L[1], Backlund A[1]

[1]Division of Pharmacognosy, Department of Medicinal Chemistry, BMC, Uppsala University, Box 574, S-751 23 Uppsala, Sweden; [2]Department of Medicinal Chemistry, AstraZeneca R&D Mölndal, S-431 83 Mölndal Sweden; [3]GDECS Computational Chemistry, AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden

Natural compounds have a unique chemical diversity occupying a different and larger space than that normally dealt with in medicinal chemistry [1, 2]. They are evolutionary selected and pre-validated by Nature with explicit biological activities, making them highly interesting for development of new drug lead candidates. Chemical space is a multi-dimensional region defined by the descriptors chosen to describe a set of chemical compounds [3]. Of utmost importance for discovery of new active compounds for future therapies is the identification and charting of a biologically relevant chemical space and a primary key to this is the coverage of the diverse natural product chemical space [1, 2, 4]. A map of chemical space can be constructed by applying the same principles as the Mercator convention in geography. Rules are corresponding to dimensions (e. g. longitude and latitude), and structures are corresponding to objects (*e. g.* cities and countries) [5]. The rules and objects together present the chemical space map, where the coordinates are t-scores from principal component analysis [6]. Here we present ChemGPS-NP, a new tool tuned for exploration of biologically relevant natural products chemical space, forming a framework for making compound comparison and selection more effec-

tive and increasing probability of hit generation when screening the vast diversity of natural products in the search for novel bioactive molecules. **References**: 1. Larsson J. *et al.* (2005), J. Nat. Prod. 68: 985 – 991. 2. Feher, M., Schmidt, J.M. (2003), J. Chem. Inf. Comput. Sci. 43: 218 – 227. 3. Dobson, C.M. (2004), Nature 432: 824 – 828. 4. Koch, M.A. *et al.* (2005), Proc. Natl. Acad. Sci. USA 102: 17272 – 17277. 5. Oprea, T.I., Gottfries, J. (2001), J. Comb. Chem. 3: 157 – 166. 6. Jackson, J.E. (1991), A users guide to principal components, Wiley, New York.

P 128

Sustainable use of the Brazilian biodiversity: Chemical and pharmacological prospection on higher plants

Wagner V[1], Monteiro Souza Brito AR[2] et al
[1]São Paulo State University - Instituto de Química de Araraquara. CP 355, CEP 14801 – 970, Araraquara, São Paulo, Brazil; [2]Campinas State University – Instituto de Biologia, Campinas, São Paulo, Brazil

Our research deals with the investigation of plant species with anti-ulcer, anti-oxidant, analgesic, immuno-stimulating, genotoxic, anti-inflammatory and antimicrobial activities. We have investigated several plant species, like *Davilla elliptica*, *Strychnos pseudoquina* and *Byrsonima fagifolia*. We performed the aforementionated biological assays and then we fractionate the active extracts in order to recognize their chemical composition, using chromatographic and spectrometric techniques. *D. elliptica* presented activity against Mycobacterium tuberculosis (MIC 62.5 ug/mL); *S. pseudoquina* presented moderate activity against gastric ulcers, which might be due to the presence of an indole alkaloid whose structure roughly resembles that of omeprezole; *B. fagifolia* presented significant activity against gastric ulcers. Therefore, Brazilian plants may be used as a potential source for compounds with biological activity. Acknowledgements: Biota-Fapesp Program, CNPq. *Acknowledgements*: Biota-Fapesp Program, CNPq

P 129

Characterization, design and synthesis of potential COX-2 inhibitors based on natural products

Jachak SM
Department of Natural Products, NIPER, SAS Nagar 160 062, Punjab

Synthetic COX-2 inhibitors (such as rofecoxib and other coxibs) have high selectivity but at the same time they are associated with thrombotic cardiovascular problems [1]. Thus, the use of COX-2 inhibitors still remains controversial and it represents challenge for the pharmaceutical industry to develop improved anti-inflammatory drugs devoid of severe side effects. Natural product-derived compounds are better candidates for lead identification and optimization in drug discovery process due to their great structural diversity which is not commonly seen in synthetic compounds. We are engaged in the characterization of natural COX-2/COX-1 inhibitors through bioassay-directed fractionations from medicinal plants [2, 3] and design, synthesis of natural product-derived analogues as potential COX-2 inhibitors e. g. curcumin and chalcone [4, 5]. In continuation of our efforts to discover COX inhibitors, design and synthesis of isoflavone analogues based on naturally-derived isoflavones are described herein. **References**: 1 Schror, K., Mehta, P., Mehta, J. L. (2005), Cardiovasc. Pharmacol. Ther. 10: 95 – 101. 2. Selvam, C., Jachak, S. M. *et al.* (2004), Tet. Lett. 45: 4311 – 4314. 3. Selvam, C., Jachak, S. M. J. (2004), J. Ethnopharmacol. 95: 209 – 212. 4. Selvam, C., Jachak, S. M. *et al.* (2005), Bioorg. Med. Chem. Lett. 15: 1793 – 1797. 5. Jachak, S. M. (2006), Curr. Med. Chem., 13, 659 – 678.

P 130

Extracts of Salvia officinalis from different growing areas and their antiviral effect against enveloped and non-enveloped viruses

Nolkemper S[1,2], Schnitzler P[2], Keppler OT[2], Reichling J[1]
[1]Institute for Pharmacy and Molecular Biotechnology, Department of Biology, University of Heidelberg, ImNeuenheimer Feld 364, 69120 Heidelberg, Germany; [2]Hygiene Institute, Department of Virology, University of Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg, Germany

In our study we compared extracts from *Salvia officinalis* L. (sage), which was grown at two different areas in Germany, for their antiviral effect. Firstly it was cultivated in an dry and hot area (area 1) and secondly in an area with a cooler climate and more rain (area 2). Different extracts were made from both samples with water/ethanol- mixtures in the ratio of 0, 20, 40, 60 and 80 % ethanol, respectively. A HPLC analysis of the extracts showed as major components apigenin-7-glucuronide, luteolin- 3-glucuronide and rosmarinic acid. The antiviral effects of these extracts were tested against the enveloped herpes simplex virus type 1 (HSV-1) and the non-enveloped adenovirus type 5. In order to determine the mode of antiviral action, the extracts were added to the cells or viruses at different times during infection. The inhibitory activity against HSV-1 was tested using a plaque reduction assay. Adenoviral GFP reporter gene expression allowed for a flow cytometry-based quantification of infection levels. When HSV-1 was pretreated with the extracts prior to adsorption, plaque formation was reduced by > 90 %. The best results were shown by the extracts from area 1. In time-response studies over a period of 2 hours, a clearly time-dependent activity for all extracts was demonstrated. Already after 20 minutes of incubation of HSV-1 with the extracts, an antiviral activity of about 70 – 80 % was shown. Pretreatment of adenovirus with the extracts for 1 hour showed an inhibitory effect on adenoviral infection from 20 % (80 % ethanol) up to 70 % (20 % ethanol). Therefore the extracts show antiviral activity independent of an envelope.

P 131

Characterisation of in vitro antioxidative properties of aqueous ethanolic (45 %v/v) extract of Lemon Balm (Melissa officinalis L.)

Dastmalchi K[1], Dorman HJD[1], Darwis Y[2], Hiltunen R[1]
[1]Faculty of Pharmacy, Division of Pharmaceutical Biology, University of Helsinki, P.O. Box 56 (Viikinkaari 5E), FIN-00014, Finland; [2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia

The plant *Melissa officinalis* (L.) has been used in European traditional system of medicine for its cognitive enhancing properties. Based on its traditional use and cholinergic properties, this plant was recently assessed for its clinical efficacy in Alzheimer's Disease (AD) in a trial conducted by the Institute of Medicinal Plant (IMP), Tehran, Iran [1]. The plant was found to be effective in the management of mild to moderate AD patients. Since it has been proposed that oxidative stress plays a cardinal role in the pathogenesis of the disease [2], we wanted to investigate if the efficacy of the plant extract in the clinical trial is due to its antioxidative properties. Therefore based on the IMP research *M. officinalis* was extracted by a similar procedure using the same solvent (45 % v/v ethanol) and plant material (cultivar). The method of extraction was Medium Pressure Solid Liquid Extraction. The total phenol content was estimated as gallic acid acid equivalents using Folin-Ciocalteu reagent method and the *in vitro* antioxidant activities assayed were iron (III) reduction, iron (II) chelation, 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonate) free radical scavenging activities, inhibiton of β-carotene-linoleic acid bleaching. The extract demonstrated activity in all the assays, however it was not as potent as the positive controls except in the β-carotene-linoleic acid bleaching assay where its antioxidant activity was superior to that of gallic and caffeic acid and statistically indistinguishable from

quercetin and butylated hydroxyanisole. *Acknowledgements*: Institute of Medicinal Plants, Iranian Academic Center for Education, Culture and Research, Tehran, Iran **References:** 1. Akhondzadeh, S. *et al.* (2003), J. Neurol. Neurosurg. Psychiatry 74: 863 – 866. 2. Varadarajan, S. *et al.* (1999), J. Struct. Biol. 309: 746 – 768.

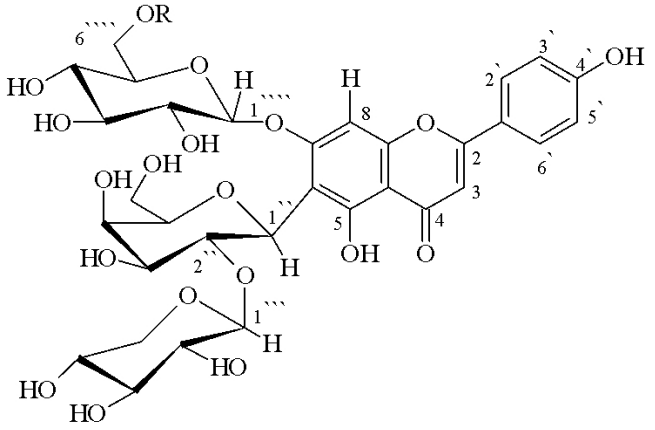
2. Recent Advances in Analysis of Secondary Metabolites

P 132

New Apigenin Triosides From The Seeds of Syzygium aromaticum

Nassar MIA
Natural Compounds Chemistry Department, National Research Centre, Dokki 12622, Cairo, Egypt

Syzygium aromaticum (L.) Merr. & Perry belongs to the family Myrtaceae. *S. aromaticum* buds (clove) are used in folk medicine as diuretic, odontalgic, stomachic, tonicardiac, aromatic condiment and condiment with carminative and stimulant properties [1]. Compounds isolated from *S. aromaticum* have been found to possess growth inhibitory activity against oral pathogens [2]. The aim of this work is the isolation and structural elucidation of flavonoid glycosides from the seeds of *S. aromaticum*. The crushed seeds were subjected to successive extraction using *n*-hexane, dichloromethane and ethanol (70%). The aqueous ethanol extract was subjected to cellulose column chromatography. The eluted polar fractions were further chromatographed on Sephadex LH-20 columns to give two new apigenin triglycosides, apigenin 6-*C*-[β-D-xylopyranosyl-(1→2)- β-D- galactopyranoside]-7-*O*-D-glucopyranoside (1) and apigenin 6-*C*-[β-D-xylopyranosyl-(1→2)-D-galactopyranoside]-7-*O*- β-D-(6-*O*-*p*-oumarylglucopyranoside) (2). The structures of the new compounds were elucidated by chemical and spectral analysis including UV, FABMS, ^{1}H, ^{13}C NMR, DEPT, HMQC, HMBC and NOESY.



1, R = H **2**, R = *p*-coumaryl

References: 1. Boulos, L. (1983), Medicinal Plants of North Africa. Ref. Publications Inc., Michigan. 2. Cai, L. *et al.* (1996), J. Nat. Prod. 59: 987 – 990.

P 133

Quantitative method development for measurement of Maesa lanceolata saponins by LC-MS

Pieters L, Theunis M, Apers S, Vlietinck A
Laboratory of Pharmacognosy and Phytochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1; B-2610 Antwerp, Belgium

Triterpene saponins are a class of plant natural products with a wide range of bioactivities, which make them an interesting research subject. The small tree *Maesa lanceolata*, growing in African countries, is used in traditional medicine against various diseases. In previous work a triterpenoid saponin mixture was isolated from the leaves of *Maesa lanceolata* and the compounds were identified [1, 2]. The compounds showed virucidal, haemolytic, molluscicidal and anti-angiogenic activity [3, 4]. Here we report the development of a quantitative LC-MS method to analyse saponin compounds in crude root and leaf extracts of *Maesa* plants. The crude extract is first purified on a C18ec SPE column. Then the compounds are separated on a reversed phase C18 column (Grace Vydac, 3.2 mm x 250 mm – 300Å) with a H_2O/ACN (0.06% formic acid) gradient before analysis with mass spectrometry (Bruker Daltonics Esquire 3000 plus). This method will be used to screen plants whether or not induced by methyl-jasmonate, or plants such as *Medicago truncatula* transformed with genes involved in saponin biosynthesis, for the presence of *Maesa* saponins. **References**: 1. Apers, S. *et al.* (1998), J. Pharm. Biomed. Anal. 18: 737. 2. Apers, S. *et al.* (1999), Phytochemistry 52: 1121. 3. Apers, S. *et al.* (2001), Planta Med. 67: 528. 4. Apers, S. *et al.* (2002), J. Pharm. Belg. 57, Hors-série 1: 47.

P 134

Proanthocyanidins from the herb of Myrothamnus flabellifolia Welw

Petereit F, Anke J, Engelhardt C, Hensel A
University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

Myrothamnus flabellifolia Welw. (Myrothamnaceae), a species growing in arid areas of Southeastern Africa, has the ability to survive regular periods of extreme dehydration to an air-dry-state (resurrection plant). In continuation of our previous results [1] we investigate the proanthocyanidin pattern from an aqueous acetone extract in more detail. Chemical investigation of the ethylacetate soluble fraction has led to the isolation and characterization of epicatechin, epigallocatechin, epicatechin-3-O-gallate and epigallocatechin-3-O-gallate as flavan-3-ol precursors. The dimeric proanthocyanidin fraction consists exclusively of procyanidins, partly substituted with gallic acid or p-hydroxybenzoic acid. A range of ten different procyanidins were identified by extensive 2D NMR studies of the peracetylated derivatives beside the known 3,4,5-tri-O-galloylquinic acid [2]. In addition, the more abundant polymeric proanthocyanidin fraction was also isolated and its chemical constitution characterized by ^{13}C NMR and optical rotation. The results are discussed briefly. *Acknowledgement*: Myro AG, CH-8606 Greifensee, switzerland, for financial support. **References**: 1. Deters, A. *et al.* (2005), 53rd Annual Congress of the Society for Medicinal Plant Research, Florence, P 130 (poster abstract). 2. Moore, J. *et al.* (2005), Biochem. J. 385: 301 – 308.

P 135

Air-transport alters the composition of essential oils in aromatic plants

Jäger AK[1], Rasmussen HB[1], van Staden J[2]
[1]Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen O, Denmark; [2]Research Centre for Plant Growth and Development, University of KwaZulu-Natal, P/Bag X01, Scottsville 3209, South Africa

A previous study has shown that aromatic plant material transported on a truck could lose all the essential oil during a half hour drive [1]. In our collaborative work, plant material is often air-freighted between continents, and it is suspected that the quality of the material is altered due to the reduced pressure in the cargo hold of an aircraft. Aerial parts of the aromatic plants *Artemisia afra* Jacq. ex Wild., *Mentha longifolia* (L.) Huds., *Ocimum basilicum* L. and *Salvia africana* L. were harvested in South Africa. Essential oils were prepared in South Africa from the fresh plant material and from material dried at 40¤C for 2 days. The oils were sealed in ampules and sent by air-freight to Denmark, along with portions of the dried plant material. In Denmark, essential oils were distilled from the air-freighted material and all the oil samples analysed by GC-MS. The most pronounced changes occurred in *S. africana* where 60 – 75 % of the monoterpenes were lost from the air-freighted material. In comparison, the sesquiterpene content did not change much for *S. africana*. Overall, there seemed to be a quantitative loss of 10 – 25 % of the monoterpenes in all the oils. Consequently, it is necessary to be careful about the interpretation of work, both chemical and biological, done on essential oils from aromatic plants that have not been processed locally before shipping. **Reference:** 1. Webber, L.N. *et al.* (2000), S. Afr. J. Plant Soil 17: 10 – 14.

P 136

Phytochemical Study of Artemisia persica Boiss. and Evaluation of its Antiplasmodial Activity

Sadeghpour O[1], Asghari G[2], Ardekani MRS[3], Jaroszewski JW[4]
[1]Herbal Medicine Dept.Research institute of Medical History,Islamic and Complementary Medicine Pirnia, Lalezar now Jomhoori Tehran IRAN; [2]Pharmacognosy Dept.Faculty of Pharmacy Isfahan Univrsity of Medical Sciences Hezarjerib St. Isfahan IRAN; [3]Pharmacognosy Dept.Faculty of Pharmacy Tehran Univrsity of Medical Sciences Enghelab St. Tehran IRAN.[4]Medicinal Chemistry Dept.Pharmaceutical University of Denmark Universitetsparken 2 DK-2100 Copenhagen Denmark

Introduction: The genus *Artemisia* is one of the largest and most widely distributed of the nearly 100 genera in the tribe Anthemideae of the Asteraceae. Considering to different compounds exist in *Artemisia* spp. They have a wide variety of biological activity. The outstanding antimalarial compound, artemisinin, isolated from *Artemisia annua* L.. The aim of this study was phytochemical investigation of "*Artemisia persica*" in order to identify bioactive compounds, which may have antiplasmodial effect [1, 2]. **Methods:** The Flowering aerial parts of plant were collected in November 2002 from Isfahan province (IRAN), and undergoes to chemical isolation of compounds by VLC, CC and HPLC, mainly using bioactivity-guided fractionation approach, and the structures of isolated compounds were determined using modern homo-and heteronuclear two-dimentional NMR experiments. The essential oil of *A. persica* was obtained by hydrodistillation and analyzed by GC/MS. **Results:** Methanolic extract of *A. persica* showed a moderate effect against the *Plasmodium* parasite compared Chloroquine. The yield of essential oil was 0.40 % Davanone, as the major constituent. Phytochemical investigation of the extract led to isolation of Friedelin, Ascaridol, Scopoletin, Scopolin and two other unknown compounds. Discussion: Davanone was the major constituent of *A. persica* while the other studies indicated that the major constituent of volatile oil is 1,8-Cineol. The results showed that although isolated compounds like Friedelin, Ascaridol, Scopoletin and etc. might have different biological effect but not high antiplasmodial. However comparing with artemisinin the compounds Ascaridol and Friedelin (specially its derivative, ß-Amyrin) showed week antiplasmodial effect. In conclusion there is improbable to find major compounds with high antimalarial effect in *Artemisia persica* or other species of *Artemisia*. *Acknowledgement*: I would like to give my best gratefulness to Majid Sairafianpour for all his efforts and kindnesses in Copenhagen. **References** 1.Marco, J.A., Barbera, O., (1990), Natural Products from Genus Artemisia L., in Studies in Natural Products Chemistry, Attaur-Rahman ed., London, Elsevier, Vol.7, 201 – 264. 2. Wright, C.W. (2002), Artemisia, New York: Taylor & Francis Inc..

P 137

Induction of naphthoquinone and flavonoid production in Dionaea muscipula and Drosera capensis

Krolicka A[1], Szpitter A[1], Gilgenast E[2], Romanik G[2], Kamiński M[2], Lojkowska E[1]
[1]Department of Biotechnology UG & AMG, Kladki 24, 80 – 822 Gdansk, Poland; [2]Technical University of Gdansk, Chemical Faculty, Analytical Chemistry Department, Narutowicza 11/12, 80 – 952 Gdansk, Poland

The secondary metabolites (naphthoquinones: plumbagin and ramantaceone, flavonoids: myricetinand quercetin) from Droseraceae plants: *Dionaea muscipula* and *Drosera capensis* 'Broadleaf' are used as anticancer drugs and antispasmodic agents. The aim of the study was to check the ability of the biotic elicitors to induce the production of secondary metabolites in *in vitro* grown *D. muscipula* and *D. capensis* 'Broadleaf'. The optimal conditions for micropropagation of both species were described as: 0.75 % agar solidified ½ MS medium with 25 mg/l ascorbic acid and 2 % sucrose, pH 5.6. Autoclaved overnight suspension of *Agrobacterium rhizogenes* and a crude elicitor from *Verticillium dahliae* Kleb. were added to ½ MS medium as elicitors, to the final concentration of 2.5 % and 0.2 – 0.4 mg%, respectively. A 4 – 6-week-old plantlets were planted on these media and after 30 days of growth they were collected and the extraction of naphthoquinones and flavonoids was performed. The extraction was carried out in an ultrasonic bath Sonic-5. Quantitative and qualitative determination of naphthoqinones and flavonoids in chloroform and methanol extracts was performed by using NP – HPLC/UV-DAD. Dihydroksypropyl stationary phases, hexane and tetrahydrofuran mixture as eluent and gradient elution were used. HPLC analysis of chloroform extracts indicated 3 times higher accumulation of plumbagin in *D. muscipula* plants elicited with *A. rhizogenes* than in the control ones. In *D. capensis* elicited with *A. rhizogenes* about 100 % increase of ramantaceone was determined. Also increase of myricetin and quercetin content was observed after biotic elicitation. Chloroform and methanol extracts obtained from *A. rhizogenes* elicited *D. muscipula* and *D. capensis* plants show antimicrobial activity. Methanol extracts of *D. muscipula* exhibit the strongest antimicrobial activity against a broad spectrum of tested human pathogenic bacteria: *Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*; MBC from 25 to 75 mg fw/mL. *Acknowledgements*: State Committee for Scientific Research, Grant No KBN 0430/P04/2004/26.

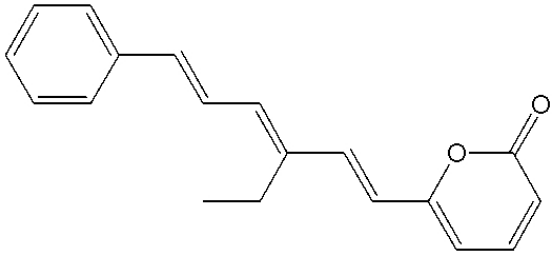
P 138

Exploring the structural diversity of myxobacterial secondary metabolism

Ohlendorf B, Erol Ö, Krick A, Kehraus S, König GM
Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, D-53115 Bonn, Germany, bohlendo@gmx.de

Myxobacteria are gram-negative bacteria commonly found in soil. They form swarm-like colonies which spread over surfaces and feed on other microorganisms. The most outstanding feature of myxobacteria is their ability to form fruiting bodies by which the different genera can be distinguished. In the last years myxobacteria have been in the focus of natural products research and have become known as potent producers of secondary metabolites. To date more

than fifty unique structural types have been isolated, among them compounds with promising biological activities, e. g. the epothilones [1] which are now in clinical studies as anticancer drugs. In our screening program the myxobacterial strain 150 (morphologically characterized as a *Polyangium* or *Nannocystis* sp.) was singled out due to the results of TLC, LC-MS and NMR analyses. The next step was the cultivation of the strain in a large scale and subsequent extraction and fractionation. HPLC separation eventually yielded compound 150E, a new metabolite with an unusual ethyl residue connected to a polyunsaturated carbon chain. The structure was elucidated by applying mass spectrometry and different 1D- and 2D- NMR techniques. Future work will address the biosynthesis and evaluation of the bioactivity of the compound.



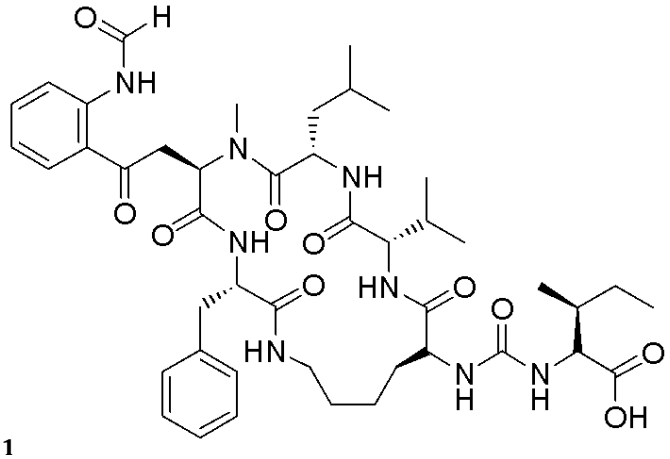
Reference: 1. Gerth, K. *et al.* (1996), J. Antibiot. 49: 560 – 563

P 139

New cyclic peptides from the cyanobacterium Tychonema sp

Mehner C, Kehraus S, Krick A, Müller D, König GM
Institute for Pharmaceutical Biology, University of Bonn, Nußallee 6, D-53115 Bonn, Germany, mehchr2@web.de

Cyanobacteria are an amazing source of new compounds of pharmaceutical interest. A specific feature of their secondary metabolite spectrum is the occurrence of cyclic peptides containing unusual amino acids. Our project focuses on a group of bioactive cyclic peptides, which are present in the methanolic extract of the cyanobacterium *Tychonema* sp. This microorganism was isolated from a field sample collected from a pond of a sugar factory near Braunschweig (Germany). To date the new cyclic hexapeptides brunsvicamides A-C were identified. Brunsvicamide C contains a unique *N*-methylated-*N'*-formyl-kynurenine moiety **1** [1]. The brunsvicamides are related to the sponge-derived mozamides.



1

Furthermore two cyclic peptides with $[MH^+]$= 1486 and $[MH^+]$= 1456 were isolated. The structure elucidation of these peptides is still ongoing using a combination of NMR methods and MS/MS spectroscopy. **Reference**: 1. König, G.M., Kehraus, S. *et al.* (2006), ChemBioChem 7: 229 – 238.

P 140

Safety Assessment and Metabolic Fingerprinting of GMO Gerberas

Pohjala L[1,2], Tammela P[1,2], Ainasoja M[3], Somervuo P[3], Teeri T[3], Vuorela P[4]
[1]Drug Discovery and Development Technology Center, Faculty of Pharmacy, P.O. Box 56, 00014 University of Helsinki, Finland; [2]Division of Pharmaceutical Biology, Faculty of Pharmacy, University of Helsinki, Finland; [3]Department of Applied Biology, Faculty of Agriculture and Forestry, University of Helsinki, Finland; [4]Pharmacy, Faculty of Mathematics and Natural Sciences, Åbo Akademi University, Turku, Finland

Genetically modified organisms (GMOs) have raised concerns in general public and involve also subjects of scientific interest, as it is still not exactly known how single genes brought about by means of genetic engineering affect other genes and thus the metabolism of the plant. In this work, effects of 228 GMO *Gerbera hybrida* lines and 42 traditional gerbera varieties on human gastrointestinal epithelium Caco-2, bronchial epithelium Calu-3 and hepatocellular Huh-7 cell lines were assessed using WST-1 cell viability assay [1]. After collection, the inflorescence gerbera samples were freeze-dried, ground and extracted with methanol. The cell viability assays were performed in automated environment by exposing the cell cultures to 40 µg/mL and 100 µg/mL extracts for 24 hours. < 80 % and > 120 % threshold values were set to mark significant effects on cell viability. The statistical analysis of the frequencies of hit extracts found no differences between GMO and non-GMO lines in any of the cell lines used. In addition to the cell viability testing, the metabolic fingerprinting of the extracts was performed with TLC. The principal component analysis did not separate GMO lines from non-GMO lines, whereas the nearest neighbour classifier method found the right neighbour in 46.3 % of samples when 42 different transgenic groups were formed. These results indicate that even though some metabolic differences between traditional and GMO gerbera lines may exist, these differences seem not to affect human cell viability in vitro. **Reference**: 1. Ishiyama, M. (1995), In Vitro Toxicol. 8:187 – 190.

P 141

Soyabean Lipoxygenase Inhibitory Activity of Flavonoids, Phenylethanoid glycosides and phenolic acids from Marrubium velutinum and M. cylleneum

Karioti A[1], Hadjipavlou-Litina D[2], Skaltsa H[1]
[1]Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece; [2]Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotelian University of Thessaloniki, Thessaloniki, 54124, Greece

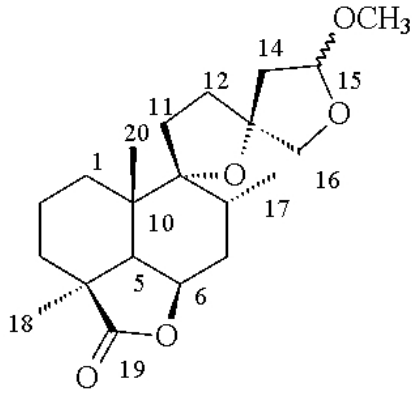
Marrubium sp. are widely used in traditional medicine in Greece in cases of inflammatory, bronchial, stomach diseases, coughs and catarrhs of the respiratory tract. In previous papers, we reported the isolation and identification of secondary metabolites from the methanol extracts from the aerial parts of *M. velutinum* Sm. and *M. cylleneum* Boiss. et Heldr. [1, 2]. In a continuation of our chemical and biological investigations on *Marrubium* species of the Greek flora, we studied the isolated phenolic compounds *in vitro f*or their interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical and for their inhibitory activity against soybean lipoxygenase. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor. The initial methanol extracts and 25 secondary metabolites have been tested. Phenylethanoid glycosides were found to be active. **References**: 1 Michelis, F., Tiligada, E. *et al.* (2002), Pharm. Biol. 40: 245 – 248. 2. Karioti, A. *et al.* (2003), Phytochemistry 64: 655 – 660.

P 142

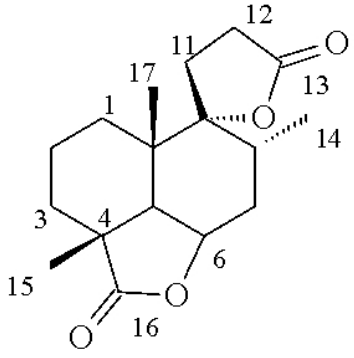
Novel cytotoxic labdane diterpenes from Marrubium cylleneum

Karioti A[1], Skopeliti M[2], Heilmann J[3], Tsitsilonis O[2], Skaltsa H[1]
[1]Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece; [2]Department of Animal and Human Physiology, School of Biology, Panepistimiopolis-Zografou, 15771 Athens, Greece; [3]Institute of Pharmacy, Department of Pharmaceutical Biology, University of Regensburg, Universitätsstrasse 31, D-93040, Regensburg, Germany

In continuation of our phytochemical investigations into *Marrubium* species of the Greek flora [1], we report on the isolation and identification of further novel secondary metabolites from the dichloromethane extract from the aerial parts of *Marrubium cylleneum* Boiss. & Heldr.. One labdane diterpene (**1**) and one labdane nor-diterpene (**2**) have been isolated along with β-sitosterol and palmitic acid. The structures of the isolated compounds were established by means of 1D & 2D NMR and MS spectral analyses. Both new and previously isolated diterpenes were tested for their immunomodulating potential in standard *in vitro* cytotoxicity assays [2]. Peripheral blood mononuclear cells (PBMC) from normal donors and cancer patients were isolated and subsequently incubated with low concentrations of each compound for 1 – 3 days. Effectors PBMC were further assayed for enhancement of their lytic ability against ^{51}Cr-labeled target cells (K562, Daudi and Jurkat) at effect or to target ratios varying between 10 – 80:1. Some of these compounds could be potentially used to enhance PBMC anticancer activity, as they enhance immune responses of human lymphocytes, inducing cell proliferation and augmenting their cytotoxicity against tumor targets, whereas at the same time they present significant antitumor activity, efficiently lysing leukemic cells.



1



2

References: 1. Karioti, A. *et al.* (2005), Phytochemistry 66: 1060 – 1066. 2. Tsavaris, N.B. *et al.* (2004), Oncology 67: 403 – 410.

P 143

Extraction and chromatographic analysis of ginsenosides occurring in roots and leaves of Panax quinquefolium grown in Poland

Ludwiczuk A[1], Wolski T[1,2], Głowniak K[1]
[1]Department of Pharmacognosy with Medicinal Plant Laboratory, Skubiszewski Medical University, 1 Chodźki Street, 20 – 093 Lublin, Poland; [2]Department of Vegetable and Medicinal Plant, University of Agriculture, 58 Leszczyński Street, 20 – 068 Lublin, Poland

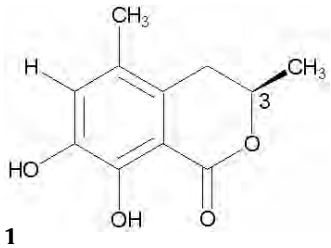
A wide variety of extraction methods has been employed for the isolation of ginsenosides from plant material. They are *e.g.*: extraction in Soxhlet apparatus [1], ultrasound-assisted extraction (UAE) [2], microwave-assisted extraction (MAE) [3], pressurized liquid extraction (PLE, ASE) [4], and supercritical fluid extraction (SFE) [5]. In presented studies, for isolation of ginsenosides from ginseng cultivated in Poland, three different extraction methods such as: ASE, UAE and mechanical shaking assisted solvent extraction was applied. The separation of compounds was achieved with water-acetonitrile gradient system using a C_{18} reversed-phase column. The highest extraction efficiency of ginsenosides in roots and leaves of *Panax quinquefolium* L. was observed during mechanical shaking with 50 % aqueous methanol (total concentration for roots – 4.3 % and for leaves – 9.1 %). Extraction efficiency of ginsenosides by ASE is comparable to that obtained by sonication. The main compound of *P. quinquefolium* roots is ginsenoside Rb_1. The amount of this compound in ginseng roots was ranged from 1.36 % to 3 % according to extraction method and extraction solvent used. Ginsenosides Rd, Rg_2 and Rb_2 are the main compounds occurring in American ginseng leaves. They content in raw material after mechanical shaking was 3.8 %, 2.4 % and 1.4 % respectively. Ginseng leaves, in comparison to ginseng roots are characterized by higher concentration of ginsenosides. Therefore, based on the concentration of major saponins, leaves can by alternative to root source of ginsenosides used in herbal preparations. **References**: 1. Chuang, W.C., Sheu, S.J. (1994), J. Chrom. A 685: 243 – 251. 2. Wu, J. *et al.* (2001), Ultrason. Sonochem. 8: 347 – 352. 3. Kwon, J.-H. *et al.* (2003), Food Research International 36: 491 – 498. 4. Choi, M.P.K. *et al.* (2003), J. Chrom. A 983: 153 – 162. 5. Wang, H.-C. *et al.* (2001), Food Chemistry 72: 505 – 509.

P 144

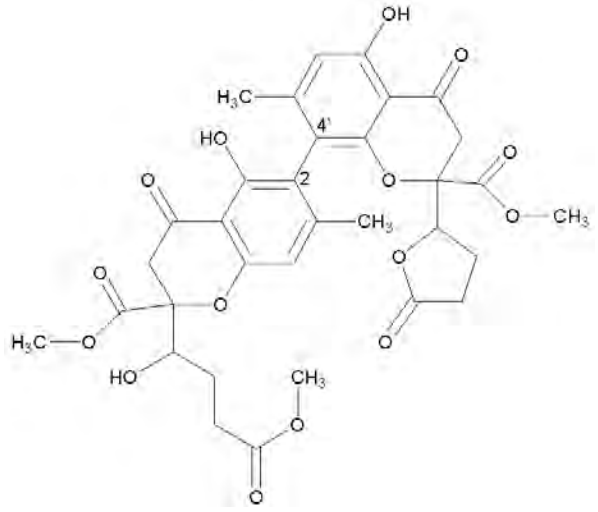
Aromatic Polyketides from the Marine Algicolous Fungus Nodulisporium sp

Pontius A, Kehraus S, Krick A, König GM
Institute for Pharmaceutical Biology, University of Bonn, Nußallee 6, 53115 Bonn, Germany, apontius@uni-bonn.de

Marine microbes, especially those living in close association with macroorganisms represent an important source of pharmacologically active natural products[1]. Investigation of the marine-derived fungus *Nodulisporium* sp. located in the inner tissue of a marine algal species led to the isolation of the new natural product (3*R*)-7-hydroxy-5-methylmellein (**1**) and a new polyketide with a dimeric xanthone structure (**2**). The new compound **1** is distinguished from other natural melleins[2] by its rare 7,8-ortho hydroxy substitution. The new dimeric xanthone **2**, presumably related to anthraquinones concerning its biosynthesis[3], consists of two subunits similar to ergochrome F. In one of these the lactone ring is cleaved to give a 4-hydroxy-butyric acid methyl ester moiety. The monomeric substructures are connected asymmetrically *via* carbon 2 and 4'. The stereochemistry of the four chiral centres and the chiral axis is still under investigation.



1



2

References: 1. König, G.M. *et al.* (2006), ChemBioChem. 7: 229 – 238. 2. Krohn, K. *et al.* (1997), Phytochem. 45: 313 – 320. 3.Tabata, N. *et al.* (1996), J. Antibiot. 49: 267 – 271.

P 145

Prevalence of three tetraene alkamide isomers in Echinacea angustifolia and Echinacea purpurea roots

Lehmann RP[1,2], Matthias A[1], Matovic N[2], Penman KG[1], Bone KM[1,3], de Voss JJ[2]

[1]MediHerb Research Laboratories, 3/85 Brandl Street, Eight Mile Plains, Brisbane, 4113 Australia; [2]School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, 4072 Australia; [3]School of Health, University of New England, Armidale, 2351 Australia

Three tetraene alkamide isomers were identified in *Echinacea angustifolia* DC. and *Echinacea purpurea* (L.) Moench roots by comparison with their synthetic cis-trans 8,10 counterparts which were synthesised using novel pathways. The three tetraenes were: (2E, 4E, 8Z, 10Z)-isobutyldodeca-2, 4, 8, 10-tetraenamide, the ZZ isomer, (2E, 4E, 8Z, 10E)-isobutyldodeca-2, 4, 8, 10-tetraenamide, the ZE isomer, and (2E, 4E, 8E, 10Z)-isobutyldodeca-2, 4, 8, 10-tetraenamide, the EZ isomer. The EZ isomer has not been previously reported to be present in Echinacea species. The relative concentration of each tetraene was examined in several commercially available samples by GCMS. The amount of each tetraene as a percentage of the total differed between the two species, with 10% and 29% of the ZZ isomer, 80% and 63%of the ZE isomer and 10% and 8% of the EZ isomer in *Echinacea angustifolia* and *Echinacea purpurea* respectively. These species differences between *Echinacea angustifolia* and *Echinacea purpurea* roots may help to explain experimental differences in the activity of preparations from either species as well as the variations in their efficacy noted in clinical trials.

P 146

Identification of spiroketal polyacetylenes as the main components of an oil extract of chamomile (Chamomilla recutita L. Rausch.) flowers

Shikov AN[1], Laakso I[3], Pozharitskaya ON[1], Dorman HJD[3], Makarov VG[1], Tikhonov VP[2], Hiltunen R[3]

[1]Interregional Center "Adaptogen", 47/5, Piskarevsky pr, 195067, St-Petersburg, Russia; [2]Open joint-stock company "Diod", 11-A, ul. Derbenevskaya, 115114, Moscow, Russia; [3]Faculty of Pharmacy, Division of Pharmaceutical Biology, University of Helsinki, P.O. Box 56 (Viikinkaari 5E), FIN-00014 Helsinki, Finland

The composition of *Chamomilla recutita* L. Raush. (family Asteraceae) flowers extract obtained by polar solvents (water, ethanol) is well investigated. They contain flavonoids, coumarins, arene carboxylic acids, sesquiterpene lactones as precursors of chamazulene, etc. However, the extracts of this plant obtained by non-polar, natural plant fixed oils (soybean, olive, etc.) are significantly enriched by more hydrophobic compounds. This report is devoted to the identification of main compounds of an oil extract from the flowers of *C. recutita*. Chamomile oil extract have been obtained by original technology at the flowers/oil ratio 1:10 [1]. In our previous studies, it has been shown that the oil extract of the flowers contains flavonoids and coumarins and two major compounds. For concentration of these compounds, flowers were ground and extracted with diethyl ether/saturated sodium hydrogen carbonate solution (1:1, v/v). The non-silylated and silylated samples were injected into a GC-MS system consisting of an HP 5980 gas chromatograph coupled with an HP 5790A quadrupole mass selective detector operating at EI mode with electron energy of 70 eV and mass range of *m/z* 40 – 400. The injector and detector were set at 250 °C. The analyses were performed on an NB-54 capillary column (15 m x 0.25 mm i.d., Nordion, Finland) with split injection mode. Identification of compounds was done by comparing the retention times and spectral data obtained from GC-MS library and literature. Total ion analyses of the sample showed excellent resolution between the two compounds. Retention times were 17.8 ± 0.1 min and 18.0 ± 0.1 min, and their molecular weights were 200. Both compounds had molecular ions at m/z 200 and other fragments at m/z 185, 170, 157, 144, 141, 128, 115, 102, 76. Based upon the library and literature data, the compounds were identified as en-yn-dicycloethers (spiroketal polyacetylenes) (*E*)-2-[2,4-hexadiyniliden]-1,6-dioxaspiro[4,4]-non-3-ene and (*Z*)-2-[2,4-hexadiyniliden]-1,6-dioxaspiro[4,4]-non-3-ene. Spiroketal polyacetylenes are powerful inhibitors of NFκB activity, and they occur in chamomile [2], where they might synergize the activity of other inflammatory principles, like the bisabolane-type sesquiterpenoids and chamazulene. **References**: 1. Shikov, A.N. *et al.* (2004), Plants oil and oily extracts: technology, standardization, properties. M, Russky vrach. 2. Redaelli, C., Formentini, L. (1981), J. Chrom. A 209: 110 – 112.

P 147

Biologically active compounds from grated cocoa and cocoa butter samples

Kosman V[1], Stanckevich N[1], Makarov VG[1], Tichonov V[2]

[1]Interregional center Adaptogen, Puskarevsky prospect, 47/5, 195067, St-Petersburg, Russia; [2]Diod- factory of ecological technique and econutrition, Derbenevskaya str. 11-a, 115114, Moscow, Russia

Cocoa-products are source of biologically active substances and render the expressed and versatile pharmacological action – raise serviceability, regulate inflammatory mediators, normalize blood pressure, influence on immune system, inhibits growth of human breast cancer cells, etc. The results of five samples of grated cocoa and five samples of cocoa butter analysis under contents of the main biologically active components such as phenolic compounds – tannins, monomeric and polymeric proanthocyanidins, individual catechins; amino acids and their biochemical derivatives – purin alkaloids and biogenic amines; lipophylic compounds – fatty acids formed trigly-

cerides and phytosterines are presented. The investigation was done by modern instrumental methods such as HPLC, GC, UV-VIS-spectroscopy, and also with application of titrimetric and gravimetric methods. Grated cocoa samples were richer then cocoa butter for content of biologically active components. In the analyzed samples contents of total phenolics changes in an interval 1.0 – 3.2 %, including monomeric proantocyanidins 0.6 – 1.35 %; pyrroloquinoline quinine (PQQ) 0.34 – 0.76 μg/g; phenyl ethylamine from 2.79 to 14.97 μg/g, tyramine from 9.56 to 71.68 μg/g, dopamine from 5.3 to 25.85 μg/g; theobromine from 3.3 to 8 %, caffeine from 0.49 to 0.70 %; among the amino acids at the greatest quantities were presented glutaminic and asparaginic acids, arginin and leucin; three main fatty acids were determined – palmitinic (31 ± 2 % rel.), oleinic (35 ± 2 % rel.) and stearinic (35 ± 2 % rel.); the main phytosterins were sytosterin (up to 192 mg%) and obtusifoliol (up to 198.5 mg%). Antiradical and protective effects of cocoa can be connected with proanthocyanidins and pyrroloquinoline quinone presence, soft stimulating action on the central nervous system and spasmolythic effect are caused by alkaloids theobromine and coffeine, emotional and angioprotective effects, increase of serviceability and sexual potention – by presence of biogenic amines (phenyl ethylamine, tyramine, dopamine). *Acknowledgements*: Prof. I.G. Zenkevich (St.-Petersburg State University, Russia), Dr. I.K. Zurkovich (Institute of Toxicology, St.-Petersburg, Russia), Dr. E.A. Protasov (Institute of high clear substances, St.-Petersburg, Russia)

P 148

Isoflavonoids in the Cannabaceae family

Koblovská R[1], Kokoška L[2], Klejdus B[3], Lapčík O[1]
[1]Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic; [2]Institute of Tropical and Subtropical Agriculture, Czech University of Agriculture in Prague, Kamýcká 129, 165 21 Praha 6, Czech Republic; [3]Department of Chemistry and Biochemistry, Mendlova University of Agriculture and Forestry, Zemìdìlská 1, 613 00 Brno, Czech Republic

Isoflavones (3-phenyl chromones) are biologically active secondary metabolites found in a limited number of taxa with unclear phylogenetic relationships. They are abundant in the Fabaceae family (about 1000 known structures) and a few others, *e.g.* Iridaceae and Moraceae. In recent study, we have tested two representatives of the Cannabaceae family, i. e. *Humulus lupulus* L. cultivars Orion and Magnum and *Cannabis sativa* L. cultivars Manitoba poison and Duke foot, for the presence of ten metabolites synthesized at the early steps of the isoflavonoid biosynthetic pathway. Six compounds of interest were aglycones (i. e. daidzein, genistein, formononetin, isoformononetin, biochanin A, prunetin) and four were glycosides (i. e. daidzin, genistin, ononin, sissotrin). Leaves and hops were lyophilized, pulverized and extracted with a mixture methanol/water. The extracts were analyzed by HPLC-MS-SIM and by specific ELISAs. Both approaches revealed a spectrum of isoflavonoids, aglycones as well as glycosides, in all plants under study. The concentrations of individual compounds ranged from units up to hundreds of micrograms per kg (dry weight). Methoxy isoflavones prevailed to non-methylated ones. Previously small amounts of isoflavonoids have been found in beer, but their origin in this foodstuff was unclear. Mazur [1] detected daidzein and genistein in several samples of barley. Our data indicate that hops may represent an additional source of isoflavonoids in beer. Moreover, this is the first report on isoflavonoids in the Cannabaceae family. *Acknowledgment:* This study was supported by the grant GACR 525/06/0864. **Reference**: 1. Mazur, W., Adlercreutz, H. (1998), Pure & Appl. Chem. 70: 1759 – 1776.

P 149

Detection of isoflavonoids in selected representatives of the Solanaceae family

Koblovská R[1], Klejdus B[2], Kokoška L[3], Lapčík O[1]
[1]Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Technická 5, 166 28 Praha 6, Czech Republic; [2]Department of Chemistry and Biochemistry, Mendl University of Agriculture and Forestry, Zemìdìlská 1, 613 00 Brno, Czech Republic; [3]Institute of Tropical and Subtropical Agriculture, Czech University of Agriculture in Prague, Kamýcká 129, 165 21 Praha 6, Czech Republic

Selected representatives of the Solanaceae family have been tested for the presence of ten isoflavonoids, namely daidzein, genistein, formononetin, isoformononetin, biochanin A, prunetin, daidzin, genistin, ononin and sissotrin. Plant material was obtained in the Botany Garden of Charles University and in Czech University of Agriculture. Following species have been tested: *Nicotiana tabacum, N. alata., N. sanderae* Hort., *N. glauca* Graham, *N. silvestris, Lycopersicon esculentum* Mill., *Solanum dulcamara* L.. Leaves and inflorescence stalks were freeze-died, pulverized and extracted with methanol/water. Extracts were analyzed by HPLC-MS-SIM and by specific immunoassays. Both approaches revealed a spectrum of isoflavonoids, aglycones as well as glycosides, in all Solanaceae plants under study. The concentrations of individual compounds ranged from units of micrograms up to two milligrams per kg (dry weight). Methoxy isoflavones (both types, *i. e.* 4'-methoxy as well as 7-methoxy) prevailed to non-methylated ones. Prunetin was the most abundant aglycone, followed by formononetin and biochanin A, sissotrin was the most abundant glycoside. Isoflavones were more abundant in the inflorescence than in leaves, the content in stalks was negligible. Traces of individual isoflavones were detected also in tomato jujice. Up to now, the only isoflavonoid described in the Solanaceae was torvanol from *Solanum torvum* (1). Our data indicate, that isoflavonoid metabolism may occur generally in the Solanaceae. *Acknowledgment:* This study was supported by the grants MSM6046137305 and GACR 525/06/0864. **Reference**: 1. Arthan, D. et al. (2002), Phytochemistry 59: 459.

P 150

Qualitative and quantitative analysis of grape seeds by HPLC-MS

Mayer R[1], Stecher G[1], Sultana T[1], Trojer L[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020, Innsbruck, Austria; [2]Bionorica AG; Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Seeds mainly consist of 35 % crude fibres, 29 % nitrogen free compounds (*e.g.* polyphenols), 15 % crude lipids, 11 % crude proteins, 3 % ash and 7 % water [1]. Especially the class of polyphenolic compounds presents an interesting source for pharmacologically active plant ingredients. The heterogeneity of phenolic compounds in the sample as well as the similarity of some compounds poses a challenge to every analyst. In literature catechin and epicatechin as well as their oligomers already are described in grape seeds [2, 3]. The aim of this work was extraction, separation and qualitative as well as quantitative analysis of phenolic compounds of grape seeds. Extraction was optimized on basis of different extraction methodologies as well as on the basis of diverse solvents. Variable forms of stationary phases were tested for their separation efficiency. Therefore not only conventional carrier material but also monolithic systems in capillaries and columns were evaluated. The results of these experiments clearly show that microwave extraction results in highest yields of phenolic compounds in connection with short duration time possible. A mixture of methanol water (50/50 v/v) was chosen as extraction solvent. Monolithic carrier systems show higher separation efficiency in comparison to other stationary phases. The separation efficiency of a new carrier material on basis of monolithic poly(*p*-methylstyrene-co-1,2-bis(*p*-vinylphenyl)ethane [4] in capillaries can be impressively demonstrated by separation of small

molecules. Qualitative analysis of grape seeds allows the identification of monomers, oligomers (e. g., stilbene-oligomers) and of glycosidic flavonoids. Finally due to the lack of commercially available standards the importance of mass spectrometry is pointed out. **References**: 1.
Dietrich, H. (2005), Der Deutsche Weinbau, pp. 34 – 39. 2. Gabetta, B., Fuzzati, N. (2000), Fitoterapia, 71: 162 – 175. 3. Peng, Z., Hayasaka, Y. (2001), J. Agric. Food Chem.) 49: 26 – 31. 4. Bonn, G. K., Lubbad, S., Trojer, L. (2005), patent pending.

P 151

Quality assessment of flavonoids and polyphenolic compounds in green tea samples belonging to different origins

Sultana T[1], Stecher G[1], Mayer R[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020 Innsbruck, Austria; [2]Bionorica AG; Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Components of green tea (*Camellia sinensis*) have been of considerable interest in recent years because of their potential utility as pharmaceutical agents [1]. So a comprehensive approach was adopted to carry out analysis for the quality assessment of flavonoids in tea samples belonging to different origins. For this purpose extraction, thermal decomposition investigations, separation and mass spectrometric detection parameters were optimized. Extraction methods tried so far include, reflux extraction, a modified accelerated solvent extraction (ASE) namely aquasolve extraction [2] and microwave assisted extraction [3] (MAE). For separation a HPLC method using different C18 stationary phases was established. In this coherence, the influence of material itself *i. e.* monolithic silica (Chromolith, Merck) and silica particles (Prontosil, Bischoff) was studied. HPLC-DAD (diode array detector) and HPLC-ESI-MS (electrospray ionization mass spectrometry) were used for simultaneous detection, rendering MS more reliable owing to high specificity and sensitivity. Results clearly demonstrated MAE in 50 % ethanol to be best extraction method giving highest yields in shortest possible time. Thermal decomposition studies for two standards, quercetin and gallic acid revealed that approx. 20 % of quercetin in aquasolve method is lost. Additionally aquasolve extraction showed high degree of epimerization [4] as compared to MAE, but it did not accompany the complete conversion of green tea epicatechins (GTE) to their corresponding epimers. Rather some irreversible degradation phenomenon was also involved. Optimized separation system was finally used for qualitative and quantitative investigation of catechin derivatives, oligomers and polymers, from different green tea samples. Within this approach a correlation to high quality products could be noticed. **Refrences**: 1. Harborne, J.B. (1998), Phytochemical Methods, Chapman & Hall, London. 2. Bonn, G., Hörmeyer, H.F., Bobleter, O. (1987), Wood Science and Technology 21: 179 – 185. 3. Pan, X. (2003), Chemical Engineering and Processing 42: 129 – 133. 4. Jin Ze Xu, (2003), Sci. Food Agric. 83: 1617 – 1621.

P 152

LC-PDA-MS-profiles of phenolic compounds in extracts of aerial parts of Urtica species

Bucar F[1], Britzmann B[1], Streit B[1], Weigend M[2]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Graz, Universitaetsplatz 4/1,A-8010 Graz, Austria; [2]Institute of Biology – Systematic Botany and Plant Geography, Freie Universität Berlin, Altensteinstr. 6, D-14195 Berlin, Germany

Extracts of the aerial parts of nettle (Urticae folium/herba) are used for adjuvant therapy of rheumatic ailments and as a diuretic in inflammatory disorders of the lower urinary tract [1]. The active constitutents are supposed to be phenolic acids and flavonoids. However, the taxonomy of *Urtica* seems to be complex and numerous subspecies and varieties of *U. dioica* L. exist. Hence we undertook LC-PDA-MS analyses of a range of *U. dioica* samples (including subspecies and varieties) and compared their profiles of phenolic acids and flavonoids with those of *U. urens* L., *U. galeopsifolia* Wierzb. ex Opiz, *U. flabellate* Kunth., *U. platyphylla* Wedd., *U. pubescens* Ledeb., *U. peruviana* Goltman and *U. mexicana* Liebm.. In all *U. dioica* samples neochlorogenic acid, chlorogenic acid and caffeoylmalic acid together with the rutinosides and glucosides of quercetin, kaempferol and isorhamnetin could be detected which was in accordance with literature [2, 3]. Additionally kryptochlorogenic acid, 2-caffeoyltartaric acid and p-cumaroylquinic acid was found. *U. urens* contained predominantly chlorogenic acid, no caffeoylmalic acid could be detected (4). Further constituents which were new for the genus *Urtica* were feruloylmalic acid (*U. flabellata, U. peruviana*), cichoric acid, feruloyltartaric acid, schaftosid and orientin (*U. peruviana*). In an in *vitro assay* on 12-LOX inhibition (5) the methanolic extract of *U. platyphylla* and *U. flabellata* showed the highest activities (% inhibition at 100 µg/mL: 53.6 ± 10.5 and 58.2 ± 16.6, respectively). **References**: 1. ESCOP Monographs, 2nd edition, ESCOP, Exeter, and Thieme, Stuttgart. 2. Budzianowski, J. (1991), Planta Med. 57:507 – 515. 3. Chaurasia, N., Wichtl, M. (1987), Planta Med. 53: 432 – 434. 4. Schomakers, J. *et al.* (1995), Dtsch Apoth Ztg 135: 578 – 84. 5. Schneider, I. *et al.* (2004), Planta Med, 70:471 – 74.

P 153

Quantitative Determination of 1-Deoxynojirimycin in Mulberry Leaves using Liquid Chromatography-Tandem Mass Spectrometry

Nuengchamnong N[1], Ingkaninan K[2], Kaewruang W[3], Wongareonwanakij S[3], Hongthongdaeng B[3]
[1]Regional Medical Sciences Center Phitsanulok, Department of Medical Sciences, Ministry of Public Health 65000 Thailand. nitra@dmsc.moph.go.th; [2]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University 65000 Thailand; [3]The Queen Sirikit Institute of Sericulture, Chatuchak, Bangkok 10900, Thailand

An HPLC-MS/MS method was developed for the quantitative determination of 1-deoxynojirimycin (DNJ), a potent glucosidase inhibitor presented in mulberry leaves (*Morus alba* L.). DNJ was separated from an extract of mulberry leaves on a TSK gel Amide-80 column using a mixture of 0.1 % formic acid and acetonitrile as the mobile phase at a flow rate of 0.6 mL/min. A triple quardupole mass spectrometry using turbo spray ionization source in the positive ion mode under multiple reaction monitoring with the $[M-H]^+$ ions, *m/z*164.4/ 109.9 was used. The detection limit (S/N = 3) was 75 pg and quantification limit (S/N = 10) was 100 pg. The DNJ was higher in young shoots of mulberry in comparison with young leaves and mature leaves.

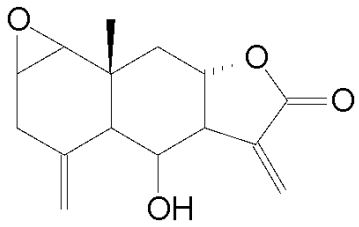
P 154

Sesquiterpene Lactones and Flavonoids from the aerial parts of Anthemis melanolepis L

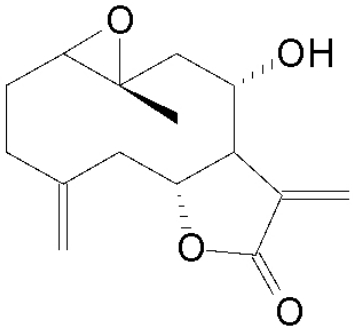
Skaltsa H[1], Saroglou V[1], Kypriotakis Z[2]
[1]Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, GR– 157 71, Athens, Greece; [2]Technological Education Institute, School of Agricultural Production, Laboratory of Taxonomy and Management of Wild Flora, Stavromenos P.O. Box 140, Heraclion-Crete, 71110, Greece

Continuing our research on the chemical constituents from the aerial parts of Greek *Anthemis melanolepis* L., a species belonging to the section Cota [1], we report here the isolation and identification of sesquiterpene lactones **1**-**6**, flavonoids **7**-**11**, p-anisic acid and protocatechic acid. The isolation was proceeded according to the Bohlmann isolation method [2]. The structures of the isolated compounds **1 – 10** were elucidated by spectroscopic methods, particularly high-field NMR spectroscopy. Compounds **1** and **2** namely melanolepin B and melanolepin C are two new naturally occurring of

sesquiterpene lactones. Besides compounds **1** and **2**, four known sesquiterpene lactones and five flavonoids were isolated, namely β-cyclopyrethrosine (**3**), β-hydroxy-1-desoxotamirin(**4**), 1α-hydroxyacetyltulirinol, 4α,5β-epoxide (**5**), deacetylludalbin (**6**), apigenin **(7)**, 7, 4' dimethylether- apigenin **(8)**, dihydrokaempferol **(9)** and 5,7,3'-trihydroxy-3,6,4'-trimethoxy-flavonol **(10)** and naringenin **(11)**.



1



2

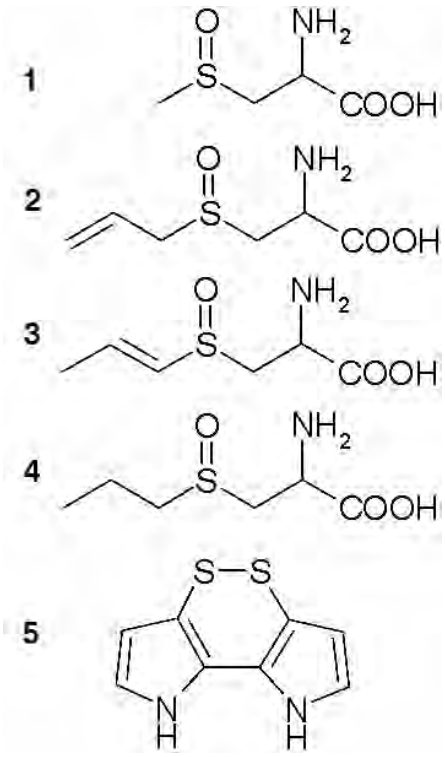
References: 1. Davis, P. H. (1975), Flora of Turkey and the East Aegean islands, Edinburgh, Vol. 5: 174 – 221. 2. Bohlmann, F. *et al.* (1984), Phytochemistry 23: 1979 – 1988.

P 155

Sulphur Chemistry of Drumstick Onions (Allium Subgenus melanocrommyum)

Jedelská J[1], Keusgen M[1], Fritsch RM[2]
[1]Philipps-Universität Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany; [2]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany

Mountainous areas of Central Asia show a high variety of plants belonging to the genus *Allium* (1). Out of this, the subgenus *melanocrommyum* is most prominent, but only a few species were noticed as ornamentals in the western world. In contrast, several species are highly estimated by local populations as food or medicinal plant. However, the concentration of sulphur compounds as shown in the Figure is usually rather low. Typically, bulbs contain the cysteine sulphoxide methiin (**1)** in noteworthy amounts. Alliin (**2)** and propiin (**4)** could be detected in trace amounts, whereas some species also produce isoalliin (**3)**. Normally, plant material exhibits no or only a weak onion or garlic like smell. However, there are exceptions: *Aliium stipitatum* Regel and *A. suworowii* Regel emit a strong (and unpleasant) smell but have lower cysteine sulphoxide contents than odourless taxa like *A. jesdianum* Boiss. et Buhse and *A. hollandicum* R.M. Fritsch. Besides cysteine sulphoxides, the activity of the enzyme alliinase must be also considered for the formation of odour compounds. In addition to cysteine sulphoxides listed above, a number of species like *A. giganteum* Regel, *A. rosenorum* R.M. Fritsch, *A. jesdianum*, and *A. komarowii* Lipsky produce a red-staining substance with the chemical structure of a sulphurpyrrol (**5**). This compound showed some bioactivity, but the function inside the plant is unknown yet.



Acknowledgements: Research was supported by the German VolkswagenStiftung as part of the "PharmAll"-project. **Reference**: 1. Fritsch, R.M., Keusgen, M. (2006), Phytochem. (in press).

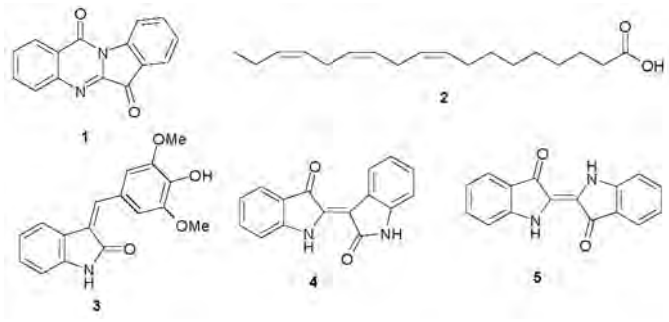
P 156

Simultaneous quantitative analysis of the active principles and pigments in leaf extracts of Isatis tinctoria by HPLC/UV/MS

Potterat O, Mohn T, Hamburger M
Institut für Pharmazeutische Biologie, Universität Basel, CH-4056 Basel, Switzerland

Woad (*Isatis tinctoria* L., Brassicaceae) has been used in Central Europe since antiquity for the treatment of inflammatory disorders [1]. Trypthanthrin (**1**), linolenic acid (**2**), and indolin-2-one **3** were shown to be active principles inhibiting COX-2, 5-LOX, the expression of i-NOS, and the release of histamine. Indirubine (**4**) is a potent inhibitor of CDK5 and GSK-2, and indigo (**5**) is a side product of indirubine formation. Lipophilic extracts showed anti-inflammatory activity in animals and in a clinical pilot study. Quantitative data on the pharmacologically active principles is essential in view of a possible develop-ment of phytopharmaceuticals. We developed and validated a HPLC procedure for the quantitative analysis of **1 – 5**. The assay combines ESI^+, ESI^- and UV detection modes and enables the determination of all compounds in a single HPLC run. The method has been applied to the analysis of extracts obtained by

accelerated solvent extraction (ASE) and supercritical fluid extraction.



Reference: 1. Hamburger, M. (2002), Phytochem. Rev. 1: 333 – 344.

P 157

An UPLC-MSTOF investigation of the leaves of Arrabidaea patellifera

Martin F[1], Hay AE[1], Gupta MP[2], Hostettmann K[1]
[1]Laboratory of Pharmacognosy and Phytochemistry, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Quai Ernest-Ansermet 30, CH-1211 Geneva 4, Switzerland; [2]Center for Pharmacognostic Research on Panamanian Flora (CIFLORPAN), College of Pharmacy, University of Panama, Panama City, Panama

As a part of our ongoing investigations on Panamean Bignoniaceae, several species were submitted to rapid TLC autobiographical tests, against *Cladosporium cucumerinum* and radical scavenging activity. *Arrabidaea patellifera* (Schlecht) Sandw, a liana which grows from lowlands to mountains forest, has been selected due to the good activity observed against *Cladosporium,* and moreover this plant has never been investigated.
The methanol extract has been prepurified by SPE, separated and analyzed by Ultra-Performance Liquid Chromatography coupled with High-Resolution Time of Flight Mass Spectroscopy (UPLC-TOF). Comparing datas (UV, exact mass) with those of *Arrabidaea samydoides* (1), several compounds with radical scavenging activity have been identified such as mangiferin, muraxanthone and other C-glucosylxanthones. The dichloromethane extract of *A. patellifera*s has been fractionated by centrifugal partition chromatography (CPC), medium pressure chromatography (MPLC) and semi-preparative HPLC. Several products have been isolated, such as a flavonoid (chrysin), an antocyanidine and triterpens (like ursolic acid).
Reference: 1. Pauletti, P. *et al.* (2003), J. Nat. Prod. 66: 1384 – 1387.

P 158

Genetic polymorphism, antitumour and antioxidant potential of Todelia asiatica on in vitro mice models

Ramaswamy V
International Centre for Bioresources Management, Malankara Catholic College Mariagiri, Kaliakkavilai. PIN 629 153. Tamil Nadu. India

The aim of the present study is to evaluate the antitumor effect and antioxidant role of *Todelia asiatica* against EAC bearing Swiss albino mice. The effect of methanol extract of *T. asiatica* on tumor growth and host's survival time was studied by the following parameters: tumor volume, packed cell volume, viable and non-viable cell count and life span of the host. Methanol extract was administered at a 125 and 250 mg/kg b.w. once a day for 14 days, after 24 h of tumor inoculation. Decrease in tumor volume, packed cell volume, and viable cell count were observed in Methanol extract treated animals when compared to EAC animals. Treatment with Methanol extract at a dose of 125 and 250 mg/kg increased the mean survival time to 29.5 ± 0.55 and 34 ± 0.2 days respectively. The extract also decreased the body weight of the EAC tumor bearing mice. Hematological studies reveal that the Hb content was decreased in EAC treated mouse, whereas restoration to near normal levels was observed in extract treated animals. There was a significant decrease in RBC count and increase in WBC counts in extract treated animals when compared to EAC treated animals. The study was also extended to estimate the liver biochemical parameters such as LPO, GSH, and antioxidant enzymes like SOD, CAT etc. Treatment with Methanol extract decreased the levels of lipid peroxidation and increased the levels of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The results suggest that the methanol extract exhibited significant antitumor and antioxidant effects in EAC bearing mice. Manganese superoxide dismutase (MnSOD) is a major enzyme that is responsible for the detoxification of reactive oxygen species in the mitochondria. A T → C substitution in the *MnSOD* gene resulting in a Val → Ala change at the -9 position of the mitochondrial targeting sequence (Val-9Ala), which alters the protein secondary structure and thus affects transport of MnSOD into the mitochondria was also analysed in the present study.

3. Genomics, Proteomics and Metabolomics in Medicinal Plant Research

P 159

Study of metabolites in benzothiadiazole treated Arabidopsis using nuclear magnetic resonance spectroscopy and principal component analysis

Thanh HDT[1,2], Puig RC[1], Kim HK[1], Choi YH[1], Verpoorte R[1]
[1]Pharmacognosy Department, Metabolomic Section, IBL, Leiden University, PO Box 9502, 2300RA Leiden, The Netherlands; [2]Traditional Pharmacy Department, Hanoi Pharmacy University, 23 Le Thanh Tong, Hanoi, Vietnam; [*]Email address: d.hien@chem.leidenuniv.nl

The resistance against pathogen infection in Arabidopsis is associated with systemic acquired resistance (SAR) gene induction. After infection, mRNAs of PR1, PR2 and PR5 genes accumulate in a coordinate manner in tissues that become resistant to subsequent pathogen infection [1]. The SAR pathway also can be activated by benzothiadiazole (BTH) [2, 3]. BTH has been shown to activate SAR in tobacco [4], wheat and Arabidopsis [5]. A powerful tool for plant metabolite analysis is high-resolution nuclear magnetic resonance spectroscopy (NMR) combined with principal component analysis (PCA). We used NMR and PCA to study the changes in metabolite profile of Arabidopsis after treatment with BTH. The results shown clear changes in the metabolite profile in the polar fraction which contain compounds such as flavonoids and phenylpropanoids... but the metabolite profile in non-polar fraction did not show any difference between BTH treated and non-treated Arabidopsis. *Acknowledgements*: Vietnamese overseas scholarship fund. **References**: 1. Uknes, S. *et al.* (1993), Mol. Plant-Microbe Interact. 6: 692 – 698. 2. Sticher, L. *et al.* (1997), Ann. Rev. Phytopathol. 35: 235 – 270. 3. Ryals, J. A. *et al.* (1996), Plant Cell 8: 1809 – 1819. 4. Friedrich, L. *et al.* (1996), Plant J. 10: 61 – 70. 5. Lawton, M.A. *et al.* (1996), Plant J. 10: 71 – 82.

P 160

Effects of Echinaforce® on differentiation and activity of antigen-presenting cells in vitro

Schoop R, Suter A
A. Vogel Bioforce AG, 9325-Roggwil, Switzerland

Extracts from different species of Echinacea are used today for treatment and prevention of upper respiratory tract diseases. No definite molecular way of action could be attributed to the observed clinical efficacy so far. Here we investigated the effects of endotoxin-free alcoholic fresh plant extract from *Echinacea purpurea* L. Moench (Echinaforce®) on different antigen-processing cells. Blood-isolated monocytes were differentiated into macrophages (MDM), into im-

mature (DDC) or mature dendritic cells (MDC). Phagocytosis of FITC-labelled bacteria was not affected in any of the above cell lineages upon incubation with Echinaforce®. Also the process of maturation from DDC into MDC was not influenced by Echinaforce® as shown by typical markers of differentiation. However Echinaforce® modulated LPS-induced cytokine expression in blood-derived monocytes. At concentrations of 1 ul/mL Echinaforce® (45 µg/mL dry mass) potently inhibited Interleukin-1 and Interleukin-6 expression after 12 h ($p < 0.05$). Minor and no inhibition were seen for TNF-alpha and Interleukin-8 respectively. The observed effects partly were amplified or reversed after 24 h of incubation indicating complex feedback regulation. As demonstrated earlier Echinaforce® did not significantly induce cytokine production even at high concentrations (50 µl/mL). LPS-induced expression of metalloproteinase (MMP-1 and MMP-9) also was reversed at 1 µl/mL of Echinaforce® after 24 h ($p < 0.01$). We postulate that Echinaforce® has no effect on differentiation- and maturation-process of various antigen-presenting cells. We further demonstrated that pyrogenic and inflammation-inducing mediators (IL-1, IL-6) in a first phase are potently down regulated by Echinacea extract. Although many cytokines show biphasic expression profiles a clear anti-inflammatory effect was shown.

P 161

Isolation of microsatellite markers in Hieracium pilosella L

Zini E[1], Komjanc M[2]
[1]Consiglio per la Ricerca e la sperimentazione in Agricoltura (CRA), Istituto sperimentale per l'Assestamento Forestale e per l'Alpicoltura (ISAFA), Piazza Nicolini, 6,38050, Villazzano (Trento), Italy; [2]Istituto Agrario di San Michele all'Adige, Via E.Mach 1, 38010, San Michele all'Adige (Trento), Italy

Hieracium pilosella L. (mouse-ear hawkweed) is a perennial, herbaceous weed belonging to the *Asteraceae* family. It grows in grassy and dry sites, on the edge of the fields and along the roads [1]. *H.pilosella* L. is mostly apomictic, but it is also known to have the potential to reproduce sexually under field conditions [2]. Additionally, populations possess high levels of genotypic variation, almost similar to outcrossing species [3]. The herb is mildly astringent, cholagogue, diaphoretic, strongly diuretic, expectorant and tonic [4]. A direct study of representative DNA would be most suitable for elucidating the genetic variability of species and establishing the genetic associations. Microsatellite or SSR (Simple Sequence Repeats) markers are co-dominant, multiallelic, highly polymorphic genetic markers and they are considered the most appropriate for genetic diversity studies. Microsatellite markers were developed in *Hieracium pilosella* using the SSR enrichment procedure (5). Three biotinylated probes $(CAA)_{10}$, $(CT)_{15}$ and $(GT)_{15}$, which were reported being rich in other plant species, were separately used to construct an enriched genomic library. Primer pairs for SSR analysis were designed on 34 different microsatellite regions detected. Eight developed SSRs were applied for genotyping 130 plants collected in 10 different locations in the Trentino region (Italy) and five of them showed polymorphism among the genotypes studied. The aim of this research was to evaluate the genetic diversity within different accessions of *Hieracium pilosella*. This is the first reported attempt of successfully SSRs isolation and characterization in this species. *Acknowledgements*: PARMA project financed by Autonomous Province of Trento, Claudio Varotto (CSBT) **References**: 1. Dalla Fior, G. (1969), La nostra flora. Monauni. Trento. 2. Houliston, G., Chapman, H. (2001), Am. J. Bot. 91: 37 – 44. 3. Chapman, H. *et al.* (2000), Heredity 84: 401 – 409. 4. Chiej, R. (1984), Encyclopaedia of Medicinal Plants. MacDonald. London. 5. He, G. *et al.* (2003), BMC Plant Biol. 3: 3.

P 162

DNA fingerprinting of medicinally used Derris species by RAPD molecular markers

Sukrong S, Phadungcharoen T, Ruangrungsi N
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, 10330, Bangkok, Thailand

DNA fingerprinting of five medicinal used *Derris* species, *D. scandens* (Roxb.) Benth, *D. elliptica* (Wallich.) Benth., *D. malaccensis* (Benth.), *D. trifoliate* Lour., and *D. reticulate* Benth., was studied using random amplified polymorphic DNA (RAPD) technique. Herbal drugs, *Derris*, were often sold in medicinal plant market as processed plant parts which often lose their original features, making them difficult to be differentiated morphologically. Accurate identification is needed in order to ensure their efficacy, thus RAPD was exploited as a molecular method. The screening of twenty deca-oligonucleotide primers allowed the selection of nine primers, OPS-03, OPS-05, OPS-07, OPS-08, OPS-12, OPS-14, OPS-16, OPS-17, and OPS-19, which revealed polymorphism. The results were reproducible. RAPD bands were scored for the presence and the absence from the photographic results and grouped by distance analysis using a pair-wise genetic similarity according to the index of Nei and Li. Dendrogram was generated by the unweighted pair-group method using arithmetic averages (UPGMA). Two clusters were revealed: the first consisted of *D. scandens, D. elliptica, D. malaccensis, and D. reticulata* and the second only *D. trifoliata*. Variation in DNA fingerprint detected among selected medicinal *Derris* species indicates the efficiency of RAPD molecular markers for the identification and construction of genetic relationship. *Acknowledgements:* Young Scientist Scholar, Ratchadapiseksompoach Research Funds, Chulalongkorn University, Bangkok, Thailand. **Reference**: 1. Sukrong, S. *et al.* (2006), Thai J. Pharm. Sci. 29: 155 – 163.

P 163

Metabolic engineering of plant cell cultures – towards the new resources of alkaloids

Häkkinen ST[1], Rischer H[1], Goossens A[2], Ritala A[1], Seppänen-Laakso T[1], Inzé D[2], Oksman-Caldentey KM[1]
[1]VTT Technical Research Centre of Finland, P.O. Box 1000, FIN-02044 VTT, Finland; [2]VIB Plant Systems Biology, University of Ghent, B-9000 Ghent, Belgium

Plants produce a wide range of secondary compounds, which have important functions for plants in survival and competing in the ecosystem. One of the major bottlenecks connected with the exploitation of plant cell cultures is that the biosynthetic pathways of secondary compounds are still poorly understood. We have designed a novel technology for unravelling the genes involved in the plant secondary metabolism. This technology called SoluCel® is based on the genome wide identification and functional analysis of genes involved in the production of phytopharmaceuticals in plant cell cultures. The advantage in this technology is that it is applicable to any plant, and no prior knowledge of the gene sequences is required, the fact which very often is encountered when it comes to exotic medicinal plants. As a model system we used *Nicotiana tabacum* L. (BY-2) cell culture to unravel the unknown steps involved in nicotine alkaloid biosynthesis. From altogether 591 differentially regulated genes discovered by cDNA-AFLP, 38 were chosen for further functional studies. Of particular interest were the genes encoding for protein kinases, signal transduction proteins, transcription factors and other master regulators. Full length cDNAs were constructed from cDNA-AFLP tags and were subsequently delivered to *Agrobacterium* for the establishment of transgenic cell suspension cultures as well as hairy root cultures. The transformed cell lines were subjected to metabolite analysis in order to determine the functional properties of the inserted gene construct. Two genes have resulted in an altered secondary metabolite profile in tobacco hairy roots. Furthermore, the genes derived from tobacco showed potential in altering the secondary metabolite

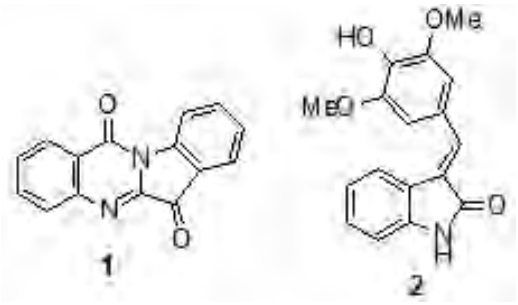
production in related (*Hyoscyamus*) or non-related (*Catharanthus*) species. These genes will be further examined to unravel their use when finding novel high-value pharmaceutical compounds from plants.

P 164

A comprehensive metabolite profiling of Isatis tinctoria leaf extracts

Potterat O, Mohn T, Hamburger M
Institut für Pharmazeutische Biologie, Universität Basel, CH-4056 Basel, Switzerland

Woad (*Isatis tinctoria* L., Brassicaceae) is an ancient indigo dye and medicinal plant, which has been used and cultivated in the temperate climate zones of Europe since antiquity. The anti-inflammatory potential of *Isatis tinctoria* was recently confirmed in a broad-based pharmacological profiling [1], in various animal models, and in a clinical pilot study. The alkaloid tryptanthrin (**1**), indolin-2-one **2,** and γ-linolenic acid were identified as inhibitors of COX-2, 5-LOX, leucocytic elastase, and of histamine release from mast cells. To further characterize the active leaf extracts, we carried out a comprehensive metabolite profiling. Extracts were analyzed by gradient HPLC combined with photodiode array (PDA), evaporative light scattering (ELSD) and mass spectrometry (ESI-MS and APCI-MS in positive and negative ion modes) detection. Over 80 peaks were resolved. A majority of peaks could be assigned to structural classes such as alkaloids, fatty acids, flavonoids, porphyrins and carotenoids, and a large number of compounds were identified with the aid of UV-vis spectra, MS and MS^n experiments, and reference compounds.



Reference: 1. Hamburger, M. (2002), Phytochem. Rev. 1: 333 – 344.

4. Health Beneficial Effects of Plant Phenolics

P 165

Phenolics, volatiles and biological activities of Salix babylonica L. leaves and stem bark

Abou Zeid AH
Pharmacognosy Department, National Research Centre, El-Tahrir St, Dokki (12622) Cairo, Egypt

Since ancient times, *Salix spp*. has been used for treatment of various diseases. The main active constituent of this species is the phenolic glycoside salicin which is responsible for pharmaceutical values of these plants. The present study deals with isolation and identification of phenolics from *Salix babylonica* L. leaves, as well as, investigation of volatile constituents of both leaves and stem bark of the same plant. The LD_{50}, anti-inflammatory (carrageen an induced rat hind baw oedema test), analgesic (Charlier,*et al.* method), antipyretic (Bush and Alexander method), antioxidant (DPPH / ESR method) and antimicrobial (paper-disc antibiotic assay method) activities of 95 % ethanol of leaves and stem bark were investigated. The dried powdered leaves of the plant were extracted with 95 % ethanol by maceration. The ethanol extract was evaporated to dryness. The residue was dissolved in water and extracted with petroleum ether followed by ethyl acetate. The phenolic compounds were isolated from the ethyl acetate extract by using polyamide column chromatography [1, 2]. Two flavonoids, luteolin and luteolin-6-C-β-D-glucopyraniside (iso-orientin) and two phenolic glycosides, trichocarpin and tremuloidin were isolated, identified by physical, chemical and spectroscopic methods [2, 3]. The volatile constituents of both leaves and stem bark of *S. babylonica* were prepared by hydro-distillation using Nikerson apparatus and analyzed by GC/MS analysis. Fifty and forty five compounds were identified representing 88.04 % and 87.38 % of the total volatiles of leaves and stem bark, respectively. The total oxygenated compounds constituted 53.25 % and 85.49 % of the volatiles of leaves and stem bark, respectively. The results of biological tests were statistically analyzed using the student's "*t*" test. Significant activities were obtained. **References**: 1. Titto, R. (1985), J. Agric. Food Chem. 33: 213 – 217. 2. Mabry, J., Markham, K. (1970), The Systematic Identification of Flavonoids, Springer Verlag, Berlin. 3. Steele, J., Weitzel, P. (1972), J. Chrom. A 71: 435 – 441.

P 166

Effect of some Teucrium species (Lamiaceae) on lipid peroxidation in rat lever microsomes

Panovska TK[1], Kulevanova S[2]
[1]Institute of Toxicology, Faculty of Pharmacy, University "Ss. Cyril and Methodius" Vodnjanska 17, 1000 Skopje, Macedonia; [2]Institute of Pharmacognosy, Faculty of Pharmacy, University "Ss. Cyril and Methodius" Vodnjanska 17, 1000 Skopje, Macedonia

Certain *Teucrium* species (Lamiaceae), *T. montanum* L., *T. polium* L. and *T. chamaedrys* L., have long been recognized in folk medicine in the treatment of gastrointestinal disorders, inflammations and diabetes [1]. The antioxidant action of diethyl ether (E), ethyl acetate (EA) and n-butanole (B) extracts – (0.01 mg/mL), obtained from the aerial parts of Macedonian *Teucrium chamaedrys* L., *Teucrium polium* L. and *Teucrium montanum* L. were studied in a lipid peroxide system using microsomes from rat liver. HPLC method is employed for quantitative determination of flavones in the extracts. The NADPH-induced lipid peroxidation, was inhibited by the addition of the extracts [2]. The order of the inhibitory potencies of the extracts tested seems to be *T. polium* (EA), 39 % > *T. polium* (B), 36 % > *T. montanum* (E and B), 35 % > *T. polium* (E), 28 % > *T. montanum* (EA), 21 % > *T. chamaedrys* (E and B), 20 % > *T. chamaedrys* (EA), 8 %. The effect of *Teucrium* extracts was compared with that of reference compounds with confirmed antioxidant activity. The activities of luteolin, thymol and BHT were as potent as that of the extracts, 19, 37 and 36 %, respectively. Caffeic and rosmarinic acid, carvacrol, silymarin and BHA showed 42, 52, 50, 54 and 49 % inhibition, respectively. Quercetin had the strongest inhibitory effect on the NADPH lipid peroxidation among all the samples tested. These findings indicate those *Teucrium* extract act as an antioxidant in lipid peroxidation carried out by rat liver microsomes. **References**: 1. Tariq, M. *et al.* (1989), Int. J. Tissue React., 11: 185 – 188. 2. Gutterige, J.M.C. (1988), Lipid peroxidation: some problems and concepts. In: Oxygen Radicals and Tissue Injury, Halliwell, B. (Ed.). Bethesda, Federation of American Societies of Experimental Biology, pp. 9 – 19.

P 167

Hepatoprotective activity of the ethyl acetate extract of Teucrium polium L. against carbon tetrachloride induced hepatic injury in rats

Kulevanova S[1], Panovska TK[2], Stefkov G[1], Gjorgoski I[3], Bogdanova M[4], Petrushevska G[5]

[1]Institute of Pharmacognosy, Faculty of Pharmacy, Vodnjanska 17, 1000 Skopje, Macedonia; [2]Institute of Toxicology, Faculty of Pharmacy, Vodnjanska 17, 1000 Skopje, Macedonia; [3]Institute of Biology, Faculty of Natural Sciences and Mathematics, P.B. 126, 1000 Skopje, Macedonia; [4]Institute of Clinical Biochemistry, Clinical Center, Vodnjanska 17, 1000 Skopje, Macedonia; [5]Institute of Pathology, Faculty of Medicine, University "Ss. Cyril and Methodius" Vodnjanska 17, 1000 Skopje, Macedonia

The hepatoprotective activity of ethyl acetate extract of *Teucrium polium* (L.) has been investigated using CCl_4-induced liver damage in rats. Specific biochemical parameters (glutathione peroxidase – GPx, superoxide dismutase – SOD, reduced glutathione – GSH and total antioxidative status – TAS) were estimated in blood and liver homogenate [1]. Lipid peroxidation (LP) in CCl_4-intoxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS) [2]. Histopatological examination of the liver were undertaken to monitor the status of the liver. Silymarin has been used as a standard to compare the activity of the extract [3]. The influence of *T. polium* extract at a dose of 25 mg/Kg, on the biochemical parameters was significant different ($p < 0.05$) with that of the CCl_4-treated group. The liver biopsy of experimental rats showed significant restoration of normal histomorfological pattern of liver cells. The study substantiates the hepatoprotective potential of ethyl acetate extract of *T. polium* which may be due to the presence of luteolin, apigenin, cirsimaritin and cirsiliol, determined by HPLC. **References**: 1. Ellenhorn, M.J. (1997), Ellenhorn's Medical Toxicology: Diagnosis and Treatment of human Poisoning. Williams and Wilkins publication, Los Angeles. 2. Ohkawa, H. *et al.* (1979), Anal. Biochem. 95: 351 – 358. 3. Valenzuela, A. *et al.* (1985), Biochem. Pharmacol. 3: 2209 – 2212.

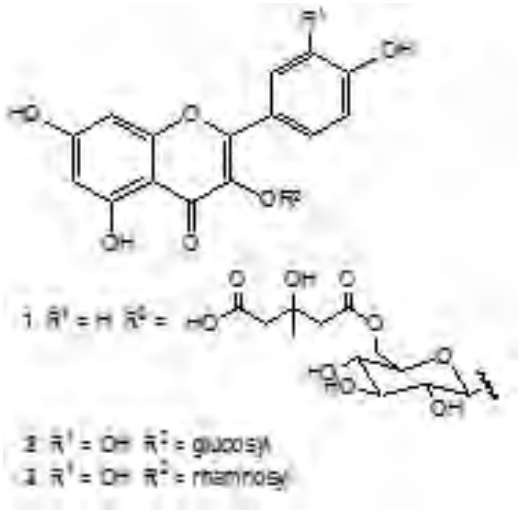
P 168

Antioxidant and anti-inflammatory phenolics from Pedilanthus tithymaloides

Abreu P[1], Matthew S[1], González T[1,2], Costa D[3], Segundo M[3], Fernandes E[3]

[1]CQFB/REQUIMTE, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, 2829 – 516 Caparica, Portugal; [2]Departamento de Farmacia, Facultad de Ciencias Naturales, Universidad de Oriente, Santiago de Cuba; Cuba; [3]REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia da Universidade do Porto, Rua Anibal Cunha 164, 4090 – 030, Porto, Portugal

Pedilanthus tithymaloids (L.) Poit. (Euphorbiaceae) is a low tropical American shrub with a wide range of healing properties such as emetic, anti-inflammatory, antibiotic, antiseptic, antihemorrhagic, antiviral, antitumoral, and abortive [1]. In Cuban traditional medicine, a tincture of *P. tithymaloides* is used as an anti-inflammatory remedy in the treatment of stomatological affections. Following a methodology of bioassay-guided fractionation using ROO, $ONOO^-$, NO, and DPPH, the flavonoids kaempferol 7-*O*-β-d-glucopyranoside-6''-(3'''-hydroxy-3'''-methylglutarate) (**1**), quercitrin (**2**), isoquercitrin (**3**), and the coumarin scopoletin were isolated from the ethanolic extract of *P. tithymaloides* stems and leaves. The results here presented indicate that these phenolic compounds constitute part of the active principles of the plant extract responsible for its anti-inflammatory and antioxidant activities [2].



Acknowledgements: REQUIMTE, Fundação para a Ciência e Tecnologia, and Programme Alβan. **References**: 1. Roig, J.T. (1974), Plantas Medicinales e Venenosas de Cuba, ed. Editorial Científico Tecnico, La Habana. 2. Abreu, P. *et al.* (2006), Life Sciences 78: 1578 – 1585.

P 169

Antioxidant activity of selected Nigerian green leafy vegetables

Odukoya OA[1], Inya-Agha SI[2], Segun FI[1], Sofidiya MO[1], Ilori OO[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria; [2]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

Green leafy vegetables (GLV) offer a cheap but rich source of micronutrients and other phytochemicals having antioxidant properties and essential for good health. The potential of 21 GLV in the cooked form as natural antioxidant supplements diets was assessed. The antioxidant activity of hot water extracts of the GLV of *Amaranthus hybridus* Linn. (Amaranthaceae), *Amaranthus caudatus (*Amaranthaceae), *Beilschmedia manni* (Meisn.) Benth. Et Hook.f. (Lauraceae), *Celosia argentea var argentea* (L.)O.Kuntze (Amarantheceae) *Celosia argentea var cristata* Linn. (Amarantheceae), *Corchorus olitorius* L. (Tiliaceae), *Crassocephalum crepidioides* (Benth). S.Moore (Asteraceae), *Gnetum bucholzianum* Welw. (Gnetaceae), *Gongronema latifolium* Benth. (Asclepiadaceae), *Heinsia crinita* (Afz.) G. Taylor (Rubiaceae), *Hibiscus callyphyllus* Cav. (Malvaceae), *Lasianthera africana* P. Beauv (Icacinaceae), *Myrianthus arboreus* P. Beauv. (Urticaceae), *Pterocarpus mildbraedii* Harms (Papilionaceae), *Pterocarpus santalinoides* DC. (Papilionaceae), *Solanum macrocarpon* L. (Solanaceae), *Solanum melongena* Linn. (Solanaceae), *Struchium sparganophora* (*Linn.*) O. Ktze (Asteraceae), *Talinum triangulare* (Jacq.) Wild. Portulacaceae, *Telferia occidetalis* Hook (Curcurbitaceae), *Vernonia amygdalina* Del. (Asteraceae) were investigated.Potential free radical scanvenging activity of these vegetables was confirmed by spraying spots of the extracts with DPPH(yellow color on purple background). Antioxidant activity was assayed in linoleic acid model system. Total polyphenols as Tannic Acid Equivalent (TAE) and ascorbic acid were evaluated spectrophotometrically.The activity of each extract was calculated as %inhibition of lipid peroxidation. The extracts showed marked antioxidant activity in linoleic acid model systems. Antioxidant values ranged from as low as 3.67 % in *A. hybridus* to as high as 68.41 % in *C. argentea var cristata.* Phenol content varied from 21.83 mg/100 g dry weight in *T. triangulare* to 546.97 mg/100 g dry weight in *G. bucholzianum*. Ascorbic acid content was from 13.41 mg/100 g dry weight in *V. amygdalina* to 187.11 mg/100 g dry

weight in *G. latifolium*. There was no correlation (R^2=– 0.432) between antioxidant activity, total phenols and ascorbic acid content.

P 170

Astringency as antisensitivity marker of some Nigerian chewing sticks

Segun FI[1], Odukoya OA[1], Inya-Agha SI[2], Agbelusi GA[3], Sofidiya MO[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, [3]Department of Preventive Dentistry, College of Medicine, University of Lagos, Nigeria.
[2]Department of Pharmacognosy, University of Nigeria, Nsukka, Nigeria

Astringents contract the tissues and canals of the body. Chewing sticks are used for oral hygiene both as an antibacterial and desensitizing agent. Astringency of cold water extracts of *Afzelia africana* Sm. ex Pers. (Caesalpiniaceae), *Dialium guineense* Willd. (Fabaceae), *Masularia acuminata* (G.Don) Bullock ex. Hoyle, *Rauwolfia vomitoria* Afz. (Apocynaceae), *Terminalia glauscens* Planch. (Combretaceae), *Vernonia amygdalina* Del. (Asteraceae) and *Zanthoxylum zanthoxyloides* (Lam.) Waterman (Rubiaceae) was measured by precipitating extracts with hemoglobin, centrifugation and loss of absorbance measured spectrophotometrically at 578nm relative to tannic acid. Tannic Acid Equivalents (TAE) were determined from standard curve obtained. Total Tannin (TT) was determined using the protein tannin precipitation method. Relative Astringency (RA) was astringency of tannin present relative to tannic acid. Activity was in the order of *Afzelia > Terminalia > Zanthoxylum >Masularia > Vernonia > Rauwolfia > Dialium*. TT ranged from 106.92 ± 0.03 mg/100 g dry plant in *Dialium* to 632.86 ± 0.42 mg/100 g dry plant in *Afzelia*. TAE was 27.37 ± 0.07 mg/100 g dry plant in *Dialium t*o 148.11 ± 0.07 mg/100 g dry plant in *Afzelia*. RA correlated positively with TAE (R^2= 0.8763); TT (R^2 = 0.9493). It is proposed that the desensitization may be due to the astringent activity; as these extracts will form a protective layer on the exposed dentine; contract / block the tube like channels that pass through teeth and connect to nerves thereby reduce the ability of the nerves to transmit pain.

P 171

Free radical scavenging activity of some Nigerian medicinal plants

Sofidiya MO[1], Odukoya OA[1], Familoni OB[2], Inya-Agha SI[3]
[1]Department of Pharmacognosy, Faculty of Pharmacy, [2]Department of Chemistry, Faculty of Science, University of Lagos, Nigeria. [3]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria

Antioxidant properties are among the first links between chemical reactions and biological activity. Phenolic natural products are of particular interest because of their antioxidant activity through scavenging oxygen radicals and inhibiting peroxidation. The *cure all* activity of Nigerian medicinal plant extracts used in traditional medicine practice has been associated with the antioxidant potential of their phenolic content in our laboratory [1]. The present work evaluated the DPPH radical scavenging, total antioxidant activities, reducing power, and total contents of phenolic compounds in methanolic leaf extracts of five Nigerian medicinal plants *Dalbergia saxatilis* Hook.f. (Papilionaceae), *Ekebergia senegalensis* A.Juss.(Meliaceae), *Hymenocardia acida* Tul. (Hymenocardiaceae), *Icacina tricantha* Oliv.(Icacinaceae) and *Salacia pallescens* Oliv.(Celastraceae)]. Free radical scavenging activity was measured spectrophotometrically as maximum fading power of DPPH at 517nm (DPPH is reduced to DPPH-H, with colour change from violet to yellow)at 0.025, 0.05, 0.1, 0.2 mg/mL concentration of extracts. Reducing power was determined using Ferricyanide Trichloroacetic acid method and total phenolic content, according to the Folin-Ciocalteu assay. Antioxidant activity of the plant extracts with the DPPH radical scavenging and reducing power method, were in the order *Hymenocardia > Ekebergia > Salacia > Icacina > Dalbergia. H. acida* and *E. senegalensis* possess very high radical scavenging activity in both assays. Potency of *H. acida* extract (97.4 % inhibition) was of the same magnitude as that of reference α – tocopherol. Total phenols in all the samples expressed as GAE (Gallic Acid Equivalent) varied from 1.83 to 15.47 mg/g of dry plant material. Free radical scavenging activity correlated with reducing power (R^2 = 0.9564) and total phenols R^2 = 0.6640 (y = 1.2281 x -103.11) respectively. This suggests that 66 % of the antioxidant capacity of these extracts result from contribution of phenolic compounds. **Reference**: 1. Odukoya, O.A. *et al.* (2005), Antioxidant activity of Nigerian Dietary Spices. Electronic J. Environ. Agricult. Food Chem. 4:1086 – 1093.

P 172

Effects of grape consumption on plasma and erythrocyte antioxidant parameters in elderly subjects

Avci A[1], Atli T[2], Ergüder IB[1], Varli M[2], Devrim E[1], Demir O[2], Durak I[1], Aras S[2]
[1]Department of Geriatric Medicine, Ankara University School of Medicine 06110, Ankara; [2]Department of Biochemistry, Ankara University School of Medicine 06100, Ankara

Aim: Effects of ingesting *Fructus vitis minuta* (black grape) on plasma and erythrocyte antioxidant parameters of elderly subjects were investigated in this study. Dried black grape (Maras uzumu for Turkish name) contain water (approx. 80 % v/w), sugars (glucose and fructose) (appprox. 15 % w/w) lipids, proteins, phenolic compounds, some minerals and vitamins. **Methods:** Thirteen subjects (mean age 74.67 ± 0.58) participated in the study. They ingested grape at the daily dose of 1 g/kg body weight for 1 month. Before and after these periods, fasting blood samples were obtained, and oxidant (malondialdehyde, MDA (nmol/mg protein), and xanthine oxidase, XO(-mIU/mL)) and antioxidant (superoxide dismutase, SOD (U/mg protein) and glutathione peroxidase, GSH-Px (IU/mg protein)and catalase, CAT (IU/mg protein)) parameters were studied in erythrocytes, and MDA levels were studied in plasma samples obtained from the subjects. SOD, GSH-Px and CAT activities were measured in erythrocyte hemolysate fraction. **Results:** In the erythrocyte hemolysate, MDA levels and SOD activities were found to be lower ($P < 0.01$) in the second samples relative to the first ones. Xanthine oxidase activity was found to be lower in the second samples, but this decrease was not statistically meaningful. Because of free radical production was decreased, SOD activities were not increased. Our results show that ingestion of grape consumption leads to significantly lowered erythrocyte MDA levels, which indicate that consumption of grape decreases oxidation reactions. It is quite possible that reduced peroxidation processes due to grape consumption may play a part in some of the beneficial effects of grape in elderly subjects.

P 173

Variation of polyphenols and antioxidant activity in mulberry leaves

Ingkaninan K[1], Kaewruang W[2], Wongthai J[1], Wongareonwanakij S[2], Hongthongdaeng B[2]
[1]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University 65000 Thailand; [2]The Queen Sirikit Institute of Sericulture, Chatuchak, Bangkok 10900, Thailand

Mulberry (*Morus alba* L., Moraceae) tea is considered as healthy beverage in Thailand. In order to identify the good source of Mulberry tea, seven cultivars grown in Thailand were studied for their total polyphenolic content and antioxidant activity using Follin-Ciocalteu and DPPH methods, respectively. The results showed that Khunphai, the native cultivar, had the highest total polyphenols (41.88 ± 0.45 gGA/kg) and antioxidant activity (with IC_{50} value of 93.42 ± 13.17 mg/L). The young mulberry leaves contained 2 – 3 fold higher level of total polyphenols as well as antioxidant activity comparing to older leaves. Seasonal variations of total polyphenolic content in mulberry leaves were also observed. The leaves were collected in three commercial harvest seasons i. e. summer (April-

July 2005), rainy season (July-September 2005) and winter (October-December 2005). The total polyphenols and antioxidant activity were not different in dry periods (winter and summer) while significantly decreased in rainy period.

P 174

Efficacy of (±)-taxifolin from Larix sibirica (Mûnchh.) Ledeb. on blood pressure in experiments in vivo

Tikhonov VP[2], Makarova MN[1], Zajtseva MA[1], Makarov VG[1]
[1]Interregional Center "Adaptogen", Piskarevsky pr. 45/5, 195067, St.-Petersburg, Russia; [2]Open joint-stock company "Diod", 11-A, ul. Derbenevskaya, 115114, Moscow, Russia

The cardiovascular pathology is one of principal causes of death around the world. P-vitamin and antioxidant effects of flavonoids are well-known, however the mechanism of their action is not clear. The aim of the present work was to investigate the efficacy of taxifolin on a vascular tone booth blood pressure (BP) in the model of spontaneously hypertensive rats (SHR). (±)-taxifolin was isolated from Larix sibirica wood (OJSC "Diod). Throughout the experiment male SHR rats (180 – 230 g; age of 3 months breeder: Rappolovo, St-Petersburg, Russia) were used. All animals were kept in a room maintained under environmentally controlled conditions of 20 – 23 °C, relative humidity – 50 – 70 %, and 12 h light– 12 h dark cycle. All animals had free access to water and standard food. Taxifolin (in starch suspension) was administered intragastrically daily in 14 days, in doses 10, 20, 50 and 150 mg/kg. Enalapril maleate (KRKA; 2 mg/kg) and atenolol (AO "Pliva"; 10 mg/kg) were used as reference preparations. The control group received vehicle only. Systolic and diastolic blood pressure, diuresis, electrocardiogram (II standard leads) were monitored in rats. It was established, that taxifolin in doses 10, 20, 50 and 150 mg/kg has no diuretic effect. Taxifolin caused a manifest decrease of blood pressure. The most positive results were observed at use of a dose of 20 mg/kg. Taxifolin had more expressed influence on the level of systolic BP. The decrease of myocardium stress was observed at the dose of taxifolin over 20 mg/kg that was shown in normalization of R wave amplitude on an electrocardiogram. The effect of taxifolin was similar to reference preparations.

P 175

In vitro antiviral assessment against DNA and RNA viruses as well as antibacterial and antifungal profiles of selected Turkish species of the Salvia genus

Orhan I[2], Özçelik B[1], Kartal M[3], Karaoglu T[4], Yilmaz G[5], Kan Y[6], Şener B[2]
[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey; [3]Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06330 Ankara, Turkey; [4]Department of Virology, Faculty of Veterinary, Ankara University, Ankara, Turkey; [5]Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, 06330 Ankara, Turkey; [6]Department of Field Crops, Faculty of Agriculture, Selcuk University, 42070 Konya, Turkey

The present study was undertaken to evaluate antibacterial, antifungal, and antiviral properties of the chloroform and methanol extracts from the aerial parts of fourteen Turkish *Salvia* species (Lamiaceae) including *S. albimaculata* Hedge & Hub., *S. aucheri* var canescens Boiss. et Heldr., *S. candidissima* ssp. *occidentalis* Vahl., *S. ceratophylla* L., *S. cryptantha* Montbret et Aucher ex. Benth., *S. cyanescens* Boiss. et Bal., *S. frigida* Boiss., *S. forskahlei* L., *S. halophila* Hedge, *S. microstegia* Boiss. & Bal., *S. multicaulis* Vahl., *S. sclarea* L., *S. syriaca* L., and *S. verticillata* L. ssp. *amasiaca* (Freyn, & Bornm.) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Acinetobacer baumannii, Staphylococcus aureus, Bacillus subtilis, Enterococcus faecalis*, and *Candida albicans* by microdilution method. Both *Herpes simplex* (DNA) and *Parainfluenza*-3 viruses (RNA) were used for the determination of antiviral activity of the abovementioned *Salvia* extracts by using Madin-Darby bovine kidney (MDBK) and Vero cell lines. The methanol extracts were found to be quite active against *S. aureus* and *E. faecalis* (2 and 4 μg/mL, respectively), while the chloroform extracts were more active against *S. aureus*, *B. subtilis*, and *E. faecalis* (1, 2, and 2 μg/mL, respectively). All of the extracts displayed the antifungal activity having the MIC value at 8 μg/mL. Maximum cytopathogenic effect (CPE) concentrations as the indicator of antiviral activities of the extracts were determined and the chloroform extracts belonging to *S. albimaculata*, *S. cyanescens*, and *S. microstegia* (1 – 64 μg/mL) along with the methanol extracts of *S. ceratophylla*, *S. halophila*, and *S. sclarea* (16 – 32 μg/mL) showed reasonable antiviral effect. The cytotoxicity of the extracts was also expressed as the maximum non-toxic concentrations (MNTC), ranging between 16 – 128 μg/mL.

P 176

Antioxidant and antimicrobial activity of lichen Pseudevernia furfuracea (L.) Zopf

Zovko M[1], Kosalec I[1], Pepeljnjak S[1], KaloPera Z[1], Partl A[2], Bakmaz M[3]
[1]Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, HR-10000 Zagreb, Croatia; [2]State Institute for Nature Protection, Savska 41, PO Box 50, HR-10144 Zagreb, Croatia; [3]Zagreb City Pharmacy, Karlovačka cesta 108, HR-10436 Rakov Potok, Croatia

Lichen *Pseudevernia furfuracea* (L.) Zopf. (*Parmeliaceae*) grows across Europe and northern Africa. Antioxidant and antimicrobial activity, as well as phenolic content of chloroformic (PFC), ethanolic (PFE) and water (PFW) extracts of *P. furfuracea* collected on Velebit mountain were investigated. Antioxidant activity of extracts was evaluated in three manners: β-carotene-linoleic acid model system, iron (III) to iron (II) reducing activity and DPPH radical-scavenging activity. PFC and PFE extracts demonstrated significant antioxidant activity. As it can be expected from the higher phenolic content, ethanolic extract exhibited higher antioxidant activity than the chloroformic extract in each of our three assays, especially in β-carotene-linoleic acid assay. Antimicrobial activity of PFE and PFW extracts was determinated using cylinder diffusion method, and macrobroth dilution method against standard and clinical isolates of 19 bacterial, 7 yeast and 4 dermatophyte species. Gram-positive bacterial species were highly susceptible to the PFE with inhibition zones (ZI) from 19 to 33 mm, and MIC values between 0.15 and 3.1 mg/mL (MMC under 4.8 mg/mL), with the *Bacillus* spp. most resistant (MIC 9.95 – 114.6 mg/mL). Dermatophytes showed ZI 13 – 17 mm and MICs between 1.19 and 2.39 mg/mL (MMC 2.39 – 4.78 mg/mL). Susceptibility of Gram-negative bacterial species, as well as antifungal activity against yeasts, was specie-dependent. On the other hand, PFW did not show noticeable antimicrobial activity. Using bioactivity guided "bioassay *in situ*" against *Bacillus subtilis* NCTC 8236, fraction rich in antimicrobial phenolic acids was isolated.

P 177

Evaluation of cytotoxic and antioxidant activity of Rhaponticum carthamoides (Willd.) Iljin extracts

Biskup E, Èojkowska E
Department of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk ul. Kladki 24, 80 – 822 Gdansk, Poland

Rhaponticum carthamoides (Willjd) Iljin is a Siberian plant used for centuries in the folk medicine. Its extracts possess immunomodulating activity and may stimulate the protein synthesis in muscles and kidneys. Their activity is mainly attributed to ecdysteroids – steroid compounds occurring in plants and in insects. This study was focused on antioxidant and cytotoxic activity of *R. carthamoides* extracts. Plant material (dry leaves) was obtained from FITOSTAR™. Secondary metabolites were extracted via sonication (30 min.), using three solvents, differning in polarity: chloroform, methanol

and water. Radical scavenging activity (RSA) of the extracts was measured in the DPPH (2,2-diphenyl-1-pichydrazyl free radical) assay and was compared with the activity of known antioxidants: ascorbic acid, α-tocopherol and butylated hydroxytoluene (BHT). Aqueous and methanolic extracts exhibited radical scavenging activity towards DPPH (IC_{50}= 25 and 45 μg/mL, respectively) and turned to be more effective than BHT (IC_{50}= 190 μg/mL), but less than ascorbic acid and alpha-tocopherol (IC_{50}= 4 and 12 μg/mL, respectively). In the same time chloroformic extract was not capable of scavenging DPPH (IC_{50} > 1 mg/mL). Cytotoxic activity of all three extracts and pure 20-hydroxyecdysone (20E) was examined against HeLa (cervical carcinoma) and HL-60 (leukemia) cell lines, using MTT assay. Aqueous extract as well as pure 20E did not influence the cell lines viability, whereas chloroformic and methanolic extracts exhibited mild cytotoxic activity. However according to the NCI recommendation (IC_{50} < 4 μg/mL) neither of them can be regarded as a potential cytotoxic agent. *Acknowledgements*: State Committee for Scientific Research Nr PBZ-KBN-092/P05/2003

P 178

Changes in the phenolic compounds composition of virgin olive Oil due to different storage conditions under accelerated ageing

Krieg C[1], Stecher G[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, Leopold Franzens University, Innrain 52a, 6020 Innsbruck, Austria; [2]Bionorica AG; Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Virgin olive oil is well known for its high amount of antioxidant phenolic compounds, such as hydroxytyrosol or oleocanthal [1], and their protective effects against cancer, coronary heart diseases and ageing by inhibiting oxidative stress [2]. The composition of these compounds depends on various parameters, the variety, environmental conditions, ripeness degree and the extraction type and storage [3]. We have special interest in the long term stability of these compounds and their concentration changes during storage after bottling. For this purpose the oil is bottled in different kind of glasses and exposed to light under monitored and recorded circumstances, to induce accelerated aging. After defined periods olive oil samples are taken and their phenolic compositions are analyzed. Next to the phenolic compounds, the free fatty acids, the peroxide values and the aliphatic alcohol contents are determined according to Pharmacopeia Europea, in order to classify the oil quality. Within this approach, different types of solid phase extraction (SPE) materials are compared to each other. The focus lays on the different properties of silica based and polymeric based materials in regard to their applications for phenolic compounds. These materials are partly commercial available and partly self synthesised and functionalised [4]. Due to this work, a greater insight is gained for the adulteration of olive oils and its health supporting components. Furthermore, a good way for the optimisations of phenolic analyses in complex biological matrices is shown by synthesising and functionalising of SPE materials. **References**: 1. Beauchamp, G. *et al.* (2005), Nature 437: 45 – 46. 2. Owen, R. *et al.* (2000), Eur. J. Cancer 36: 1235 – 1247. 3. Owen, R. *et al.* (2000), Food Chem. Tox. 38: 647 – 659. 4. Sultan, M., Stecher, G. *et al.* (2005), Curr. Med. Chem. 12: 573 – 588.

P 179

Immunomodulatory effects of flavonoids in vitro

Jasprica I[1], Dumić J[2], Mornar A[1], Medić-Šarić M[1]
[1]Department of Medicinal Chemistry, [2]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

Flavonoids, a group of very popular phytochemicals, exhibit numerous biological activities. We investigated immunomodulatory effects of 16 flavonoids (eriodictyol, rhamnetin, isorhamnetin, sakuranetin, isosakuranetin, pinocembrin-7-methylether, kaempferide, tamarixetin, tectochrysin, flavone, flavanone, quercetin, myricetin, morin, kaempferol and apigenin) *in vitro* (using cell culture models) by measuring the production of cytokines (TNFα, IL-1β, IL-2) that play crucial roles in innate and adaptive immune responses. The inhibitory effects of chosen flavonoids (30, 10 and 3 μM) on IL-2 production by Jurkat cells (a cell line derived from human T-cell leukemia) stimulated with polymyristate-acetate and phytohemaglutinine was determined after 24 hrs using ELISA. The level of IL-2 produced by stimulated cells was considered as a maximal (100 %), while un-stimulated cells do not produce IL-2. TNFα and IL-1β production by differentiated THP-1 cells (human macrophage-like cells) was measured using ELISA 24 hrs after the addition of flavonoids (*E. coli* lipopolysacharide was used as a positive control; all other reagents were LPS-free). To investigate the effects of tested compounds on cell proliferation, [6-H^3] thymidine incorporation was determined. Flavonoids that significantly (> 50 %) inhibited IL-2 production suppressed the proliferation of Jurkat cells as well, except sakuranetin (SKN) and pinocembrin-7-methylether (P7ME). When applied even in the highest concentration these compounds did not affect Jurkat cell proliferation, but inhibited IL-2 production for 68.3 % and 76.5 %. All flavonoids that induced TNFα and IL-1β production for ≥ 50 % of the positive control significantly inhibited proliferation of differentiated THP-1 cells. Interestingly, SKN and P7ME did not stimulate cytokine production, but suppressed the cell proliferation. Although all tested flavonoids exhibited immunomodulatory effects the most intriguing compounds for further investigation are SKN and P7ME.

P 180

Protective effects of catechin and epicatechin from Smilax china rhizome on amyloid β protein (25 – 35)-induced neurotoxicity in cultured neurons

Bana JY[1], Songb KS[1], Seonga YH[2]
[1]College of Veterinary Medicine and Research Institute of Herbal Medicine, Chungbuk National University, Cheongju, Chungbuk 361 – 763,South Korea; [2]College of Agriculture and Life-Sciences, Kyungpook National University, Daegu, 702 – 701, South Korea

We previously reported that *Smilax china* L. rhizome inhibits amyloid β protein (25 – 35) (Aβ (25 – 35))-induced neurotoxicity in cultured rat cortical neurons [1]. Here, we isolated catechin and epicatechin from *S. china* rhizome and also studied their neuroprotective effects on Aβ (25 – 35)-induced neurotoxicity in cultured rat cortical neurons. Catechin and epicatechin inhibited 10 μM Aβ (25 – 35)-induced neuronal cell death at a concentration of 10 μM, which was measured by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay and Hoechst 33342 staining. Catechin and epicatechin inhibited 10 μM Aβ (25 – 35)-induced elevation of cytosolic calcium concentration ($[Ca^{2+}]_c$), which was measured by a fluorescent dye, Fluo-4 AM. Catechin and epicatechin also inhibited glutamate release into medium induced by 10 μM Aβ (25 – 35), which was measured by HPLC, generation of reactive oxygen species (ROS) and activation of caspase-3. These results suggest that catechin and epicatechin prevent Aβ (25 – 35)-induced neuronal cell damage by interfering with the increase of $[Ca^{2+}]_c$, and then by inhibiting glutamate release, generation of ROS and caspase-3 activity. Furthermore, these effects of catechin and epicatechin may be associated with the neuroprotective effect of *S. china* rhizome. *Acknowledgements:* This work was supported by a grant from BioGreen 21 Program, Rural Development Administration, Republic of Korea. **Reference**: 1. Ban, J.Y., Cho, S.O. *et al.* (2006), J. Ehthnopharmcol. (in press).

P 181

Organic extract of flowers from a chamomile species eliminates complaints resulted from hemorrhoid disease

Kavutcu M[1], Arhan M[2], Aytaç B[3], Çetin R[4], Durak I[3]
[1]Gazi University Medical Faculty, Department of Biochemistry, Besevler Ankara-Turkey; [2]Ankara Oncology Teaching and Research Hospital, Department [2]Gastroenterology & [4]General Surgery, Ankara-Turkey; [3]Ankara University School of Medicine, Department of Biochemistry, Sihhiye Ankara-Turkey

Molecular mechanisms leading to hemorrhoid mainly consist of degeneration of connective tissue, stagnation and stasis of blood in the vascular plexus of the anal cushions, activation of white cells, release of inflammatory substances and toxic free radicals, which are followed by oxidation reactions and tissue damage [1]. Purified flavonoid fraction has been used to prevent inflammatory reactions resulting from the leukocyte-endothelium interaction [2]. We aimed to establish possible therapeutic effect(s) of the chamomile flower which contains several types of flavonoids and other flavones in the patients with hemorrhoid. A trial of 24 patients with acute hemorrhoid bleeding was performed by using organic extract of flowers from a chamomile species. Patients consumed extract fraction for 2 months at the daily dose of 200 µl/kg body weight. It has been observed that the frequency and severity of the hemorrhoid attacks are significantly reduced in 21 of 24 patients during the study period and, that all the complaints are almost eliminated after use of a month period. In this regard, significant reductions were observed in the prelopsus by the rectal touche inspection. Bleeding was also lessened (Mean ± SD, 2.2 ± 0.4 before and 0.2 ± 0.01 after), pain and itching frequencies decreased after extract use (Mean ± SD, 5.22 ± 0.6 before and 1.6 ± 0.3 after for pain and, 9.6 ± 3.1 before and 2.4 ± 0.6 after for itching). It seems possible that constituents in the extract fraction of chamomile flowers have significant potential to inhibit inflammation and, to increase micro circulation in the bowel, thereby ameliorating hemorrhoid and bleeding. **References**: 1. Haas, P.A. *et al.* (1984), Dis Colon Rectum 27: 442 – 450. 2. Ho, Y.H. *et al.* (2000), Dis Colon Rectum 43: 66 – 69.

P 182

Polyphenols are of special relevance for the multiple mechanisms of action of the willow bark extract STW 33-I (Proaktiv®)

Kelber O[6], Abdel-Aziz H[1], Elstner EF[2], Fiebich BL[3], Heinle H[4], Jaeggi R[5], Khayyal MT[1], Metz J[7], Müller J[6], Okpanyi SN[6], Weiser D[6]
[1]Department of Pharmacology, Faculty of Pharmacy, Cairo University, Kasr el-Aini Str, 11562 Cairo, Egypt; [2]Phytopathology, Laboratory for Applied Biochemistry, TU Munich, Am Hochanger 2, 85350 Freising, Germany; [3] VivaCell Biotechnology GmbH, Ferdinand-Porsche-Str. 5, 79211 Denzlingen, Germany; [4]Institute for Physiology, University of Tübingen, Gmelinstr. 5, 72076 Tübingen, Germany; [5]Department Bioassays, Vitaplant AG, Benkenstrasse 254, 4108 Witterswil, Switzerland; [6]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany; [7]Institute of Anatomy and Cell Biology III, University of Heidelberg, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany

The pharmacological profile of the willow bark extract STW 33-I (water extract, 16 – 23:1) and the contribution of its fractions to it were studied in a number of pharmacological models *in vitro* and *in vivo* for elucidating its clinical effects. In Interferon-γ/LPS treated monocytes, STW 33-I reduced expression of iNOS, COX-2, the anti-apoptotic protein Bcl2, Il-1β, Il-6 and TNF-α, measured by real time PCR, with IC_{50} between 10 and 200 µg/mL. It inhibited PGE_2, Il-6 and MMP-3 in chondrocytes. Activities of 5-LOX, hyaluronidase, elastase (HLE), COX-1 and -2 and oxidation in AAPH and XOD reactions were inhibited. Five fractions of the extract, obtained by sequential extraction with solvents of increasing polarity and analytically characterized by HPLC, were tested as well, showing that the fractions containing the different groups of polyphenols were responsible for the main part of the effect, while the fraction containing the main part of the salicylates showed only a minor contribution. In vivo, STW 33-I (50 to 150 mg/kg b.w.) was effective in writhing test in mice, Randall-Sellito model, brewers yeast model, paw edema, adjuvant arthritis and air pouch model in rats. In the latter, PGE_2 and LTB_4, Il-1β, Il-6, TNF- α, TxB4, COX-2 and the antioxidative parameters MDH were decreased, GSH increased. These studies show multiple mechanisms of the willow bark extract, including anti-inflammatory, -oxidative, -pyretic, joint protecting, and analgesic actions. These were mainly not due to salicylates, but to polyphenols, which therefore seem to be the group most relevant for the therapeutic efficacy of STW 33-I (Proaktiv®) in back pain.

P 183

Role of endogenous SHs and NO on Vernonia ferruginea Less induced gastroprotection

Barbastefano V[1], Cola-Miranda M[1], Luiz-Ferreira A[1], Farias-Silva E[1], de Paula Michelatto D[1], Camargo EES[2], Hiruma-Lima CA[3], Vilegas W[2], Souza Brito ARM[1]
[1]Departamento de Fisiologia e Biofísica, IB, UNICAMP, Campinas, SP, Brazil; [2]Departamento de Química Orgânica, IQ, UNESP, Araraquara, SP, Brazil; [3]Departamento de Fisiologia, IB, UNESP, Botucatu, SP, Brazil

The methanolic crude extract (MeOH) obtained from aerial parts of *Vernonia ferruginea* Less. (Asteraceae), a Brazilian savannah plant popularly known as "assa-peixe", was investigated for its antiulcerogenic proprieties and mechanisms employing three experimental models. Preliminar phytochemical screening showed that glycoside flavonoids are the major compounds present in this extract. To obtain MeOH, aerial parts of V. ferruginea were air dried (7 days at 40°C) and powdered. The powder (100 g) were exhaustively extracted with methanol at room temperature (3 times for 72 h) and then concentrated in a vacuum rotator evaporator. The previous administration of MeOH (50, 100, 250 and 500 mg/kg) significantly inhibited the gastric mucosa damage, from the 100 mg/kg dose (91 % of inhibition), caused by absolute ethanol oral administration in rats. This antiulcerogenic propriety of MeOH (100 mg/kg) depends in part on endogenous NO, once its gastroprotection was lightly attenuated by pretreatment with NG-nitro-L-arginine methyl-ester, a NO-Synthase inhibitor. Besides, when animals were pretreated with N-ethylmaleimide, a thiol blocker, including mucosal nonprotein sulfhydryl groups (SHs), the gastroprotective effects of MeOH have been extinguished. These results show that MeOH gastroprotective proprieties are due to the cytoprotective proprieties of NO and mainly of endogenous sulfhydryls compounds. *Acknowledgements:* FAPESP, CNPq and CAPES.

P 184

Hypotensive and vasorelaxant effect of the procyanidins complex from Guazuma ulmifolia bark, in normotensive and hypertensive rat

Magos GA[1], Mateos JC[2], Páez E[1], Fernández G[1], Márquez C[2], Lobato CE[2], Enríquez RG[2]
[1]Departamento de Farmacología, Escuela de Medicina, Universidad Nacional Autónoma de México, 04510 México D.F, México, D. F; [2]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, 04100 México D.F, México, D. F

Guazuma ulmifolia Lam. bark is used in Panama traditional medicine for the treatment of arterial hypertension [1]. The phytochemical studies carried on with *G. ulmifolia* bark, have led to the isolation of procyanidins. These compounds have also been related to antihypertensive activities. The hypotensive and vasorelaxant effect of a procyanidin complex (PACC) prepared from *G. ulmifolia* bark was investigated in normotensive and hypertensive rats, and in rat aortic rings. The oral administration of PACC (10 mg/kg) to conscious normotensive or hypertensive rats decreases the systolic arterial pressure (SAP) and the heart rate (HR). The intravenous administration of same fraction (10 mg/kg) in anesthetized rats induced arterial

hypotension that was attenuated by pre-treatment with N^G-nitro-L-arginine-methylester (L-NAME 31 mg/kg). In isolated aortic rings of normotensive and hypertensive rats, the PACC reduced the contraction induced by norepinephrine ($1X10^{-7}$ M). The IC_{50} doses was 35.3 ± 12 ng/mL and 101 ± 57 ng/mL in isolated aortas of normotensive and hypertensive rats, respectively. This relaxant activity was inhibited by either removal of vascular endothelium or pre-treatment with L-NAME (30 μM), while indomethacin (10 μM) or atropine (10 μM) had no effect. Preliminary analysis of the PACC by HPLC-PAD-/MS and FAB^+ mass spectrometry allowed detection of the main components as a complex of procyanidin oligomers consisting mainly of type B procyanidins. These findings suggest that cardiovascular effects from *G. ulmifolia* are due to the procyanidin complex, while the antihypertensive mechanism was found to be linked to endothelium related factors, where nitric oxide is know to be involved. *Acknowledgements:* Consejo Nacional de Ciencias y Tecnología, CONACYT, México for financial support through projects MO292 and Q42096. **Reference**: 1. Caballero-George, C. *et al.* (2001), Phytomedicine 8: 59 – 70.

P 185

Antioxidant activity of an aqueous fraction obtained from Indigofera truxillensis against ischemia-reperfusion-induced gastric lesions

Barbastefano V[1], Farias-Silva E[1], Cola-Miranda M[1], Calvo T[2], Luiz-Ferreira A[1], Pimentel FO[1], de Paula Michelatto D[1], Hiruma-Lima CA[3], Vilegas W[2], Souza Brito ARM[1]
[1]Departamento de Fisiologia e Biofísica, IB, UNICAMP, Campinas, SP, Brazil; [2]Departamento de Química Orgânica, IQ, UNESP, Araraquara, SP, Brazil; [3]Departamento de Fisiologia, IB, UNESP, Botucatu, SP, Brazil

The aqueous fraction (FAq) from the methanolic crude extract obtained from aerial parts of *Indigofera truxillensis* Kunth. (Fabaceae), a Brazilian savannah plant popularly known as "Indigo", was investigated for its antioxidative proprieties in gastric lesions induced by ischemia-reperfusion (IR) in rats. Preliminar phytochemical screening showed that flavonoid glycosides are the major compounds present in this fraction. male Wistar rats (180 – 220 g, n > 4) were fasted during 24 h, orally treated with FAq (100 mg/kg), and submitted to gastric lesions by IR of the celiac artery. After, the stomach was removed, lesion areas were counted, gastric mucosa was scraped, homogenized, and frozen to posterior analysis of: a) DNA fragmentation, b) LPO and c) GSH content. FAq protected the gastric mucosa in 91 % in IR model, but presented no significant changes over LPO and GSH content. Indeed, one of the antioxidative mechanisms observed in FAq was its capacity to protect cells from IR induced DNA fragmentation. Flavonoids should be responsible for this antiulcerogenic activity of FAq. *Acknowledgements:* FAPESP, CNPq and CAPES.

P 186

Antioxidant activities of Punica granatum as determined by FRAP assay method

Hajimahmoodi M[1], Oveisi MR[1], Sadeghi N[1], Jannat B[1], Davoodabadi E[1]
Drug and food control department, faculty of pharmacy, Tehran university of medical science, 14155, Tehran, Iran

The fruits are rich sources of various vitamins, minerals and fibers required by human body for optimal health. In the recent years, more attention has been paid to the antioxidants contained in fruits. Epidemiological studies revealed that high fruit intake was associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer and one of possible mechanisms was attributed to the antioxidant activity presented by the fruits. Pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively in the folk medicine of many countries. The presence of antioxidants has been reported from pomegranate in juice, peel, pulp and seed fractions [1 – 3], however, no literature was found reporting the antioxidant activity in the pomegranate of Iran. In the present study, the ferric reducingântioxidant power assay (FRAP assay) was employed and the FRAP value of peel and pulp fractions 10 cultivar of pomegranate produced in Saveh was determined in an attempt to make a systematic comparison among their antioxidant activities and identify the fractions with high antioxidant power for further studies. On the other hand the stability of antioxidant power was determined for three weeks. The results showed that sweet white peel and black peel cultivars was high in antioxidant power in peel and pulp fractions respectively and both peel and pulp fractions are quite stable in antioxidant activity for three week in refrigerator. **References**: 1. Navindra, P. *et al.* (2005), J. Nut. Biochem. 16: 360 – 367. 2. Negi, P. S. *et al.* (2003), Food Chemistry 80: 393 – 397. 3. Yunfeng, L. *et al.* (2006), Food Chemistry 96: 254 – 260.

P 187

Antioxidants from fruits and leaves of Eugenia jambolana, an edible Myrtaceae species from Atlantic Forest

Silva DHS, Plaza CV, da S. Bolzani V, Cavalheiro AJ, Castro-Gamboa I
Instituto de Química, Universidade Estadual Paulista CP 355 CEP 14800 – 900 Araraquara, SP, Brazil

Eugenia jambolana Lam. belongs to Myrtaceae, a plant family widespread in Brazil, known for its edible fruits as guava (*Psidium guajava* L.), jambo (*Syzygium jambos* (L.) Alston), araçá-rosa (*Psidium cattleianum* Sabine), pineapple guava (*Feijoa sellowiana* Berg.), pitanga (*Eugenia uniflora* L.), grumixama (*Eugenia brasiliensis* Lam.), scrub cherry (*Syzygium australe* (Link.) B. Hyland), most of which present astringent properties due to their tannin content. There are few phytochemical studies on such species, although preliminary evaluation of some Myrtaceae edible fruits has evidenced high contents in vitamin C and phenolic compounds. Additionally, the extract from seeds of *E. jambolana* has been used as traditional medicine in India, Brazil and other tropical countries for its hypoglycemic and antidiabetic properties, which have been associated with its antioxidant and anti-inflammatory activities. As part of our studies on species from Tropical Rain Forest (Atlantic Forest), fruits and leaves of *Eugenia jambolana* were collected and their ethanol extracts showed positive results when screened for antioxidant (bleaching of beta-carotene TLC autographic assay [1]) and cytotoxic (MTT assay for MDA/MB-435, SF-295 and HCT-8 cell lines [2]) activities. Phytochemical work on the EtOH fraction led to the isolation of polyphenols, including flavonoid glycosides and phenolic acids from the leaves, and anthocyanins cyanidin and delphinidin glycosides from the fruits extract. The isolates were submitted to evaluation of their antioxidant properties and showed strong free radical scavenging activity towards DPPH. These results support the use of extracts of *E. jambolana* in traditional medicine and suggest their actions as possible chemopreventive agents or phytoceuticals. *Acknowledgements:* This work was sponsored by the program BIOTA-FAPESP, BioProspecTa, CAPES and CNPq. **References**: 1. Pratt, D. E., Miller, E. E. (1984), *J. Am. Oil Chem. Soc.* 61: 1064 – 1067. 2. Skehan, P., Storeng, R. *et al.* (1990), *J. Natl. Cancer Inst.* 82: 1107 – 1112.

P 188

Aromatic plants from Valsesia (Italy): bioassay-guided isolation of flavonoids with antioxidant activity from Achillea species

Tomè F[1], Fico G[1], Visioli F[2], Iorizzi M[3], Vitalini S[1]
[1]Dipartimento di Biologia, via Celoria 26, 20133, Università degli Studi di Milano, Italy; [2]Dipartimento di Scienze Farmacologiche, via Balzaretti 9, 20133, Università degli Studi di Milano, Italy; [3]Dipartimento di Scienze e Tecnologie per l'Ambiente e il Territorio, Via Mazzini 8, Università degli Studi del Molise, Isernia, Italy

Valsesia is an alpine area of Northern Italy whose autochthonous flora has been maintained over the years due to the almost com-

plete absence of imported varieties. This area maintains many of its traditional habits, including the use of wild plants for culinary and pharmaceutical purposes. In a previous work we have investigated some methanolic extracts of different species collected in Valsesia [1]. Among the analysed plants, two species belonging to the *Achillea* genus (*Achillea distans, Achillea moschata*) have shown significant antioxidant activity. Aim of this work is therefore the identification of pure compounds responsible of the activity of crude extracts. The MeOH extracts have been separated with Sephadex LH-20; 22 and 25 fractions have been obtained from *A. distans* and *A. moschata* respectively. The fractions with different TLC profiles were tested for the antioxidant activity, evaluated as scavenging activity of the stable radical DPPH, antioxidant capacity (Cu++ to Cu+ reduction) and LDL oxidation. Subsequently we have separated the selected fractions with HPLC: 5 active compounds were isolated. *Acknowledgements*: supported by MIUR grant PRIN 2004038183/004, programma 2004 **Reference**: 1. Vitalini, S. *et al.* (2006), Phytoter. Research, in press.

P 189

Structure and biological activities of antimicrobial compounds recently isolated from southern African Combretum and Terminalia species

Eloff JN
Phytomedicine Programme, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa

Combretum and *Terminalia* species have recently been identified as among the 50 most important medicinal plant species in Africa. These widely distributed species are traditionally used for a wide range of indications in Africa and Asia. By antibacterial and antifungal bioassay guided fractionation more than 20 terpenoids, flavonoids and bibenzyls have been isolated by our group during the last decade [MIC 16 – 50 μg/mL] Several biological activities of these compounds and of crude plant extracts against several bacteria, fungi, parasites and cell lines were determined *in vitro* [MIC some extracts against fungi 20 μg/mL] and also *in vivo* in animal experiments. Toxicity and the application of some of these compounds or extracts in animal production systems were also evaluated. The results support many of the ethnomedicinal uses of these genera, but there is a poor correlation between activities of extracts of different species and use against microbial infections. The reason is probably the relatively non-polar character of the antimicrobial compounds, making it difficult for poor rural people to extract. Many species with excellent antimicrobial activities growing widely are not used. It may be possible to use these species in primary health care. For example, leaf extracts of *C. erythrophyllum* growing widely along river banks have good activity against *Vibrio cholera* [MIC 25 μg/mL] and may be used during cholera outbreaks. There appears to be no correlation between sections of *Combretum* and chemical composition or biological activity. Some of the results indicate that at least in some of the species active speciation is still taking place.

P 190

Phenolic extracts of strawberry fruits, leaves and cell cultures – analysis and biological activities

Nohynek L, Seppänen-Laakso T, Jääger K, Oksman-Caldentey KM, Puupponen-Pimiä R
VTT Biotechnology, Tietotie 2, FI-02044 VTT, Finland

Berry fruits are rich in phenolic compounds, such as flavonoids, phenolic acids, lignans and complex polymeric tannins, with wide variation in their contents depending on the plant species and environmental factors. The main phenolics in strawberry fruits are anthocyanins and ellagitannins. We have shown earlier that ellagitannins possess strong antimicrobial properties against human gastrointestinal pathogens. As an alternative to use of strawberry fruits, plant leaves and cell cultures are interesting choice for production of strawberry phenolics, as well as other, potentially novel secondary metabolites with interesting biological effects. As far as we know, strawberry cell cultures have not been used for production of berry and plant phenolics earlier. In this study, sterile *in vitro* strawberry plants were germinated from sterilized seeds. Cuts of the *in vitro* leaves were treated with plant growth hormones for production of callus, and the most productive calli were used for establishment of strawberry cell cultures. Analysis of phenolic extracts of strawberry fruits, leaves and cell cultures were performed using HPLC-DAD and LC-MS, and comparison of phenolic compound patterns of *in vivo* strawberry fruits and leaves to those of *in vitro* leaves and cell cultures revealed interesting differences. Also antimicrobial activities against selected human pathogenic bacteria and anti-oxidative activities, measured as radical scavenging activity, varied depending on the origin of the material of phenolic extract. These results showed that strawberry leaves are interesting and rich sources of bioactive phenolic compounds.

P 191

Are cinnamic acids responsible for in vitro neuroprotection exerted by Bryothamnion triquetrum (S.G.Gmelin) Howe aqueous extract?

Fallarero A[1,4], Tammela P[2], Loikkanen J[3], Vidal A[4], Vuorela P[1]
[1]- Department of Biochemistry and Pharmacy, Åbo Akademi University, FIN-20520 Åbo, Finland; [2]- Viikki Biocenter, Faculty of Pharmacy, University of Helsinki, FIN-00014, Helsinki, Finland; [3]- Department of Pharmacology and Toxicology, University of Kuopio, FIN-7021, Kuopio, Finland; [4]- Department of Biochemistry, Faculty of Biology, University of Havana, CP 10400, Ciudad Habana, Cuba

Three cinnamic acids: ferulic (FA), *p*-coumaric (*p*-CA) and *trans*-cinnamic acids (*t*-CA) have been identified as constituents of *Bryothamnion triquetrum* (S. G. Gmelin) Howe aqueous extract, a product that has been reported to exert different *in vitro* neuroprotective properties [1, 2, 3]. In current study, it was analyzed the effect of these three cinnamic acids in different models of oxidative stress, with the purpose of elucidating their contribution to the *in vitro* neuroprotective properties of *B. triquetrum* extract. GT1 – 7 cells were exposed to chemical agents that induce oxidative neuronal death: H_2O_2; H_2O_2 + $FeSO_4$; 3-morpholinosydnonimine hydrochloride (SIN-1) and methyl mercury (MeHg) and the protective effect of cinnamic acids, when added immediately before toxic compounds, was assessed. At the end of the insulting period, GT1 – 7 cells viability was measured by using propidium iodide fluorometric assay [2]. Treatments were compared using ANOVA and Tukey's Multiple Comparison tests. The protective effect of FA was proved to occur in all the 4 examined cytotoxicity models. Results showed that FA can at least partially mimic the neuroprotective effect of *B. triquetrum* extract, although some other antioxidant compounds are still required to reach the extract maximal protective effect. However, no protection was observed after exposure to *p*-CA or *t*-CA, and no increase in FA protection was registered when adding *p*-CA and *t*-CA to FA, as they naturally occurs in the extract. In summary, this investigation showed evidences of the contribution exerted by FA to the *in vitro* neuroprotective effect of *B. triquetrum* aqueous extract, in the models of neuronal cell death induced by H_2O_2; H_2O_2 + $FeSO_4$; SIN-1 and MeHg. **References**: 1. Vidal, A., Motidome, M. *et al.* (2001), Braz. J. Pharm. Sci. 37: 373 – 382. 2. Fallarero, A., Loikkanen, J.J. *et al.* (2003), Phytomedicine. 10: 39 – 47. 3. Fallarero, A., Peltoketo, A. *et al.* (2006). Phytomedicine. 13: 240 – 245.

P 192

Effects Of Carvacrol Upon The Liver Of Rats Undergoing Partial Hepatectomy

Canbek M[1], Uyanolu M[1], Aral E[2], Baser KHC[3]
[1]Eskişehir Osmangazi University, Faculty of Science, Biology Department, 26480, Eskişehir, Turkey; [2]Eskişehir Osmangazi University, Medical Faculty, Histology Department, 26480, Eskişehir, Turkey; [3]Anadolu University, Faculty of Pharmacy, 26470, Eskişehir, Turkey

There are several studies reporting effects of volatile oils upon human health extracted from origanum (kekik). The present study aims to investigate the possible effects of purified carvacrol obtained from origanum and silymarin upon the regenerative feature of the liver subsequent to partial hepatectomy in rats. The carvacrol was tested in comparison with silymarin ale Wistar Albino rats, weighting 230 ± 30 g, were divided into 3 experiment groups. First group rats, called Group 1 (n = 8) were used as control group. Rats in Group 2 (n = 8) were applied carvacrol and hepatectomy (73 mg/kg). Silymarin and hepatectomy (100 mg/kg) were applied to the last group of the rats, Group 3 (n = 8). One dose of test materials was injected to Groups 2 and 3 one hour before 68 % partial hepatectomy. At the end of the experiments, blood and organs were removed intra cortically. The liver regeneration ratio of the rats was calculated measuring the half weights of their liver before and after the hepatectomy. H&E, IL-6 and PCNA treatments were applied to liver sections. AST, ALT, TNF-α and IL-6 levels were determined in serum samples. In AST, ALT, TNF-α and IL-6 levels, there were no statistically significant difference. Mitotic index and PCNA index comparisons were displayed significant differences; between Group 1 and 2, $p < 0.001$ between Group 1 and 2, $p < 0.05$ between Group 2 and 3. Histological evaluations were also similar with these results of PCNA and Mitotic indexes. According to these results, it is concluded that carvacrol increases the liver regeneration ratio.

P 193

Black Grape Extract Protects Against Cyclosporine A Nephrotoxicity

Durak I[1], Çetin R[2], Çandır Ö[3], Devrim E[1], Kılıçolu B[4], Avci A[1]
[1]Ankara University School of Medicine, Department of Biochemistry, Ankara-Turkey; [2]Ankara Oncology Education and Search Hospital, Department of General Surgery, Ankara-Turkey; [3]Süleyman Demirel University School of Medicine, Department of Pathology, Isparta-Turkey; [4]Ankara Education and Search Hospital, Department of General Surgery, Ankara-Turkey

The aim of this study was to determine if dried black grape protect against cyclosporine nephrotoxicity. Twenty eight Sprague-Dawley rats were given Cyclosporine A (CsA) orally for 10 days, with the black grape (Kalecik karasi, total phenolic content of the grape was approx. 96.25 ± 2.03 mg gallic acid equivalent/gr) supplementation begun three days before CsA treatment and continued during the study period (totally 13 days). In each group (control, CsA alone, CsA plus black grape, and black grape alone), there were 7 animals. At the end of the study period, the animals were sacrificed; their kidneys were removed and prepared for biochemical investigations. Oxidant (xanthine oxidase enzyme and malondialdehyde) and antioxidant (superoxide dismutase, glutathione peroxidase and catalase enzymes) parameters were measured in the kidney tissues of the groups. It has been found that CsA creates oxidant load to the kidneys through both xanthine oxidase activation and impaired antioxidant defense system, which accelerates oxidation reactions in the kidney tissue. Supplementation dried black grape led to reduced malondialdehyde level in the kidney tissue possibly, by preventing oxidant reactions. In conclusion, the results suggest that impaired oxidant/antioxidant balance may play part in the CsA-induced nephrotoxicity, and black grape may ameliorate this toxicity, in agreement with studies with antioxidant vitamins.

5. Clinical Studies with Herbal Medicinal Products

P 194

The flavonoidal constituents of Limoniastrum monopetatum and their biological activity

Radwan HM, Hassan RA
Chemistry of Medicinal Plants Dept, National Research Centre, Dokki, Cairo, Egypt, 12311

Investigation of *Limoniastrum* species (Family *Plumbaginaceae*) revealed the presence of flavonoids, coumarins, terpenes and alkaloids [1, 2]. *Limoniastrum* species are represented in Egypt by only one species [3]. The present work deals the study of the flavonoidal constituents of *L.monopetatum* and evaluation their hepatoprotective and the anti- oxidant activity of both the total extracts and the isolated compounds, using DPPH free radical. The aerial parts of *L. monopetatum* was dried, powdered and extracted with pet.ether and then with 80 % ethyl alcohol. The alcoholic extract after partitioner with chloroform, ethyl acetare and n-butanol yielded crude extracts containing flavonoids [4]. The previous extracts were subjected separately to preparative PC (3MM, 20 % acetic acid) and the main flavonoidal bands were cut and eluted separately with 90 % methanol. The eluted fractions were further purified by using Sephadex LH-20 column. The isolated flavonoid compounds were identified as myricetin, rutin, kaempferol-7-*O*-glucoside and myricetin-3-*O*-glucoside. Their identities were verified by TLC, PC, m.p, UV, ^{1}-H-NMR, MS and FAB mass spectrum. Acute toxicity studies of pet. ether and alcoholic extracts of the aerial parts of the plant, showed that the alcoholic is more safe than that of pet.ether extract and both extracts have a hepatoprotective effect on the hepatocytes against CCl_4 cytotoxicity at concentration of 40 μg/mL and 50 μg/mL, respectively. On the other hand, all the isolated flavonoid compounds showed significant antioxidant activity compared to Trolox.
References: 1. Rizk, A.M. (1986), The Phytochemistry of the flora of Qatar, Scientific and Applied Research Centre, University of Qatar. 2. Rizk, A.M. (1982), Fitoterapia 52: 35. 3. Tackholm, V. (1974), Students Flora of Egypt, 2nd ed., Published by Cairo Univer., Cooperative Printing Co., Beirut. 4. Radwan, H.M., Shams, K.A. (2005), J. Egypt. Pharmac. 4 (2).

P 195

Effect of garlic during and before administration of lead acetate on lead content of some tissues in mouse

Pourjafar M[1], Karimi I[1], Kojouri G[1], Kheiri S[2], Aghbolaghi PA[3]
[1]School of veterinary medicine, Shahrekord University, Shahrekord, Iran; [2]School of medical science, Shahrekord University of medical science, Shahrekord, Iran; [3]Educated of Shahrekord University, Shahrekord, Iran

Garlic (*Allium sativum* L.) has been found to possess heavy metal chelator activity. Prophylactic and therapeutic effects of garlic and garlic tablets on chronic administration of lead in mouse were investigated. Eighty mature mice (body weight of 35 – 40 g) were divided into eight groups and each group was made up of ten mice. Group D as a negative control group received placebo garlic tablet. Groups A1, A2 and A3 respectively received 500,250 and 125 mg/Kg/day garlic in first four weeks, and in second four weeks they received 5 mg/kg/day lead acetate and 500,250 and 125 mg/kg/day garlic respectively. Groups B1, B2 and B3 respectively received 1/4, 1/8 and 1/16 garlic tablet/kg/day (equal to 500 mg of fresh garlic) in first four weeks and in second four weeks received 5 mg/kg/day lead acetate and also respectively 1/4, 1/8 and 1/16 garlet tablet/kg/day (equal to 500 mg of fresh garlic). Group C as a positive control group received a quarter of a placebo garlic tablet/kg/day in first four weeks and in second four weeks they received 5-mg/kg/day lead acetate and a quarter of a placebo garlic tablet/kg/day. Reduction in lead content of kidney, liver and bone as a result of administration of garlic or garlic tablet in studied groups was significant compared with group C ($p < 0.05$) and reduction in lead content of blood

in all groups was significant except group A3. Results of comparison of lead content between different groups showed that fresh garlic and garlic tablet had the same effects on lead intoxication and lead deposition in tissues.

P 196

Cardioprotective effect of Thevetia neriifolia Jusss glycoside in male white rat

Saikia G
Department of Zoology, Gauhati University, Guwahati. India

The plant *Thevetia neriifolia* Jussex Steud. (TN, yellow oleander) is widely distributed in Assam, India either in wild or in garden. The root has been in used against heart heart trouble [1]. The seed kernels were used as common position for suicidal purpose. The defatted seed kernel contains glycoside [2] and the basic carbon skeleton is cyclopentanophenanthrene nucleus is a reduced form with an unsaturated gamma lactone ring attached to the 3 – 17 position. It has been aimed to identify the glycoside present in the seed kernel extracted in ether (EE) and its possible effect on blood glucose and blood cholesterol. The melting point of the extract was determined at 210 °C and contain C, H and O in the percentage proportion of 65.89, 9.01 and 25.10 respectively. The mass spectral analysis suggests the molecular formula as $C_{22}H_{36}O_6$. It has been ascertained that the EE fraction is the aglycone part of the glycolyside. The EE extract (2 mg) was dissolved in 0.1 mL absolute alcohol and was made 1.00mL in redistilled water injected intramuscularly(per animal) into the male Sprague Dawley rat (b.w. 120 g) and the blood was collected after 60 minutes following the animal ethical protocol. A control group receiving 0.1mL alcohol as in above was standard procedure and the chemicals used were either from Sigma or Hi Media, India Ltd. The blood glucose was reduced by 30 % after 60 minutes in the treated group. The serum lipid profile viz total cholesterol, triglycerides LDL and HDL were estimated following the CHOD – PAP, GPO and Friedwads method. The LDL and TC were reduced by 30 % and 80 % compared to hyperlipidaemic rats maintaine separetly on 14th day. The HDL was recorded as higher 25 % compared to control and the results were compared with Gamfibrazil on 21st day of treatment. The fall of blood glucose might be for its rapid utilization while the cholesterol might be degraded rapidly under the influence of glycoside. The reduction of LDL and the rise of HDL is a positive character. Therefore, it is suggested that the aglycone, present in the TN may be a potential cardiac glycoside as cholesterol controlling agent, however further characterization is necessary. **References**: 1. Mazumdar, R. *et al.* (1978), *J. Crude Res.* 16: 185. 2. Rangaswami, S. *et al.* (1959), *J. Indust. Res.* 18B: 443.

P 197

Hypolipidaemic effect of Clerodendron colebrookianum Walp glycosides in C_3H mice

Sharma DK, Bhuyan SK
Department of Zoology, Gauhati University, Guwahati – 781 014, Asom: India

Clerodendron colebrookianum Walp leaves (Verbenaceae; CC) are traditionally used in the folklore medicine of the Mizo people of India. The CC leaf possesses antihypertensive property [1, 2]. Magnesium is related to aldosteronism and hypertensive. The nexus between deficiency and disturbances in plasma lipid profile favors atherosclerosis. Therefore, it is aimed to quantify the leaf Mg and to evaluate the effect of methanolic extract and isolated glycosides (sitosterol) [3] of CC in the lipid profile of rats. 30 male C_3H mice were used and grouped into 1)with only basal diet as control; 2) with high fat (11 %) diet; 3) with basal diet and oral dose of methanol extract (40 mg'kg b.w.); 4) high fat diet and CC glycoside (20 mg/kg/ b.w.) and 5) with high fat diet and Gemfibrozil (20 mg/kg b.w). Dried finely powered leaves of CC were extracted in Methanol for 18 h and the presence of glycosides was qualitatively tested. 500 mg of weighed liver tissue was added with 10mL of 1:2 mixtures of HNO_3 and $HClO_3$ and digested to get a clear solution for Mg analysis. The cholesterol (TC) and HDL was assayed by the enzymatic CHOD – PAP method while the triglyceride (TG) was obtained by the GPO method. LDL was noted to be 40 % and 90 % respectively, after 7 days of introduced rats on and after 28th day. Elevation of Mg was noted in serum as well as in liver in the treated group. CC glycoside and high Mg extends antihyperlipidaemic activity. **References**: 1. Devi Rajlakshmi, Sharma, D.K. (2004), J. Ethnopharmacol. 90: 63 – 68. 2. Hui Yang *et al.* (2000), Fitopteria 71: 641 – 648. 3. Goswami, P. *et al.* (1995), Phytochemistry 41: 279 – 281.

P 198

Anti-dog tick herbal shampoo from Thai medicinal plant

Gritsanapan W[1], Ratanasak W[2], Ratanatham T[1], Carithape A[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand; [2]Faculty of Veterinary Science, Mahidol University, Salaya, Nakornpratom, Thailand

Dogs are always infested with ticks and flea which cause itching, skin irritation, inflammation and skin diseases. Some chemicals and drugs used for killing dog parasites cause irritation to dogs and dog keepers. Medicinal plants such as Custard apple, Stemona, Pyrethrum and Neem tree have been used to kill insects, head-lice and dog ticks [1, 2]. This study is aimed to investigate antiparasitic activity of Thai herbal shampoo preparations against dog ticks and find out the satisfaction of the dog keepers after using the herbal shampoo for cleaning their dogs. Anti-dog tick activity of the root extract of *Stemona tuberosa*; the seed extracts of Siamese neem tree and custard apple; and citronella oil was investigated *in vitro*. The 5 – 15 %w/w Stemona extract in polyethylene glycol gave the best killing effect within 30 – 40 minutes and was further incorporated with a shampoo base. The herbal shampoo with different concentrations of the Stemona extract (2, 4, 6, 8, 10 % w/w) were tested *in vitro* for the killing effect against dog ticks by direct contaction. The herbal shampoos with selected concentrations were used for shampooing 30 dogs infested with brown dog ticks. After 4 treatments, once a week, continuing for 4 weeks, the satisfaction of the dog keepers was investigated by answering questionnaires concerning decreasing of the dog ticks, the shampoo appearances and odor, cleaning and softness of dog hairs, skin irritation, etc. The shampoos with 2 – 5 % w/w of *Stemona* extract were found to be the preferred preparations. **References**: 1. Muanwongyart, P. (1994), Samunprai kaw mai. T.P. Print. Bangkok. 2. Gritsanapan, W. *et al.* (1998), Studies of stability and effectiveness of intensive hair masks from *Annona squamosa* seed extract. 50th IPC and 17th FAPA Congress, Mumbai, India.

P 199

Comparison of alkamide pharmacokinetics between equivalent liquid and tablet echinacea preparations

Lehmann RP[1], Matthias A[1], Watson K[2], Bone KM[1,3]
[1]MediHerb Research Laboratories, 3/85 Brandl Street, Eight Mile Plains, Brisbane, 4113 Australia; [2]School of Biological, Biomedical and Molecular Sciences, University of New England, Armidale, 2351 Australia; [3]School of Health, University of New England, Armidale, 2351 Australia

The traditional liquid preparations of echinacea suffer poor compliance due to the strong, characteristic tingling sensation associated with the alkamides. Tablet preparations are a dose form which offers much better compliance, but there have been concerns that alkamides from these are not as well absorbed. To investigate this concern, the relative oral bioavailability of alkamides from two different echinacea formulations (liquid and tablet) were compared in a small two way crossover study in humans (n = 3) and their pharmacokinetics parameters compared. The liquid preparation was a mixture of *Echinacea purpurea* L. (Moench) root (300 mg/mL) and *Echinacea angustifolia* DC. root (200 mg/mL) extracted in 60 % etha-

nol. The tablet preparation was also a mixture of *Echinacea purpurea* root (675 mg/tablet) and *Echinacea angustifolia* root (600 mg/tablet) but was prepared from the dried ethanolic extracts of the two Echinacea species. 9 mL of the liquid, containing 9.1 mg of the tetraene alkamide, was diluted to 25 mL with water and then swallowed immediately. 3 tablets, containing 7.8 mg of the tetraene alkamide were swallowed with 25 mL of water. Alkamides were rapidly absorbed and measurable in plasma from both preparations. No significant differences in the tetraene alkamide pharmacokinetic parameters for $T_{1/2}$, $AUC_{t\text{-}lin}$ and C_{max} in the two different preparations were found. T_{max} increased from 20 minutes with the liquid to 30 minutes with the tablet which is not unexpected as the tablet required time for disintegration before absorption could occur. By swallowing the liquid product immediately, the normal method of dosing for this product, any buccal absorption should have been minimised. These results suggest that there is no significant difference in the bioavailability of alkamides from liquid and tablet echinacea formulations and that both any alkamide loss due to digestive processes and the absorption site is similar in both preparations.

P 200

Positive influence of a Harpagophytum procumbens preparation on different rheumatic complaints – results from clinical trial

Suter A[1], Whittaker P[2], Dickson S[2], McIntyre L[2], Tan J[2]
[1]A. Vogel Bioforce AG, 9325-Roggwil, Switzerland; [2]Clinical Trial Department, Bioforce UK, Irvine, United Kingdom

Preparations of Devil's claw (*Harpagophytum procumbens* Burch. (DC.)) are nowadays widely used in the treatment of rheumatic diseases. Several clinical trials carried out in recent years show harpagophytum may improve symptoms of back pain and of osteoarthritis. Nevertheless, it is not known if there are differences in the efficacy of Devil's Claw in small or large joints affected by osteoarthritis. We carried out an open clinical trial with patients who reported a mild to moderate rheumatic disorder in at least one joint or body area. The main goal was to investigate if in the end there were differences in pain relief in the assessed joints and body areas. For 8 weeks, patients took 2 x 1 harpagophytum tablet daily (A. Vogel Rheuma Tabletten®), one tablet contained 480 mg harpagophytum extract (DER 1.5 – 3:1; extractant 60 % V/V ethanol). For each affected joint pain was assessed with a 10 point rating scale, additionally for knee and hip osteoarthritis (OA) the WOMAC score, for hand OA the hand algofunctional index and for patients suffering from back pain, the finger-floor distance were evaluated together with global assessments of safety and efficacy and of quality of life (SF-12). A total of 259 patients were treated and 222 analyzed in the intention-to-treat population. Osteoarthritis was the most common arthritic condition amongst patients. From 154 patients taking pain medication for their rheumatic disorder, at the end of the treatment 44.8 % could decrease their dosage, 16.9 % remained the same and 26 % could stop pain medication completely. Global pain, stiffness and function improved significantly. Pain reduction was similar in all joints. Pain decreased on average -35 % and statistically significant ($p < 0.05$). Treatment was well tolerated and 92 % of all patients would take the treatment again.

P 201

Effects of Garlic Consumption on Plasma and Erythrocyte Antioxidant Parameters in Elderly Subjects

Ergüder IB[1], Avci A[1], Atli T[2], Varli M[2], Devrim E[1], Aras S[2], Durak I[1]
[1]Department of Biochemistry, Ankara University School of Medicine 06100, Ankara; [2]Department of Geriatric Medicine, Ankara University School of Medicine 06110, Ankara

Aim: Effects of ingesting garlic on plasma and erythrocyte antioxidant parameters of elderly subjects were investigated in this study. **Methods:** Thirteen subjects (mean age 70.69 ± 4.23) participated in the study. They ingested garlic at the daily dose of 0.1 g/kg body weight for 1 month. Before and after this period, fasting blood samples were obtained, and oxidant (malondialdehyde, MDA and xanthine oxidase, XO) and antioxidant (superoxide dismutase, SOD and glutathione peroxidase, GSH-Px and catalase, CAT) parameters were studied in erythrocytes, and MDA levels were studied in plasma samples obtained from the subjects. **Results:** In the plasma fraction and erythrocyte hemolysate, MDA levels significantly were found to be lower, but erythrocyte GSH-Px and SOD activities significantly higher in the second samples relative to the first ones. Xanthine oxidase activity was found to be lower in the second samples, but this decrease was not statistically meaningful. Our results show that ingestion of garlic consumption leads to significantly lowered plasma and erythrocyte MDA levels, and to increased activities of some antioxidant enzymes, which indicate that consumption of garlic decreases oxidation reactions. It is quite possible that reduced peroxidation processes due to garlic consumption may play a part in some of the beneficial effects of garlic in elderly subjects.

Table 1: Oxidant and antioxidant parameters in erythrocytes (RBC) and plasma from elderly subjects who consumed garlic (Mean ± SD; n = 13).

Groups	G SH-Px (RBC)IU/mL	CAT (RBC)IU/mL	SOD (RBC)U/mL	MDA (RB)nmol/mL	XO (RBC)IU/mL	MDA (Plasma) nmol/mL
Before garlic	8.34 ± 1.07	57462 ± 17533	1668 ± 495	381.7 ± 39.8	2.21 ± 0.86	2.2 ± 1.4
After garlic	9.32 ± 0.78*	565787 ± 17904	2065 ± 298*	352.2 ± 38.4*	1.76 ± 0.93	1.2 ± 0.8*

* $p < 0.05$; Paired t-test

P 202

Destenotil – a combination of troxerutin and aescin to treat inner ear perfusion disturbances

Siegers CP[1], Schulze J[2]
[1]Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Ratzeburger Allee 160 D-23538 Lübeck; [2]Office of the Dean, Johann Wolfgang Goethe-University Frankfurt/Main, Theodor Stern Kai 7, D-60590 Frankfurt/Main, GERMANY

Destenotil is a fixed combination of troxerutin (450 mg) and aescin (25 mg) per capsule. Indications for this combination are inner ear perfusion problems of different aetiology. The efficiency of destenotil (5 x 450 mg troxerutin plus 25 mg aescin; n = 34 patients) versus pentoxyfyllin (600 mg/die; n = 34 patients) was tested in a randomized clinical study in a randomized group comparison design; end point was hearing improvement after 40 – 44 day treatment as compared to pretreatment values. The study included patients with hypacusis for ore than 6 month; patients with sound transmission disturbances were excluded (difference between bone and air conduction < 10 dB). Hearing was measured by threshold audiometry at 1000, 2000 and 4000 Hz before and after treatment; a difference of 10 dB or more was judged as significant improvement. After destenotil treatment hearing was significantly improved, in 23 of 34 patients the threshold was changed by more than 10 dB (significantly different). With the comparison treatment pentoxyfyllin hearing was also improved, albeit to a lesser degree. Both drugs were well tolerated, major adverse drug effects were not observed with either treatment.

P 203

Antibacterial and anti-inflammatory activity of Byrsocarpus coccineus and its constituents

Ahmadu AA[1], Haruna AK[1], Sule MI[1], Pateh UU[1], Akpulu IN[2]
[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria; [2]Department of Pharmaceutics and Pharmaceutical microbiology, Ahmadu Bello University, Zaria-Nigeria

In our continuous search to find bioactive compounds from Nigeria medicinal plant *Byrsocarpus coccineus* Schumach. & Thonn. was studied. The plant is used locally to treat diarrhea, venereal disease, inflammation and wound healing [1], but there is no hitherto report on the chemical constituents. The ethanol leaf extract, n-butanol and aqueous fraction of the leaf extracts were investigated for in-vitro antibacterial activities by agar diffusion technique [2]. The n-butanol extract inhibited the growth of standard and local strain of bacteria, including *Pseunomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis.* The minimum inhibitory concentration of the extract range 1.25 mg/mL to 5 mg/mL was studied. Fractionation of this extract over silica gel G and repeated purification over sephadex LH-20 led to the isolation of flavonoid: quercitin, quercetin 3-*O*-α-arabinoside, and quercetin 3-*O*-β-D-glucoside. Anti-inflammatory activity of the n-butanol extract by egg albumin induced edema in rats was assayed [3]. The extract at 100 mg/kg and 200 mg/kg ***i.p*** showed significant activity ($p < 0.05$) in comparism to the standard acetysalicyclic acid. The observed activity supports the traditional uses. **References**: 1. Dalziel, J.M., Hutchinson, J. (1955), Useful plants of West Africa. Crown Agents for Oversea Publication, London pp. 88 – 90. 2. Perez,C, ***et al.*** (1990), Acta Biol. Med. Exp. 15: 113 – 115. 3. Akah, P.A., Nwambie, A.I. (1994), J. Ethnopharmacol. 42. 179 – 182.

6. Other related topic

P 204

In vitro anti-fungal activity of a plant-based ear gel and its essential oils

Ketzis JK[3], Nolard N[1], Ryder NS[2]
[1]Scientific Institute of Public Health, Mycology Section, 14 rue Juliette Wytsman, 1050 Brussels, Belgium; [2]Infectious Diseases, Novartis Institutes for Biomedical Research, Inc, 100 Technology Square, Cambridge, MA 02139, USA; [3]Novartis Animal Health, Inc, Basel, Switzerland (currently at Charles River Laboratories Biolabs Europe Ltd, Carrentrila, Ballina, County Mayo, Ireland)

The dog and cat ear gel (PID 02027020, produced by Oystershell NV, Belgium) used to maintain auricular health, contains a mixture of essential oils (11 % v/w) in a non-natural base. While the ear gel contains oils from 15 plants, five of the essential oils make 9 % v/w of the product. These are listed in order of highest to lowest concentration in the ear gel with those with equal quantities listed together: *Calophyllum inophylum* L. and *Hypericum perforatum* L., *Calendula officinalis* L., *Melaleuca alternifolia* (Maiden & Betche) Cheel, and *Origanum compactum.* Two *in vitro* studies were conducted to determine the anti-fungal activity of the gel. In Trial 1, the fungal inhibition properties of the formulated gel (rates of 100 mg and 250 mg) were tested against 7 fungi using agar diffusion. The minimum inhibition concentration (MIC) also was tested with 6 fungi using a broth macrodilution assay. Given difficulties of using the gel, a second trial was done with 2 fungi (*Candida albicans* and *Malassezia pachydermatis*; 3 strains of each) using the mixture of essential oils in the gel. In Trial 2, a broth microdilution method was used and the MIC (read visually and with a spectrometer) and minimum fungicidal concentration (MFC) was determined. In Trial 1, the formulated gel, at 100 mg, showed 80 % (*100 %) inhibition against *C. albicans* (*against one strain), *Saccharomyces cerevisiae**, *Cryptococcus neoformans**, *Aspergillus fumigatus*, *Trichophyton mentagrophytes**, *Trichophyton rubrum**. It was not effective against *Malassezia furfur* and *M. sympodialis* and the MIC in the macrodilution assay was > 1280 µg. In Trial 2, the MIC 80 % values ranged from 0.25 to > 2 % concentration (visual) and 0.5 to 1 % concentration (spectrometer), with more activity against *C. albicans.* The MFC values ranged from 0.5 to > 2 % concentration with more inhibition against *M. pachydermatis. Acknowledgements*: Novartis Animal Health Inc., Switzerland, Oystershell NV, Belgium **References**: 1. Timoney, J.F. ***et al.*** (eds) (1998), Hagan & Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th Ed. Comstock Publishing Association. Ithaca, NY. 2. Griffin, S.G. ***et al.*** (2000), J. Essent. Oil Res. 12: 249 – 255. 3. Jansen, A.M. ***et al.*** (1987), Planta Medica 53: 395 – 398.

P 205

In Vivo Testing of the Wound-healing Activity of a Natural-based Skin Cream for Dogs and Cats

Jia S[1], Mustoe T[1], Ketzis JK[2]
[1]Wound healing research laboratory, Northwestern University, 303 E. Chicago Ave, Tarry 4 – 723, Chicago, IL 60611; [2]Novartis Animal Health, Inc, Basel, Switzerland (currently at Charles River Laboratories Biolabs Europe Ltd, Carrentrila, Ballina, County Mayo, Ireland)

PID 02027030 (Oystershell NV, Belgium), a skin cream for minor abrasions for dog and cats, was tested in a rat incision and rabbit excision model to determine wound-healing activity. The product contains plant extracts (*Arnica Montana* L., *Calendula officinalis* L., *Echinacea purpurea* (L.) Moench, *Hamamelis virginiana* L.) and essential oils (*Lavandula officinalis* Chaix, *Melaleuca quinquenervia* (Cav.) S.T. Blake, *Salvia lavendulifolia* Vahl and *Thuja occidentalis* L.) (8.25 % v/w) in a non-natural excipient. The extracts and oils conform with the EU or French pharmacopeia (when available) and/or are standardized using marker compounds. In the incision model, 10 Sprague-Dawley rats were used to determine the effect of the product on wound breaking strength. Each rat served as its own control and had a total of 6 incisions. Peak breaking strength was measured using a tensometer and 50 N load cells. Six New Zealand White rabbits were used in the excision model to determine histologic changes. Each rabbit served as its own control and had a total of four 6-mm full-thickness dermal punches on the inner surface of the ear down to bare cartilage. Any wound with evidence of infection, desiccation, or necrosis was excluded from the study. Using Masson's trichrome stain, histologic measurement of hypertrophic scar was determined by light microscopy using two blinded observers. In both models, the treated wounds had the test product applied at a rate sufficient to cover the area, while the control wounds were left untreated. Skin was harvested 7 days post-application of product. All wounds were created and harvested in a matched fashion, and the data collected in a manner to allow paired analysis. Statistical analysis using a paired two-tailed Student's t-test was performed (significance set at $p < 0.05$). Histological data also were analyzed using Chi-square. In the rat model, the product significantly increased tensile strength. In the rabbit model, the product significantly increased granulation, but decreased epithelialisation. *Acknowledgements*: Novartis Animal Health Inc., Switzerland, Oystershell NV, Belgium

P 206

In vitro anti-microbial activity of the essential oils and extracts in a plant-based skin cream

Ketzis JK, Laux MT[1,2]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, New York, 14853 USA; [2]Novartis Animal Health, Inc, Basel, Switzerland (currently at Charles River Laboratories Biolabs Europe Ltd, Carrentrila, Ballina, County Mayo, Ireland)

The investigational dermal cream (PID 02027030) is designed to soothe skin lesions of dogs, cats, and horses. The cream contains a mixture of plant extracts (*Arnica Montana* L., *Calendula officinalis* L., *Echinacea purpurea* (L.) Moench, *Hamamelis virginiana* L.) and es-

sential oils (*Lavandula officinalis* Chaix ex Villars, *Melaleuca quinquenervia* (Cav.) S.T. Blake, *Salvia lavendulifolia* Vahl. and *Thuja occidentalis* L.) (8.25 % v/w) in a non-natural excipient. The extracts and oils conform with the EU or French pharmacopeia (when available) and/or are standardized using marker compounds. In order to determine the anti-microbial activity of the cream, the mixture of plant-based oils and extracts (not in the excipients) was tested *in vitro* against 12 microorganisms (*Candida albicans* (ATCC#90028), *Epidermophytum floccosum*, *Malassezia pachydermatis*, *Microsporum canis*, *Psuedomonas aeruginosa* (ATCC#27858), *Saccharomyces cerevisiae* (ATCC#2601), *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Bacillus cereus* (ATCC#11778), *Escherichia coli* (ATCC#25922), *Helicobacter pylori* and *Staphylococcus aureus* (ATCC#25923); Organisms without ATCC numbers were isolated from infected animals presented at the Cornell University Veterinary Clinics [1]). A modified National Committee for Clinical Laboratory Standards (NCCLS) method was used. To increase solubility of the oils, the growth media was prepared with 0.5 % Tween 20 [2, 3]. Six mm diameter filter-paper discs were treated with 20 µl of the mixture. Ethanol and chloramphenicol were used as controls. Plates of all bacteria were incubated for 24 h at 37 °C. Plates with fungi were incubated for 48 h at 35 °C. *Microsporum canis* was incubated for 72 h at 35 °C. The mixture was active against all test organisms at 20 µl. It was more active against the fungi than the bacteria, with rings of growth inhibition ranging from irregular (*Malassezi pachydermatis*) to 1.5 cm for the fungi and 0.8 for all of the bacteria. Based on these results, the dermal cream could be useful in preventing secondary infections in lesions. *Acknowledgements*: Novartis Animal Health Inc., Switzerland, Oystershell NV, Belgium **References**: 1. Timoney, J.F. *et al.* (eds) (1998), Hagan & Bruner's Microbiology and Infectious Diseases of Domestic Animals, 8th Ed. Comstock Publishing Association. Ithaca, NY. 2. Griffin, S.G. *et al.* (2000), J. Essent. Oil Res. 12: 249 – 255. 3. Jansen, A.M. *et al.* (1987), Planta Medica 53: 395 – 398.

P 207

Evening primrose oil (EPO) quality changes dependent on storage temperature and storage time

Ghasemnezhad A, Cergel S, Honermeier B

Evening primrose (*Oenothera biennis* L.) is a seed drug plant and a rich source of γ-linolenic acid (GLA), an unusual fatty acid with proven value as a nutrient and prescription pharmaceutical. EPO contain high amount of unsaturated fatty acids, which makes it more susceptible to the oxidative deterioration. Therefore a suitable storage condition can improve the seed oil quality and delay it's spoliation (rancidity). Present trail was executed to clarify the effects of different storage temperatures (4 °C, 21 °C, 35 °C) and storage time (0 – 4 months) on *Oenothera biennis* extracted crude oil and unextracted oil (seed) fatty acid composition and their stability during storage period. The results showed that the oil and protein content in the whole seed (unextracted oil) were significantly influenced by storage time. The lowest amount of seed oil content was observed at the last storage month (24,3 % in compare to 26,0 % in control). In contrast to that, the highest amount of protein content was measured in the last two months (15,4 %) of storage. γ-linoleic acid and linolenic acid content were influenced by storage time both in unextracted oil and extracted crude oil. The amount of these two fatty acids shows a linear decrease during storage time. Free fatty acid was influenced by all treatment factors used for both oil samples. The highest amount of free fatty acid was observed when seeds were stored under high temperature during four months (3,4 % FFA). The lowest free fatty acid content was observed in the low temperature. The content of oleic acid, linoleic acid and γ-linolenic acid of extracted oil did not change under different combination of light and temperature. According to the obtained results it can be concluded that extracted and unextracted oil compositions are more stable under cold temperature during a short time and extracted crude oil has more stability than unextracted oil.

P 208

Development of skin whitening preparations from kaffir lime oil (Citrus hystrix)

Hongratanaworakit T[1], Tapaneeyasin P[1], Nuamlert J[1], Chansiri A[1], Hongrattanavorakit N[2]
[1]Faculty of Pharmacy, Srinakharinwirot University, Nakhon-nayok 26120, Thailand; [2]Department of Cosmetic Sciences, Fairleigh Dickinson University, Teaneck, NJ, USA

Kaffir lime oil (*Citrus hystrix* L., Rutaceae) has been used in medications and cosmetics. The oil showed antioxidant and skin whitening effects [1]. The main objective of this study was to develop topical whitening formulations from essential oil of *C. hystrix* grown in Thailand. Tyrosinase inhibition was used as an indicator of the whitening effect. The dopachrome microplate assay was used for determination of enzyme activity [2, 3]. The percentage of tyrosinase inhibition of fruit peel oil and leaf oil (0.25 % w/v) was 54 and 55, respectively. The oil was extracted and analyzed by GC-FID and GC-MS. Topical formulations from the essential oil of *C. hystrix* were developed into two different dosage forms, i. e. cream and gel. The formulations were developed in terms of physicochemical properties and organoleptic feature. Skin irritation test in rabbits and human volunteers were performed. The results showed that both topical cream and gel formulations had no irritation to either rabbits or human volunteers. In addition, stability studies of the formulations were evaluated and the formulations were found to be physically and chemically stable over a period of 1 year at 4 and 25 °C. In conclusion, this study demonstrated the strong potential of health products containing essential oil of *C. hystrix* in cosmetic application. **References**: 1.Manosroi, A. *et al.* (2003), Proceedings in the 3rd World Congress on Medical and Aromatic Plants for Human Welfare. 2. Isao, K. *et al.* (1999), Planta Med. 65: 19 – 22. 3. Koichi, I. *et al.* (1995), Planta Med. 61: 425 – 428.

P 209

Effects of nutrient elements on the production of bioactive volatile compounds from Citrus oils

Hongratanaworakit T[1], Kanna C[1], Mulsri N[1]
[1]Faculty of Pharmacy, Srinakharinwirot University, Nakhon-nayok 26120, Thailand

The kaffir lime tree (*Citrus hystrix* L., Rutaceae) is a native plant in Asia and grows best in the tropical area, especially in Thailand. It is a small and upright tree. The kaffir lime leaf is dark green color and glossy. The kaffir lime fruit is nearly spherical or lime-shape with a rough surface. The main aim of this study is to investigate the effects of nutrient elements on the production of bioactive volatile compounds from kaffir lime oils. This study was performed to obtain the highest quality of the oils by using ten different nutrient formulas. The treatments were NPK (nitrogen-phosphorous-potassium), NP, NK, PK, 2P, 2N, 2K, N, P, and K. The quality of the oil was indicated in terms of the amount of the main bioactive component of the oil. The kaffir lime oils were extracted from leaf and fruit peel of *C. hystrix* by hydrodistillation in a Clevenger apparatus. The distillation time was 2 hours. All samples were analyzed by GC-FID and GC-MS. Essential oil yield varied between 0.90 – 1.80 % w/w. The most abundant component of leaf oil was beta-citronellal [1, 2]. The results showed that the amount of beta-citronellal in leaf oil significantly increased in all nutrient formulas as compared to the control formula. Four main components, i. e. beta-pinene, limonene, beta-phellandrene and beta-citronellal, were prominent in kaffir lime fruit peel oil [1, 2]. The results revealed that the amount of beta-pinene in fruit peel oil significantly increased in all nutrient formulas as compared to the control formula. The amount of limonene in fruit peel oil significantly increased in N, K, and NK formulas

as compared to the control formula. Also, the amount of beta-citronellal in fruit peel oil significantly increased in all nutrient formulas except NK and N formulas as compared to the control formula. In contrast, the amount of beta-phellandrene in fruit peel oil significantly decreased in all nutrient formulas as compared to the control formula. *Acknowledgements:* Srinakharinwirot University, Thailand **Reference**: 1. Akiyoshi, S. *et al.* (1990), J. Essential Oil Res. 2: 179 – 183. 2. Ibrahim, J. *et al.* (1996), J. Essential Oil Res. 8: 627 – 632.

P 210

Antibacterial activity evaluation of Tunisian Thymus capitatus essential oils

Bounatirou S[1], Smiti S[1], Rejeb MN[2], Neffati M[3], Costa MM[4], Faleiro ML[5], Miguel MG[5], Figueiredo AC[4], Barroso JG[4], Pedro LG[4]
[1]Faculté des Sciences de Tunis, Université Tunis el Manar, Campus Universitaire, 2092 Tunis, Tunisia; [2]Institut National de Recherche en Génie Rural, Eaux et Forêts, 2080 Tunis, Tunisia; [3]Institut des Régions Arides, 4119 Mednine, Tunisia; [4]Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749 – 016Lisbon, Portugal; [5]Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8005 – 139 Faro, Portugal

Thymus capitatus L. (Hoffm.) commonly used in Tunisia for culinary purposes and locally known as "zaâtar" is a perennial, herbaceous shrub (Lamiaceae). *Th. capitatus* essential oils were isolated by hydrodistillation from the aerial parts of plants collected during the different phases of the plant development at different locations (Jendouba, Aîn Tounine and Haouaria) in a total of 16 oils. The main components of the essential oils were carvacrol (62 – 83 %), *p*-cymene (5 – 17 %) and γ-terpinene (2 – 14 %). In this work, we evaluated using the disc agar diffusion technique the antibacterial ability of all *Th. capitatus* essential oils against: 1) *Bacillus cereus* (C1060), *Salmonella* sp. and *Listeria innocua* [0.8 µL/disc] 2) Three different strains of *Staphylococus aureus* (C15, ATCC 6538 and ATCC 25923) [0.4 µL/disc] The most effective oils (8) were assayed against: 3) *S. aureus* C15, CFSA-2 and ATCC 25923 and one multi-resistant form of *S. aureus* (MRSA-2) [0.8 µL/disc]. *Listeria innocua* was the most susceptible of the three tested bacteria in assay (1). Of the 16 oils assayed, the flower buds and flower oils from Jendouba had a similar effect to that of the antibiotic. Of the three *Staphylococus* strains studied in assay (2), ATCC 6538 was more susceptible than C15 and ATCC25923. Of the 16 oils, 12 were the most effective, all from the flowering and the fructification phases, showing a diameter of inhibition zone 1.3 times higher than that of the antibiotic. Multi-resistant form of *S. aureus* studied in assay (3) was most vulnerable to TC11 (Jendouba, flowering buds oil). *Th. capitatus* essential oils seem to constitute an effective biocide to either combat foodborne pathogens or serious clinical pathogens such as MRSA.

P 211

Composition and Antimicrobial Activity of the Essential Oil From Satureja edmondi

Masumeh K
Department of Chemistry, Kermanshah ACECR, Iran

The genus Satureja belongs to the family Apiaceae [1]. Since ancient times satureja species have been used as spice and flavoring. Literature search showed wildly researches about this genus. The antibacterial antiviral, antimicrobial impacts of some type were Significant. [2, 3]. *Satureja.edmondi* Briquet as a member of this genus belongs to west part of Iran. In the folk medicines of the west of Iran, besides it's used in food as spice and flavoring, it's used as digestive, carminative, stimulant. To date we know of no published report concerning the volatile constituents and antimicrobial property of *S.edmondi* the present work was under taken to study the chemical composition and antimicrobial screening of this plant. Aerial parts of *S.edmondi* were collected from Dalahoo, province of Kermanshah, Iran, in Jun 2005.the yield of the yellow oil that was obtained by Hydrodistilation in a Clevenger-type apparatus was 5.26 (w/w). The essential oil was analyzed using a GC-MS Analysis to determine main constituents and micro-dilution broth susceptibility assay [4] was used for the antibacterial evaluation of the oils. 9 bacteria were used as test microorganism. 40 components were identified constituting 91.21 % of oil P-cymene (15.09 %), γ–terpinen (16.24 %) and α–terpinen (16.24 %) were the major components of the oil. Others are in small amounts and evaluated for their antimicrobial properties against the standard antimicrobial agent choloramphenicol. Results showed that the essential oil of *S.edmondi* has a minimal inhibitory concentration (MIC) value of 62.5 µg/mL against the pathogenic yeast *Candida albicans*. *Eshericia coli* and *Pseudomonas aeruginosa* were best inhibited by the *S.edmondi* oil with MIC value of 31.22 – 125 µg/mL stronger than the standard Chloramphenicol. *Enterobacter aerogenes*, *Salmonella typhimurium* and *Aspergillus flavus* were inhibited as good as the standard antimicrobial agent. The results of the present study support the folkloric usage of the studied plant. In conclusion furthermore, antibacterial activities, especially against various plant pathogenic microorganisms, and essential components of this plant, have also been reported here for the first time. **References**: 1. Rechinger, K.H. (1999), Flora Iranika. Pp. 162. 2. Saadat, M., Pournourmohammadi, SH. *et al.* (2004), J. Pharm. Pharmaceut. Sci. 7: 327 – 331. 3. Suarez, A., Echandi. M.M., *et al.* (2003), Rev. Biol. Trop. 51. 247 – 252. 4. Koneman, E.W. *et al.* (1997), Color Atlas and Texbook of Diagnostic Microbiology. Lippincott-Raven Publ., Philadelphia, pp. 785 – 856.

P 212

Antioxidant Evaluation and Stability of Guava (Psidium guajava Linn.) Dried Extract

Ingkatawornwong S, Pinsuwan S, Itharat A, Sukying S, Puripattanavong J
Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai Campus, Songkhla 90112, THAILAND

Thai guava leaves were extracted, using 50 % ethanol in water. The DPPH radical scavenging activity of the extract was determined along with its total phenolic content using caffeic acid as a standard. It has been found that the extract of Thai guava leaves possessed high antioxidant activity with EC_{50} values of 3.31 (r^2= 1) mcg /mL and total phenolic contents of 35.39 ± 2.63 %w/w. Since the dried extract was hygroscopic under high humidity, its preparation was formulated by combining 3.35 % w/w Aerosil® with the extract before drying. This provided a more physically stable extract product. The stability evaluation of the extract product was carried out under accelerated conditions (45, 60 and 70°C, 75 % RH). Quercetin in the extract was determined as a marker using HPLC. Under accelerated conditions, the overall loss of quercetin in the extract product displayed a non-linear profile. The initial phase of degradation exhibited an apparent second-order kinetic behavior where the half-life was dependent on the initial concentration and the degradation rate constant at each condition. The initial lag time was also observed in the degradation profile, suggesting the protective effect of the additive in formulation. No degradation was observed in the product heated at 100°C for 3 minutes. Although the EC_{50} of the extract product increased to about 10 mcg/mL under accelerated conditions, it is still better than the activity of the positive control, BHT (EC_{50} 19.92 mcg/mL). *Acknowledgements*: Thailand Research Fund, Thailand **References**: 1. Qian, H., Nihorumbere, V. (2004), J. Zhejiang University Science 5: 676 – 683. 2. Yamasaki, K. *et al.* (1994), Chem.-Pharm. Bull. 42: 1663 – 1665.

P 213

Bactericidal and fungicidal activity of plant extracts from endemic plants of the Chihuahuan Desert of northern Mexico

Lira RH[1], Hernández M[1], Pineda G[1]
[1]Centro de Investigación en Química Aplicada (CIQA), Blvd. Enrique Reyna 140, CP 25100, Saltillo, Coah, México

A large number of compounds are produced by plants endemic to the arid zones of Mexico. These compounds, which are stored in the roots and aerial parts, include phenolics, terpenoids, flavonoids, alkaloids and amino acids with pharmacological potential. In this work we determined the microbicidal effect of three plants from the Chihuahuan Desert. Methanol and ethanol extracts from the aerial parts of *Larrea tridentata* (Ses. et Moc ex DC.) Felger & Lowe (Zygophyllaceae), *Flourensia cernua* DC. (Asteraceae) and *Lippia graveolens* Kunth. (Verbenaceae) yielded nordihydroguiaretic acid, dehydroflourensic acid [1] and timol. The MeOH and EtOH extracts showed bactericidal and fungicidal activity in a variety of *in vitro* assays. *L. tridentata* EtOH extracts were more active against seven bacteria and fourteen fungi that cause infection in humans, plants and their products. The ANOVA showed highly significant differences ($P \leq 0.01$) within the extracts, doses, and the interaction extract x dose. At the low concentration of 125 µL/L the antibacterial activity of *L. tridentata* EtOH extract was evident against *Escherichia coli*; at 1000 µL/L all other six bacteria were inhibited by the same extract. On the other hand, aflatoxins produced by the fungi *Aspergillus flavus*, *A. parasiticus* and *A. niger* have received great attention because of their potent and acute toxicological effects in humans. Our results showed that at 1000 µL/L *A. niger* totally inhibited their mycelia growth. For the same effect, *A. flavus* and *A. parasiticus* required 4000 µL/L. These results provide evidence that *L. tridentata* extracts may offer a promising option to antibiotics and synthetic pesticides. *Acknowledgements*: This work was partially supported by the Government of Campeche State, Mexico and CONACYT through the project CAMP-2005-C01 – 045. **Reference***:* 1. Jasso de Rodríguez, *et al.* (2006), J. Ind. Crops (In press).

P 214

Composition and nutritive value of protein in some Macedonian edible wild Russulaceae mushrooms

Bauer Petrovska B, Kulevanova S
Institute of Pharmacognosy, Faculty of Pharmacy, Vodnjanska 17, 1000 Skopje, Republic of Macedonia

Increased protein requirements stimulated the interest in the introduction and use of new protein sources, which could compete as a substitute and addition to the ordinary protein food items of animal origin. Most literature data consider edible mushrooms as protein food products of the future, because the protein content of most of the species is higher than in many other natural products, the cultivation takes a short time and is inexpensive [1]. Nutritional quality of the mushroom protein varies and is strongly affected from the relative proportion of each amino acid. Thus, the purpose of this study was to estimate the concentration of the amino acid level present in the mushroom proteins, in order to evaluate the protein nutritional value of four mushroom samples of *Russulaceae* family collected in Macedonia. After acid hydrolysis and pre-column derivatization with phenyl isothiocyanate (PITC) determination of seventeen amino acids was carried out by HPLC method [2]. In the alkaline hydrolisates tryptophan was determined spectrophotometrically [3]. Evaluation of the protein quality was achivied by comparison of the essential amino acid content with the reference FAO/WHO pattern [4]. Essential amino acids made up 49 – 73 % of all determined amino acids depending on the origin and the species of the fruit body. Lysine was the most often found limiting amino acid. The nutritional value of proteins calculated by biological value, protein ratio, chemical score and essential amino acid index was very high. The biological value of the mushroom proteins varied from 55.31 to 82.87 %. *Russula xerampelina* could serve as a source of high quality proteins (PER = 2.8) with high biological value (BV = 82.87 %) similar to beefmeat (BV = 85 %; PER = 2.9). *Lactarius deliciosus* samples contains medium-quality proteins (PER = 1.54) with lower biological value (BV = 57.94 %) similar to soybean (BV = 59.9 %; PER = 1.6). **References**: 1. Friedman, M. (1996), J. Agric. Food Chem. 44, 6 – 29. 2. Bidlingmeyer, B. (1984), J. Chrom. A 336: 93 – 104. 3. Shamanthaka, M.C. Sastry, D.R. (1986), J. Sci. Food Agric. 37: 535 – 538. 4. FAO/WHO Protein quality evaluation, Rome (1991).

P 215

Assessment of antileishmanial and cytotoxic activities of some phenolic acids using the MTT assay – a critical evaluation

Kram D[1], Thäle C[2], Kiderlen AF[2], Kolodziej H[1]
[1]Freie Universität Berlin, Institute of Pharmacy, Pharmaceutical Biology, Germany; [2]Robert Koch-Institut, Cellular Immunology Unit P22, Berlin, Germany

The MTT assay, based on the reduction of a tetrazolium salt into a blue formazan product by dehydrogenase activity in viable cells, is widely used for measuring cell viability. Problems with MTT in the presence of plant phenolic acids and related reports [1] prompted the critical re-evaluation of a method that we routinely use for screening antileishmanial compounds and evaluating their potential toxicity for host cells. For this, we used caffeic, benzoic, p-hydroxybenzoic, and gallic acid, and the methyl and ethyl esters of gallic acid. We show that gallic acid and its tested esters could reduce MTT in the absence of living cells. In a cell viability assay, this apparent metabolic effect would lead to false positive results. Also, serum proteins interfered with the samples in a time-dependent manner. For example, prolonged pre-incubation of gallic acid in medium reduced its cytotoxicity (RAW 264.7 cells; IC_{50} of 230 µM at 30 min → 380 µM at 7 h). Further, when a standardized cell suspension was added to the sample, the IC_{50} values of some phenolics were conspicuously smaller compared to when the samples were given to an existing cell monolayer. Parallel investigations by FACS analysis confirmed these findings. For testing antileishmanial activity with the MTT assay, a starting concentration of 2×10^5 parasites/well was found useful. No significant differences in sensitivity were seen with *L. donovani* and *L. major*. We conclude that cell-free controls are essential when cell viability is to be tested. On the other hand, in our assay for antileishmanial activity against intracellular amastigotes [2], samples are fully removed before host cell lysis and addition of MTT, thus rendering this assay much less prone to irregular results. **References**: 1. Rollino, C. *et al.* (1995), J. Immunol. Methods 185: 141 – 143. 2. Kiderlen, A.F., Kaye, P.M. (1990), J. Immunol Methods 127: 11 – 18.

P 216

The in Vitro Antibacterial Activity of a Multiherbal Formula used in Yemeni Traditional Medicine for Topical Treatment of Impetigo

Alasbahi R[1], Al-Helali MF[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University. P.O. Box 19065, Sana'a, Yemen; [2]Department of Biology, Faculty of Science, Sana'a University, P.O. Box 13499, Sana'a, Yemen

Impetigo – a contagious superficial pyogenic infection caused by specific strains of *Staphylococcus aureus* and *Streptococcus pyogenes*– is spreading among preschool and young school age children in Yemen especially in late summer. A number of indigenous multiherbal formulas have been appreciated in Yemeni traditional medicine and claimed to be useful for the treatment of impetigo. The present study was aimed at evaluating the antibacterial activity of an empirically applied indigenous aqueous multiherbal formula for the treatment of impetigo. Different amounts (25 µL 50 µL, and 100 µL equivalent to 5 mg, 10 mg, and 20 mg of the dried extract)

of the aqueous extracts of an indigenous multiherbal formula -composed of a mixture of equal quantities of leaves from *Meriandra benghalensis* Benth., *Ruta chalepensis* L., and *Thymus laevigatus* L. and of these individual components, as well as of 80% ethanol extracts of the aforementioned plant materials individually and as a mixture of them, designated as alcoholic multiherbal formula were tested for antibacterial activity by using a modified agar diffusion assay [1, 2] against the pathogenic *Staphylococcus aureus* strains (1), (2), (3), and *Streptococcus pyogenes* strains (1) & (2) isolated from patients with impetigo. Indigenous aqueous multiherbal formula was found ineffective against all tested bacteria. On the other hand only the high concentration (100 µL equivalent to 20 mg of the dried extract) of the aqueous extracts of *R. chalepensis*, and *T. laevigatus* showed antibacterial activity approaching as well as exceeding those exhibited by the positive controls against pathogenic *Streptococcus pyogenes* strain 1. One or more of the tested concentrations (equivalent to 5. 10, and 20 mg extracts) of the 80% ethanol extracts of the alcoholic multiherbal formula as well as of two of its individual components (*R. chalepensis*, and *T. laevigatus*) demonstrated antibacterial effect similar or higher than those produced by the positive controls against the tested pathogenic bacteria. Consequently the antibacterial activity demonstrated by the 80% ethanol extracts of *Ruta chalepensis* leaves, and *Thymus laevigatus* leaves suggest these components could be utilized as an alcoholic multiherbal formula or individually as alcoholic extracts for the treatment of impetigo instead of the ineffective indigenous aqueous multiherbal formula. *Acknowledgements*: the authors thank Dr. Saeed Shibani, Director of the Central Laboratory- Sana'a, Yemen- for providing laboratory facilities. The authors also wish to thank Dr. Huda Al-Shami for isolating pathogenic bacteria from the patients and her persistent help. **References**: 1. Bauer, A.W. *et al.* (1996), Am. J. Clin. Path. 45: 493 – 496. 2. Wilkins, T.D. *et al.* (1972), Chemotherapy 1:451 – 496.

P 217

Degradation of Amyloid β-peptide (Aβ) by NEP-induction is increased by selected natural products

Ayoub S, Melzig MF
Freie Universität Berlin, Institute für Pharmazie, Königin-Luise-Str. 2+4, D-14195 Berlin, Germany

Neutral endopeptidase (EC 3.4.24.11, NEP) contributes to the degradation of amyloid beta-peptide in the brain [1]. Amyloid beta-peptide can be deposited as senile plaques in the brain leading to Alzheimer's disease (AD) [2]. The up-regulation of NEP in the brain may prevent AD development by increasing Amyloid beta-peptide clearance, resulting in a decrease of amyloid beta-peptide levels [3]. The aim of the present study was to investigate the cellular regulation of NEP expression in human neuroblastoma cell line SK-N-SH, focusing on the role of cyclic nucleotides. We studied the changes in the NEP activity after long-term treatment with substances, which increase the level of cyclic adenosine monophosphate (cAMP). The assay of NEP activity was determined according to Bormann and Melzig [4]. We determined the influence of some flavonoids as apigenin and luteolin, which are able to inhibit phosphodiesterase enzyme (PDE) [5], dibutylryl-cAMP (as protein kinase A activator [6]), forskolin (an adenylate cyclase activator [7]), and rolipram (a specific inhibitor of the phosphodiesterase type 4 isoform [8]) on the NEP activity. We could show that apigenin and luteolin induced NEP activity (up to 580%) with inhibition of cell proliferation. Whereas dibutylryl-cAMP, forskolin and rolipram induced the cellular NEP activity (up to 150%) and did not influence the proliferation. It is suggested that the enhancement of the cellular NEP activity might be correlated with an elevated level of cyclic adenosine monophosphate (cAMP) [9]. The results indicate, that the enhancement of the cellular NEP activity by apigenin and luteolin not only depends on the differentiation improvement but also on the direct influence on NEP gene expression via elevated level of intracellular cyclic Adenosine monophosphate (cAMP). The present data provide evidence for a cAMP-mediated increase of NEP activity in human neuroblastoma cells [10]. **References**: 1. Iwate, *et al.* (2001), Science 292: 1550 – 1552. 2. Selkoe, *et al.* (1998), Trends. Cell. Biol. 8: 447 – 453. 3. Iwate, *et al.* (2005), Pharmacol. Ther. 108: 129 – 148. 4. Bormann, H., Melzig M.F. (2000), Pharmazie 55: 129 – 132. 5. Ko, *et al.* (2004), Biochem. Pharmacol. 68: 2087 – 2094. 6.Graf, *et al.* (1995), Peptides 16: 1273 – 1278. 7. Wan Kim, *et al.* (2004), J. Am. Soci. Nephrol. 15: 2998 – 3005. 8. Vitolo, *et al.* (2002), Proc. Natl. Acad. Sci. USA 99: 13217 – 13221. 9. Ajiro, *et al.* (1990), J. Biol. Chem. 265: 6494 – 6500. 10. Ayoub, S., Melzig M.F. (2006), J. Pharm. Pharmacol. 58: 495 – 501.

P 218

Analgesic and topical anti-inflammatory activity of terpenoids and flavonoids from species of the genus Teucrium and Salvia in mice

Bonkanka CX, Rabanal RM, Sánchez-Mateo CC
Departamento de Farmacología, Facultad de Farmacia, Universidad de La Laguna, c/ Astrofísico Francisco Sánchez s/n, 38071, La Laguna, Tenerife, Spain

It is known that terpenoids and flavonoids exhibit interesting biological activities, including anti-nociceptive and anti-inflammatory properties [1, 2]. Nevertheless, up to date no reports have been found concerning the in vivo pharmacological action of the diterpenes Teucrin A, 19-Acetylgnaphalin, Eriocephalin, Teucvin and Teuflin isolated from different species of the genus *Teucrium* [3 – 5] as well as the hydroxymethoxyflavone Salvigenin isolated from various species of the genus *Salvia*, where it is a usual component [6, 7]. Therefore, the present study was undertaken to evaluate their potential analgesic and anti-inflammatory activities, using acetic acid-induced writhing test, tail-flick test (both of them at a dose of 10 mg/kg i.p.), and the tetradecanoylphorbol acetate (TPA)-induced ear inflammation model in mice (at a dose of 1 mg/ear). Statistical analysis was performed with the Student's t-test. Our findings showed that all products under study significantly inhibited acetic acid-induced writhing with values ranging from 28.23 to 51.24%. The most significant activity was observed with 19-Acetylgnaphalin and Teucrin A, which showed inhibition values (51.24 and 48.75%, respectively) close to that of Indomethacin (64.51%), the reference drug. Only Teucrin A was significantly active in the tail-flick assay, suggesting that it may have central analgesic properties. Moreover, the topical treatment of all products tested significantly reduced the TPA-induced ear oedema, with values ranging from 37.41 to 61.43%, being Salvigenin the best one (61.43%) with activity values similar to those found for Indomethacin (63.66%). In conclusion, the results demonstrate that all the products studied show analgesic and topical anti-inflammatory activities in mice. *Acknowledgements:* Caja Canarias- University of La Laguna, Dr. D. Benjamín Rodríguez **References**: 1. Ghisalberti, E.L. (1997), Fitoterapia 68: 303 – 325. 2. Di Carlo, G. *et al.* (1999), Life Sci. 65: 337 – 353. 3. Fayos, J. *et al.* (1979), J. Org. Chem. 44: 4992 – 4994. 4. Savona, G. *et al.* (1979), Tetrahedron Lett. 20: 379 – 382. 5. Savona, G. *et al.* (1982), Phytochemistry 21: 721 – 723. 6. Miana, G.A. *et al.* (1985), J. Chem. Soc. Pak. 7: 67 – 68. 7. Topcu, G. *et al.* (1995), Phytochemistry 40: 501 – 504.

P 219

Radical scavenging activity of ethanolic extracts from six species from genus Achillea

Nickavar B, Haj Yahya M, Kamalinejad M
Department of Pharmacognosy, School of Pharmacy, Shaheed Beheshti Medical University, P. O. Box: 14155 – 6153, Tehran, Iran

Antioxidants and free radical scavengers are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases. Due to the toxic effects of some synthetic compounds, natural-plant derived antioxidants have received growing attention. They are known to function as chemopreventive agents

against oxidative damage. The aim of the present study was to examine the radical scavenging capacity of the ethanolic extracts of six *Achillea* species (*A. micrantha* Willd., *A. filipendula* Lam., *A. millefolium* L. subsp. *millefolium*, *A. tenuifolia* Lam., *A. vermicularis* Trin. and *A. wilhelmsii* C. Koch) found in Iran. *Achillea* species are well known in Iranian traditional medicine as highly effective medicinal plants for various purposes such as the treatment of abdominal pain, inflammation, hemorrhage, rheumatic pain, menstrual disorders, etc. The in vitro radical scavenging property of the ethanolic extracts was examined using the DPPH test. The total flavonoid content (TFC) in the extracts were determined by $AlCl_3$ method and calculated as rutin. All botanical extract exhibited significant radical scavenging activityies. *A. micrantha* showed the greatest capacity (IC_{50} = 58.17 μg/mL), whereas the lowest IC_{50} value of 118.90 μg/mL was detected in *A. wilhelmsii*. The herb of *A. vermicularis* contained the highest content of flavonoid (58.17 μg/mg).

P 220

Free radical scavenging activity of ethanolic extracts from some Apiacean species

Nickavar B, Abolhasani FS, Kamalinejad M, Reza Khani MR, Mojab F
Department of Pharmacognosy, School of Pharmacy, Shaheed Beheshti Medical University, P. O. Box: 14155 – 6153, Tehran, Iran

It is well known that naturally occurring substances in higher plants have antioxidant activity. Recently there has been increased interest in free radicals in biological systems and their roles as causative agents in a variety of chronic disorders. Accordingly, attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them. The aim of this work was investigation of free radical scavenging activity (FRSA) of seven seeds from Apiacean plants (*Carum carvi* L., *Coriandrum sativum* L., *Cuminum cyminum* L., *Foeniculum vulgare* Mill., *Heracleum persicum* Desf. ex Fischer, *Pimpinella anisum* L. and *Trachyspermum copticum* (L.) Link). All of these plants are used in food industry and Iranian's traditional Medicine. FRS activities of the ethanolic extracts are evaluated by DPPH method. The total flavonoid content (TFC) in the extracts was determined by $AlCl_3$ method and calculated as rutin. The results showed that all examined extracts have FRSA activity. The highest scavenging activity was obtained with the extract of *P. anisum* (IC_{50} = 96.46 μg/mL). The largest quantity of the TFC was determined in the extract *C. cyminum* (FC = 56.92 μg/mg). However, a favorable correlation was not found between the FRSA and TFC of the extracts. Besides, four different fractions of *P. anisum* seed (as the most active radical scavenger) were studied for their FRSA. The ethyl acetate fraction exhibited the strongest activity with inhibition percentage value (IP = 93.39 %).

P 221

The Effects of Ginger Oils on Rat Uterine Contraction

Kupittayanant S, Buddhakala N
Institute of Science, Suranaree University of Technology, Muang District, Nakhon Ratchasima 30000, Thailand

Ginger, the rhizome of *Zingiber officinale* Roscoe (Zingiberaceae), has been extensively studied for its pharmacological activities [1]. It produced inhibitory effects on vascular and gut contractility [2, 3]. However, its effects on uterine contractility have not been elucidated. The aims of the study were to investigate the effects of ginger on rat uterine contraction. We examined the effects of ginger oils on phasic contractions arising either spontaneously or $PGF_{2\alpha}$ stimulation and the mode of action. Ginger oils were obtained by water distillation. Rats were killed by asphyxiation with CO2 and longitudinal uterine smooth muscles isolated. Isometric force was measured and the effects of ginger oils studied. The results showed that at each concentration (10 – 100 microL/100mL) ginger oils reduced spontaneous contractions, and that the effect was dose dependent. The $PGF_{2\alpha}$-induced contractions were significantly reduced by ginger oils. Increases in external calcium concentration completely reversed the relaxant effects of ginger oils. In conclusion, ginger oils are potent inhibitors of phasic activity in rat uterus, irrespective of how it is produced, and our data suggest their effects lie at the surface membrane. **References**: 1. Langner, E. *et al.* (1998), Adv. Ther. 15: 25 – 44. 2. Hashimoto, K. *et al.* (2002), Planta Med. 68: 936 – 939. 3. Borrellia, F. *et al.* (2004), Life Sci. 74: 2889 – 2896.

P 222

Two New Cyclic Amino Acids from the seeds and Antiviral Activity of methanolic extract of the roots of Zizyphus spinachristi

Said A[1], Huefner A[2], Tabl ESAA[3], Fawzy G[1]
[1]Department of Pharmacognosy, National Research Centre, Dokki, Cairo, Egypt; [2]Institute for Pharmaceutical Chemistry and Pharmaceutical Technology, University of Graz, Schubertstr.1, A8010 Graz, Austria; [3]Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt

Increased attention has been paid to Genus *Zizyphus* Family Rhamnaceae due to its significant medicinal uses viz hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antioxidant, antitumor, liver protective and improves the immune function. In this study we isolate two new cyclic amino acids from the seeds of *Zizyphus spinachristi* (L.) Willd. 70 % methanolic extract. The two compounds were identified by means of ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC and GC-MS as 4-hydroxymethyl-1-methyl pyrrolidine-2-carboxylic acid (less polar and major compound) and 4-hydroxy-4-hydroxymethyl-1-methyl pyrrolidine-2-carboxylic acid (more polar and minor compound) of the ratio 4:3. Antiviral activity was shown to be 94 %, 99 % with the concentration of 20, 50 μg of the 70 % methanolic extract of *Zizyphus spinachristi* roots respectively. This was done using Plaque reduction assay against *Herpes Simplex* virus (HSV)[1]. **Reference**: 1. Farag, R.S. *et al.* (2004), Phytother. Res. 18: 30 – 35.

P 223

Valerian extract prepared with methanol but not with ethanol or ethyl acetat inhibits the postsynaptic potentials in rat cortical neurons indicating an adenosine like action

Brattström A[2], Nieber K[1], Sichardt K[1], Vissiennon Z[1], Koetter U[2]
[1]Inst. Pharmacy, Univ. Leipzig, D-04103 Leipzig, Germany; [2)]ax Zeller Söhne AG, CH- 8590 Romanshorn, Switzerland

Valerian binds to adenosine receptors and acts as a partial agonist thereon. Investigation on brain slices revealed an agonistic activity on the central adenosine A1 receptors which can be blocked by an adenosine A1 receptors antagonist. Manufacturing the extract by means of different solvents might influence the extract composition and alter its biological action. Therefore, the aim of the present experiments was to compare the central action of an ethanolic (EtOH 63 %) and a methanolic extract (MeOH 45 %, Ze 911) prepared from the identical starting material. For that purpose the brain slice technique was used again. Coronal slices were cut with a vibratome from a block of rat brain including the cingulated cortex. Intracellular recordings were obtained from pyramidal cells of the cingulated cortex in layer V. Post-synaptical potentials (PSP) were evoked by electrical stimulation (0.2 Hz, 1 – 2 ms, 20 – 120 V) with a concentric bipolar tungsten electrode placed in layer I. The stimulation voltage was adjusted individually for each slice to yield PSP amplitudes which were approximately 80 % of maximum. The ethanolic extract did not modulate the PSP. However, the methanolic extract clearly inhibited the PSP in a dose related manner (range: 0.1 – 15 mg) with an IC_{50} value of 0.8 mg/mL. The maximal inhibition induced by 10 mg Ze911/mL was completely antagonized by 0.1 μM of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), an adenosine A1 blocker indicating that adenosine A1 receptors mediate the pharmacological action of the methanolic valerian extract Ze911. The extraction solvent is important for extract composition and influences

the pharmacological action. Exclusively, the methanolic extract Ze 911 acts at central adenosine A1 receptors whilst an ethanolic extract failed in this respect.

P 224

Valerian extract modulates the $GABA_A$-action on its receptors

Brattström A[2], Baburin I[1], Khom S[1], Hering S[1], Koetter U[2]
[1]Dept Pharmacol Toxicol, Univ. Vienna, A-1010 Vienna, Austria; [2]Max Zeller Söhne AG, CH- 8590 Romanshorn, Switzerland

Valerian act at the central GABA system which mediates inhibitory actions. A modulation of the GABA induced chloride channels conductance (I_{GABA}) has been tested for different valerian extracts manufactured with either ethanol (C1), methanol (C2) or ethyl acetate (C3) to obtain extracts with different constituents, and in addition an ethyl acetate extract from C2 residue (C4) as well as the combination of C2 + C4 (C5). From anaesthetized female *Xenopus laevis* parts of the ovaries were removed. Follicel membranes from isolated oocytes were enzymatically digested with collagenase. Chloride channels conductance were studied 1 to 5 days after microinjection of approximately equimolar cRNA mixtures of α_1-, β_2- and γ_{2S}- subunits of the rat $GABA_A$ receptors in a ratio 1:1:10. Experiments were carried out at room temperature in bath solution containing (mM): 90 NaCl, 1 KCl, 1 MgCl2, 5 Hepes, 1CaCl2, adjusted to pH 7.4 with NaOH. Ionic influx was measured by means of the conventional two-microelectrode-voltage-clamp technique using a Turbo Tec 01C Amplifier (NPI Electronic, Germany). Voltage-recording and current-injecting microelectrodes were filled with 2 M KCl and had a resistance of 1 – 5 MΩ. GABA was solved freshly in bath solution every day immediately before the experiments. Valerian extracts (C1 – C5) were solved in DMSO (20 mg/mL) and the stock solution diluted to 50 μg/mL and 100 μg/mL, respectively. The extracts were either co-applied with GABA (EC_{3-10}) or applied alone. Almost no stimulatory effects were observed if the extracts were applied alone. An enhancement of I_{GABA} (EC_{3-10}) of 134 % and 123 % respectively, could be observed for extracts C3 and C4. The following order of the activity was obtained: C4 = C3 > C1 = C5 > C2, indicating that the different active components by the solvents used are distinguishable solved.

P 225

Effects of some Hypericum reflexum L. fil. extracts in the forced swimming test in mice

Sánchez-Mateo CC, Bonkanka CX, Prado B, Rabanal RM
Dpto. de Farmacología, F. Farmacia, Universidad de La Laguna, c/ Astrofísico Francisco Sánchez s/n, 38071, La Laguna, Tenerife, Spain

We previously reported that the infusion and the methanol extract from the aerial parts of *Hypericum reflexum* L. fil., an endemic species of the Canary Islands, showed antidepressant activity in mice [1, 2]. Preliminary phytochemical analysis carried out with the methanol extract of this species reveals the presence of flavonoids, tannins, saponins and anthraquinones. The presence of hypericin could not be detected in the crude methanol extract. On the basis of these results, the present study was undertaken to evaluate the antidepressant activity of the aqueous, butanol and chloroform fractions obtained from the methanol extract of this plant on the forced swimming test in mice. Also the effects of these fractions on locomotor activity, body temperature and sleep potentiation were evaluated. Student's t-test was used to verify the statistical significance. The fractions under study (500 mg/kg *p. o.*) did not have a significant effect on the spontaneous motor activity, with the exception of the butanol fraction which significantly reduced this activity by 32.81 % at the first hour after administration. Furthermore, only the chloroform fraction produced a slight but significant hypothermia, being maintained up to the second hour after administration. Moreover, none of the different fractions assayed significantly prolonged pentobarbital induced sleeping time. In the forced swimming test, it was found that the butanol and chloroform fractions (500 mg/kg *p. o.*) significantly shortened the immobility time of mice by 17.05 and 22.83 %, respectively. It could be concluded that the butanol and chloroform fractions assayed have a certain antidepressant activity in the forced swimming test. *Acknowledgements:* Consejería de Educación, Cultura y Deportes del Gobierno de Canarias (PI2000/105) project, Caja Canarias- University of La Laguna. **References**: 1. Sánchez-Mateo, C.C. *et al.* (2002), J. Ethnopharmacol. 79: 119 – 127. 2. Prado, B. *et al.* (2002), Phytother. Res. 16: 740 – 744.

P 226

Volatile constituents of Scutellaria rubicunda Hornem subsp. linnaeana (Caruel) Rech. (Lamiaceae) endemic in Sicily

Formisano C[1], Rigano D[1], Senatore F[1], Bruno M[2], Rosselli S[2]
[1]Dipartimento Chimica Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano, 49, I-80131 Napoli, Italy; [2]Dipartimento di Chimica Organica, Università degli Studi di Palermo, Parco d'Orleans II, I-90128 Palermo, Italy

The genus *Scutellaria* comprises about 350 species of herbs or subshrubs and rarely shrubs, some of which are used for their antitumor [1] or anti-feedant properties [2]. The essential oils of some *Scutellaria* species were also investigated and showed antimicrobial and antifungal activities [3, 4]. *Scutellaria rubicunda* Hornem. subsp. *linnaeana* (Caruel) Rech. is a herbaceous, endemic species growing wild in the central part of Sicily in the Parco delle Madonie. An earlier phytochemical study revealed that aerial parts contained Scutecyprol B and Scutalbin C, active against five species of lepidopteran larvae [5]. No reports on the essential oil of *S. rubicunda* subsp. *linnaeana* has been found in the literature so far. Therefore we report on the chemical composition of the essential oil of this plant. Flowering aerial parts were collected at Piano Battaglia, 1600 m s/l, 80 km south of Palermo (Italy) in July 2005. The oil was isolated by hydrodistillation [6]. The GC and GC/MS analyses evidenced 31 compounds, accounting for 92.7 % of the oil that consisted mainly of terpenoids. The oxygenated monoterpenes (44.5 %) represented the main fraction with linalool (27.8 %) as dominant component. Other representative compounds were α-terpineol (6.7 %) and nerol (4.2 %). Sesquiterpenes (39.0 %) were composed of caryophyllene (28.7 %), caryophyllene oxide (4.2 %) and α -cedrol (2.3 %) as main components. **References**: 1. Chan, J. *et al.* (2006), Planta Med. 72: 28 – 33. 2. Bruno, M. *et al.* (2002), Biochem. Syst. & Ecol. 30: 793 – 799. 3. Skaltsa, H.D. *et al.* (2000), Planta Med. 66: 672 – 674. 4. Yu, J. *et al.* (2004), Phytochemistry 65: 881 – 884. 5. Bruno, M. *et al.* (1999) Phytochemistry 50: 973 – 976. 6. *European Pharmacopoeia* 4th ed. (2002), Council of Europe.

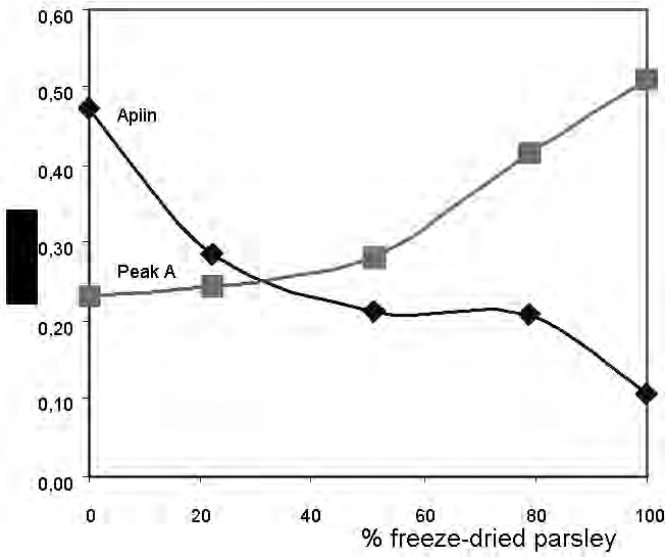
P 227

Evaluation of analytical markers characterising different drying methods of parsley leaves (Petroselinum crispum L.)

Lechtenberg M[1], Zumdick S[1], Engelshowe R[1], Gerhards C[2], Schmidt TJ[1], Hensel A[1]
[1]University of Münster, Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstraße 56, 48149 Münster, Germany; [2]University of Applied Science – Hochschule Wädenswil, Department of Food Technology, Gruental, CH-8820 Wädenswil, Switzerland

Parsley (*Petroselinum crispum* L.) is a typical spice native to Mediterranean countries. It is widely used to enhance the flavor of different foods. On the other hand there is evidence for the assumed diuretic effect of parsley in folk medicine [1, 2]. In literature effects of different drying methods are described mainly on the volatile components [3, 4]. The aim of our study was to include further analytical parameters in order to determine the used drying method of unknown parsley samples. At best we should be able to identify blends of air-dried parsley in an excess of freeze-dried material. Our study included a variety of different analytical methods: microscopy

and colorimetric measurements (L*a*b colour space) of dried samples, UV-spectroscopy of crude extracts (CH_2Cl_2, methanol, H_2O), GLC of the essential oil (extraction and steam distillation), TLC and HPLC of crude methanolic extracts and determination of enzymatic activities (APIzym®assay). The principal component analysis (PCA) was used to analyse ^{1}H-NMR-data of crude extracts. Experiments showed great influence of parsley varieties and the kind of cultivation (green-house vs. field-grown) on analytical markers. With respect to the drying method, we found that the amount of apiin (as determined by HPLC) was dependent on the amount of freeze-dried parsley in mixtures of freeze- and air-dried material. A yet unidentified flavonoid (peak A) showed an inverse behaviour (Figure). However, our results also indicate that a combination of methods would be more useful to unambiguously determine the used drying method.



HPLC analysis of a mixture of air-dried and freeze-dried parsley

Acknowledgements: Dr. R. Kruse, Freeze Dry Foods, Am Eggenkamp 8 – 10, 48268 Greven, Germany **References**: 1. Kreydiyyeh, S., Usta, J. (2002), J. Ethnopharmacol. 79:353 – 357. 2. BAnz Nr.43 (1989), Komm. E monograph "Petroselini herba/-radix" 3. Diaz-Maroto, M.C. *et al.* (2002), Eur. Food Res. Technol. 215: 227 – 230. 4. Diaz-Maroto, M.C. *et al.* (2003), Eur. Food Res. Technol. 216: 227 – 232.

P 228

Chemical composition of the essential oil from aerial parts of Micromeria fruticulosa (Bertol.) Grande (Lamiaceae) growing wild in Southern Italy

Rigano D[1], Formisano C[1], Senatore F[1], Bellone G[2], Bruno M[2], Rosselli S[2]
[1]Dipartimento Chimica Sostanze Naturali, Università di Napoli "Federico II", Via D. Montesano, 49, I-80131 Napoli, Italy; [2]Dipartimento di Chimica Organica, Università degli Studi di Palermo, Parco d'Orleans II, I-90128 Palermo, Italy

Micromeria is a genus of fragrant plants which comprises Mediterranean perennial sub shrubs or herbs or chamaephytes common in open habitats and in rocky coasts. Several extracts from these plants are used in folk medicine against heart disorders, headache, wound skin infections and as antispasmodic, stimulant and expectorant [1, 2]. The extracts of some *Micromeria* sp. exhibited significant antibacterial activity [3]. *Micromeria fruticulosa* (Bertol.) Grande is a suffruticous plant 8 – 15 cm tall, endemic of Campania and Sicily (Southern Italy) where it is locally named *issopo marittimo*. The isolation of the flavonoids naringenin and neoponcirin in this plant was previously reported [4]. Here for the first time, we report on the essential oil composition of the aerial parts of *M. fruticulosa* collected at the full flowering stage from plants wild growing on the Lattari mountains, Sorrento (NA, Southern Italy) in July 2005. The oil was isolated by hydrodistillation [5] and analyzed with GC and GC/MS. 64 constituents, representing 91.7 % of the total oil have been identified. Monoterpenes (43.3 %), almost entirely hydrocarbons, predominate over sesquiterpenes (31.0 %). γ-Terpinene (14.5 %), β-caryophyllene (12.6 %), *p*-cymene (8.9 %), α-pinene (8.2 %) and β-bisabolene (7.2 %) were the main components. The phenolic compounds amounted to 5.9 % with carvacrol (5.3 %) as the major one. The presence in this essential oil of compounds with known biological activity such as α-pinene, γ-terpinene and *p*-cymene [6] could account for the use of *Micromeria* in folk medicine. **References**: 1. Ali-Shtayeh, M.S. *et al.* (1997), J. Ethnopharm. 58: 143. 2. Kirimer, N. *et al.* (1997), 11th International Symposium on Plant-originated Crude Drugs. M. Coskun ed., Ankara, pp. 130 – 138. 3. Duru, M.E. *et al.* (2004), J. Ethnopharm. 94: 43. 4. Bellino, A. *et al.* (1980), Fitoterapia 10: 163. 5. *European Pharmacopoeia* 4th ed. (2002), Council of Europe, 183. 6. Mitsuo, M. *et al.* (2006), Flavour fragrance J 21: 198.

P 229

Molecular cloning and heterologous expression of a progesterone 5β-reductase (5β-POR) from Isoplexis canariensis

Herl V, Fischer G, Mueller-Uri F, Kreis W
LS Pharmazeutische Biologie der Friedrich-Alexander-Universität Erlangen-Nürnberg, Staudtstraße 5, D-91058 Erlangen, Deutschland

Isoplexis (Lindl.) Lindl. ex Benth., endemic to the Canary Islands and Madeira, is a plant genus closely related to *Digitalis* L.. *Isoplexis canariensis* (L.) Lindl. ex. G. Don contains 5α- and 5β-cardenolides, together with cardenolides containing a Δ^4 -or Δ^5 -double-bond and saponins [1, 2]. The biosynthesis of cardenolides in *Digitalis* is well established [1, 3], whereas the biosynthesis in *Isoplexis* needs further investigation. A full-length cDNA clone that encodes progesterone 5β-reductase (5β-POR) was isolated from *Isoplexis canariensis* leaves. The reading frame of the 5β-POR gene is 1170 nucleotides corresponding to 389 amino acids. For expression, a *Sph* I/*Sal* I 5β–POR fragment was cloned into the pQE vector system and was transformed into *Escherichia coli* strain M15[pREP4]. The recombinant gene was functionally expressed and the recombinant His-tagged gene product was purified under native conditions on a Ni-nitrilotriacetic acid (Ni-NTA) matrix. Its size was determined by SDS-Page to be about 45 kDa. The purified recombinant protein was enzymatically active, as proven in a standard enzyme assay, using progesterone and NADPH as a substrate and cosubstrate, respectively. Biochemical parameters were determined; the K_m – and V_{max} -values for the putative natural substrate progesterone were calculated to be 0.215 mM and 46.4 nkat/mg protein, respectively. Kinetic constants for cortisol, cortexone, 4-androstene-3,17-dione and NADPH were determined [4]. The 5β-POR from *I. canariensis* shares considerable homology with other progesterone 5β-reductases including those of various *Digitalis* species and *Arabidopsis thaliana*. **References**: 1. Luckner, M., Wichtl, M. (2000), Digitalis, WVG Stuttgart. 2. Schaller, F., Kreis, W. (2006), Planta Med. submitted. 3. Kreis, W. *et al.* (1998), Planta Med. 64: 491 – 499. 4. Herl, V. *et al.* (2006), Planta Med. submitted.

P 230

Characterization of Jatropha curcas L. seed polysaccharides and their influence on primary human keratinocytes

Zippel J, Deters A
University of Münster, Pharmazeutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

Jatropha curcas L. (Euphorbiaceae), traditionally used as medical plant in India and South America, is well known for its physiological effects on human skin. Especially the irritant potential of seed oil and latex is part of investigations, but no research is done to the influence of seed polysaccharides on human keratinocytes yet. Poly-

saccharides were isolated by water extraction from the seed endosperm and the monosaccharide composition was elucidated by GC FID. Galactose, Rhamnose, Arabinose, Mannose and Glucose were the main components, in a ratio of 1: 1,2: 1,7: 1,8: 1,6. Additionally minor amounts of Fucose, Ribose and Xylose were found. Galacturonic acid was detected by Dionex HPLC and TLC. Protein content (10%) of the crude extract was determined by Bradford Test [1]. Treatment of primary human keratinocytes with the crude extract (10 µg/mL) showed a strong enhancement of proliferation determined by BrdU- incorporation ELISA. Cell viability, measured by the MTT-Test, was improved and no necrotic cytotoxicity could be observed. Immuno blotting showed an increased production of Keratin and Involucrin, which characterizes an initiated differentiation as consequence of the intensified proliferation. **Reference**: 1. Bradford, M.M. (1976), Anal. Biochem. 72: 248 – 254

P 231

Antioxidants from Xylocarpus granatum

Wangensteen H[1], Duong GM[1], Alamgir M[2], Sarder M[2], Samuelsen AB[1], Malterud KE[1]

[1]Dept. of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, P. O. Box 1068 Blindern, N-0316 Oslo, Norway; [2]Pharmacy Discipline, Khulna University, Khulna-9208, Bangladesh

Xylocarpus granatum Koeg. (Meliaceae) is growing in mangrove forests in Southeast Asia, tropical Australia and East Africa The bark has been used in traditional medicine to treat diarrhea, cholera, fever and abdominal troubles, and it is well known for its astringent properties. Chemically, the tree contains a number of xyloccensins (triterpenoids of limonoid type). Content of tannins has also been reported, but their chemical structures appear to be unknown. The aim of this study was to isolate and identify chemical substances from *X. granatum* and to evaluate the DPPH radical scavenging activity and the inhibitory effect towards 15-lipoxygenase (15-LO). Total phenolic content was quantified by the Folin Ciocalteu method. *X. granatum* was collected in the Sundarbans mangrove forest in Bangladesh. The 80% methanol extract of the stem bark was suspended in distilled water and successively extracted with chloroform, ethyl acetate and n-butanol. The extracts were fractionated by different chromatographic techniques, compounds were identified by NMR and by degradation with phloroglucinol/HCl. High amounts of procyanidins in addition to the monomeric compounds catechin and epicatechin were isolated. The structures of the procyanidins were procyanidin B1 (epicatechin (4β→8) catechin), epicatechin (4β→8) epicatechin (4β→8) catechin and epicatechin (4β→8) epicatechin (4β→8) epicatechin (4β→8) epicatechin (4β→8) catechin. The limonoids gedunin, xyloccensin O, xyloccensin P and xyloccensin Q were isolated, as well. The procyanidins showed high DPPH radical scavenging and 15-LO inhibitory activities, whereas the limonoids were inactive as radical scavengers and showed only weak inhibition of 15-LO.

P 232

Direct and indirect antimicrobial activity of Cordia gilletii extracts

Okusa PN[1,2], Penge O[2], Devleeschouwer M[3], Duez P[1]

[1]Université Libre de Bruxelles, Institut de Pharmacie, Laboratoire de Pharmacognosie, de Bromatologie et de Nutrition Humaine, CP 205/9, 1050 Brussels, Belgium; [2]Université de Kinshasa, Faculté de Pharmacie, Laboratoire de Pharmacognosie, BP 212 Kin XI, Dem. Rep. Of Congo; [3]Université Libre de Bruxelles, Institut de Pharmacie, Laboratoire de Microbiologie Pharmaceutique et Hygiène, CP 205/2, 1050 Brussels, Belgium

The alarming incidence of antibiotic resistance causes an increasing need for new products that can act either by a direct antimicrobial activity or by inhibiting resistance mechanisms of germs of medical importance. Plants represent a potential source for this kind of compounds [1, 2]. Root barks of *Cordia gilletii* De Wild (*Boraginaceae*), a Congolese plant traditionally used for antimicrobial properties, were extracted successively by n-hexane, dichloromethane, ethyl acetate, methanol and water. These extracts were tested for direct antimicrobial activity against eight microbial species (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, Serratia marcescens and Candida albicans*) and for effect on antibiotic resistance by broth microdilution methods [3, 4]. The methanol extract showed direct antimicrobial activity against all the strains with MIC values ranging between 125 µg/mL and 1000 µg/mL, whereas the ethyl acetate and dichloromethane extracts showed activity on two (*Staphylococcus aureus* and *Escherichia coli)* and three (*Klebsiella pneumoniae, Enterobacter cloacae* and *Serratia marcescens*) microbial species respectively. 200 µg/mL of the n-hexane and dichloromethane extracts decreased the MIC of penicillin, amoxicillin, ampicillin and streptomycin 4 – 64 fold for *S. aureus* methicillino-resistant. *Acknowledgement:* Dr Lerson (CHU Charleroi, Belgium), Belgian Technical Cooperation. **References**: 1. Chariandy, C.M. *et al.* (2000), J. Ethnopharmacol. 64: 265 – 270. 2. Hatano, T. *et al.* (2005), Phytochemistry 66: 2047. 3. NCCLS (2003), Approved Standard, 6th edition. 4. NCCLS (2002), Approved Standard, 2th edition.

P 233

Stimulation of LAL-test by LPS-free arabinogalactan-protein preparations from Echinacea purpurea

Blaschek W[1], Zager A[1], Classen B[1], Ulmer AJ[2]

[1]University of Kiel, Institute of Pharmacy, Gutenbergstr. 76, 24118 Kiel, Germany; [2]Research Center Borstel, Parkallee 22, 23845 Borstel, Germany

While investigating the immunological activities of Echinacea preparations, the question of microbiological contamination arises, because microorganisms or parts and products of them may influence the test results. Especially LPS from gram-negative bacteria and lipopeptides from gram-negative and gram-positive bacteria may stimulate the immune-system already at very low concentrations. Arabinogalactan-proteins (AGPs) from *Echinacea purpurea* [1] were tested in the LAL-test, using the gel-clotting-method, before and after LPS-removal. Since this test responses not only to LPS, but also to β-1,3-glucans [2], it seemed possible that there could also be a response to other polysaccharide containing polymers such as AGPs. After removal of LPS by two different methods (affinity chromatography and treatment with sodium hydroxide), absence of LPS and bacterial lipopeptides was proven by testing the LPS-free AGP preparation for interaction with toll-like receptors (TLR) 2- or TLR4-transfected HEK293 cells. There was no reactivity of AGP preparations with these receptors, indicating that LPS and lipopeptides have been successfully removed. After blocking of possible glucan-activity in the LAL-test by a special buffer, the LPS-free AGP preparations still led to coagulation. The remaining activity in the test therefore has to be considered as the activity of AGPs. Thus, AGPs are able to activate the LAL-test in a µg-concentration range. This is lower, but considerable activity compared to the activity of glucans (ng-concentration range) or LPS (pg-concentration range). *Acknowledgement:* The authors thank the Madaus AG, Köln, for financial support of this work. **References**: 1. Classen, B. *et al.* (2000), Carbohydr. Res. 327: 497 – 504. 2. Blaschek, W. *et al.* (1992), Pharm. Pharmacol. Lett. 1: 118 – 122.

P 234

Phytoestrogenic Activity of Morinda citrifolia L. Fruits

Basar S, Iznaguen H, Zeglin A, Westendorf J

Institute of Experimental and Clinical Pharmacology and Toxicology, University Clinic Hamburg Eppendorf, Vogt-Kölln-Straße 30, D-22527 Hamburg, Germany

It is well known that certain plant metabolites can exert estrogenic activity. There are an increasing number of studies concerning the effects of phytoestrogen-rich diets which demonstrate that these

plant metabolites have protective effects on estrogen-related conditions, such as menopausal symptoms, and estrogen-related diseases, such as prostate and breast cancers, osteoporosis and cardiovascular diseases [1]. Because beneficial effects on menopausal symptoms are often reported by women drinking regularly Noni fruit juice (*Morinda citrifolia* L.), we investigated the estrogenic capacity of Noni fruits in two *in vitro* assays, the estrogen receptor binding assay with both estrogen receptors, ER-α and ER-β, and the estrogen-receptor dependent induction of alkaline phosphatase in Ishikawa cells. Hexane extracts prepared from Noni fruit puree exhibited high activity in both systems. A preferential binding for ER-β was observed (ED_{50}(ER-α)/ED_{50}(ER-β)= 2.33). Further analysis and fractionation of these extracts by HPLC showed that one compound is responsible for almost all the activity. The isolation of this constituent was performed by HPLC and will be followed by its characterisation with 1- and 2-dimensional NMR techniques and Mass spectrometry. **Reference**: 1. Cos, P. *et al.* (2003), Planta Med. 69: 589 – 599.

P 235

Cytotoxicity of β-aescin/agrostin mixtures in different cell lines depends on their growth characteristics

Weng A, Melzig MF
Freie Universität Berlin, Institut für Pharmazie, Königin-Luise-Str. 2+4, D-14195 Berlin

Agrostin (Mr: 27kDa), a ribosome-inactivating- protein (RIPs) from *Agrostemma githago* L., cleaves an essential adenin residue from the rRNA, leading to inhibition of protein syntheses [1]. In previous studies it was shown, that only the combination of specific triterpenoid saponins such as *Saponinum album* L. with a formyl function attached to position C_4 together with agrostin was cytotoxic in ECV-304 cells [2]. This enhancement in cytotoxicity by *Saponinum album* L is due to an enhanced penetration of agrostin through the cell membrane, indicating the induction of endocytosis, because the treatment with latranculin A und bafilomycin A_1 inhibited the cytotoxicity in ECV-304 cells [3]. β-Aescin is also a triterpenoid saponin and the major compound of aescin, a mixture of glycosids synthesized by *Aesculus hippocastanum* L.. In this study we investigated the cytotoxic effect of β-aescin (10 – 2.5 μg/mL) /agrostin (150 ng/mL) mixtures in ECV-304, Hep-G2, SK-N-SH, U-937 and H-2171 cells. In contrast to the non-adherent U-937 and H 2171 cells the proliferation of adherent growing cell lines like ECV-304, Hep-G2 and SK-N-SH was significantly reduced by the mixture of β-aescin and agrostin. It is therefore concluded that the stimulation of endocytosis of the cytotoxic agrostin by β-aescin depends on specific membrane structures present on the cell surface of adherent growing cells. Especially the difference in the caveolin-1 expression between adherent growing (high) and in suspension growing cells (low) was demonstrated [4]. Caveolin-1 is an integral membrane protein and correlates with the number of caveolae in the cell membrane. The caveolae, flask shaped invaginations in the cell membrane, are necessary for internalization of endocytotic markers and by that strongly involved in endocytosis. **References**: 1. Stirpe, F. (2004), Toxicon 44: 371 – 83. 2. Melzig, M.F. *et al.* (2005), Planta Med. 77: 1088 – 1090. 3. Hebestreit, P. *et al.* (2006), Toxicon 47: 330 – 35. 4. Sunaga, N. *et al.* (2004), Canc. Res. 64: 4277 – 4285. 5. Kiss, L.A. *et al.* (2002), Micron 33: 75 – 93.

P 236

An assessment of the use of an in vitro cell based model of the intestinal tract to investigate the bioavailability of the Chinese herbal remedy Oldenlandia diffusa

Panagiotou E, Jones LA, Opara EI
Biomedical and Pharmaceutical Sciences Research Group, School of Life Sciences, Kingston University, Penryhn Road, Kingston upon Thames, Surrey, KT1 2EE, UK

Certain Chinese herbal remedies (CHRs) are used to treat cancer. However, their mode of action has yet to be fully elucidated. An earlier study of the anti-cancer activity of one such CHR, *Oldenlandia diffusa* (Willd.) Roxb (OD) indicated that its cell cytotoxicity: may occur via apoptosis; be cancer cell specific; and involve more than one constituent [1, 2]. However, as this study was carried out *in vitro*, no information about the impact of the intestinal tract on the anti-cancer activity of this remedy, which is commonly ingested in the form of a tea, was obtained. Thus the aim of this study was to assess the use of a 21 day *in vitro* model of the intestinal tract to investigate the bioavailability of this CHR. The colon adenocarcinoma cell line Caco-2, in the form of a monolayer, was used to mimic the intestinal tract. The integrity of this model was assessed using the trypan blue exclusion test, transepithelial electrical resistance (TEER), phenol red exclusion and scanning electron microscopy (SEM). Cytotoxicity assessment using trypan blue showed that the model was not adversely affected by OD (ranging in concentration from 15 – 70 %). Phenol red exclusion and TEER measurements showed that the model's integrity was maintained before and after exposure to OD. Furthermore, SEM showed that the monolayer retained its microvilli expression and tight junctions formation before and after exposure to OD. These results demonstrate that this model can be used to study the bioavailability of this CHR. **References**: 1. Willimott, S. *et al.* (2005), Isolation of tumour modulatory compounds in Chinese herbal remedies through activity guided fractionation. Poster at the International Conference and 53rd Annual Meeting of the Society for Medicinal Plant Research; Florence August 2005. 2. Willimot, S., Barker J., Jones L., and Opara EI. The Chinese herbal medicine *Oldenlandia diffusa* induces cell-cycle-independent apoptosis in the leukaemic HL60 cell line and growth arrest in PHA-stimulated blood lymphocytes. (in preparation).

P 237

Variations in extraction protocol lead to differences in monosaccharide composition and bioactivity on human keratinocytes as shown by polysaccharides from banana and plum fruits

Deters A, Maas M, Kemper M, Lamerding F, Klenke A
Institute for Pharmaceutical Biology and Phytochemistry, Westfaelische Wilhelms University Muenster, Hittorftsr. 56, 48149 Muenster, Germany

In precede investigations polysaccharides have been shown to exhibit different bioactivities on the cell physiology of primary keratinocytes dependent on their monosaccharid composition related to the plant, which was used for extraction. The intention of the present study was that the way of polysaccharide precipitation leads to a varition of monosaccharide composition and different bioactivities, too. For the investigations water extracts from banana (*Musa paradisica* var. *sapientum*, Musaceae) and plum (*Prunus domestica* L., Rosaceae) fruits were splitted and carbohydrates were obtained by two ways of precipitation: 1. the water extracts were dropped into ethanol and 2. ethanol was dropped into the extracts to a final ethanol concentration of 80 %. Subsequently polysaccharides were examined by chemical and chromatographic methods and incubated with human keratinocytes. The analysis of monosaccharides by Dionex HPLC, GC/FID revealed that the polysaccharides obtained after dropping of ethanol in the extracts contained up to 20 % more uronic acid determined as galacturonic acid by TLC than the polysaccharides obtained by method 1. Further the amounts of fructose, galactose and arabinose rose up to 26 % (Fruc), 20 % (Gal) and 10 %

(Ara). Differences in the protein content were not observed. Investigation of keratinocyte cell physiology after incubation with the polysacchrides exhibited that the polysaccharides obtained after dropping of ethanol in the water extract triggered the cell proliferation and cell viability to a greater extend than the others, which had no or only minor effects. Further polysacchrides aquired by method 2 reduced the amount of necrotic cells. The obtained data show that slight variations in extrac

P 238

Polysaccharides from Glycyrrhiza glabra L. exert significant anti-adhesive effects against Helicobacter pylori and Porphyromonas gingivalis

Wittschier N[1], Faller G[2], Beikler T[3], Stratmann U[4], Hensel A[1]
[1]Institute of Pharmaceutical Biology and Phytochemistry, University of Münster, Hittorfstr. 56, D-48149 Münster, Germany; [2]Institute of Pathology, University of Erlangen-Nürnberg, Krankenhausstrasse 8 – 10, D-91054 Erlangen, Germany; [3]Department of Periodontology, University of Washington, Seattle, USA; [4]Institute of Anatomy, University of Münster, Vesaliusweg 2 – 4, D-48149 Münster, Germany

Glycyrrhiza glabra L. (Fabaceae), one of the oldest medicinal plants of the world, is a ligneous perennial shrub growing in Mediterranean region and Asia. Because of the expectorant, anti-spasmodic and anti-inflammatory effects extracts from roots are used therapeutically against bronchitis and gastric ulcer. In order to investigate new modes of action we investigated the influence of isolated polysaccharides from Liquorice roots with regard to their capacity to reduce bacterial binding to host cells. In the present study an *in-situ* adhesion model with *Helicobacter pylori* and *Porphyromonas gingivalis* on sections of human gastric mucosa resp. rat esophagus mucosa was used as screening model for anti-adhesive activity. Preincubation of Helicobacter-suspensions with a solution of the raw polysaccharide resulted in a significant decrease in the bacterial adhesion to gastric mucosa of 40 % compared with the non-treated control. Fractionation of the raw polysaccharide via anion ion-exchange chromatography (AEX) yielded 5 subfractions. The strongest reduction of adhesion exhibited the 0.25 molar fraction (60 % inhibition) while the other fractions were inactive. The AEX-fractions were further separated by gel permeation chromatography. Respective polysaccharide structures were elucidated. Considerable anti-adhesive effects of liquorice root polysaccharides were also observed after pre-treatment of *P. gingivalis*. Furthermore the agar diffusion-test revealed absence of any cytotoxicity of the raw polysaccharides against *H.pylori* and *P.gingivalis*. Thus, data show that polysaccharides from *Glycyrrhiza glabra* L. are a potent agent against bacterial adhesion and are able to block the initial step of an infection.

P 239

Pelargonium sidoides extract EPs 7630 inhibits adhesion of Helicobacter pylori to human gastric mucosa

Wittschier N[1], Faller G[2], Hensel A[1]
[1]University of Münster, Institute of Pharmaceutical Biology and Photochemistry, Hittorfstr. 56, D-48149 Münster, Germany; [2]University of Erlangen-Nürnberg, Institute of Pathology, Krankenhausstrasse 8 – 10, D-91054 Erlangen, Germany

Pelargonium sidoides (DC.), belonging to the family of Geraniaceae, originates from the southern parts of Africa. In traditional medicine extracts from roots are used in diseases of the respiratory system and gastrointestinal complaints. Nowadays a root extract (Umckaloabo®) is used therapeutically as antimicrobial agent against infections of the respiratory system. In order to elucidate possible modes of actions we investigated the influence of the extract EPs7630 concerning its influence on microbial adhesion. As model microorganism *Helicobacter pylori* was used, a germ with a strong adherence to human stomach tissue via its highly specific surface adhesions. In an *in-situ* anti-adhesion assay intact human stomach tissue from patient resectates was incubated with fluorescent-labelled bacteria. Epithelial adhesion occurred in untreated samples and was quantified by fluorescent microscopy. Pre-treatment of the bacteria with EPs 7630 showed good anti-adhesive activity, being less than that obtained by the positive control blocker sialyllactose. The antiadhesive effect was clearly dose-dependent in a range from 0.001 to 10 mg/mL. Using an agar diffusion-test it was shown that EPs7630 had no direct cytotoxicity against *Helicobacter pylori* over the concentration range used in the adhesion assays. The results show that the extract from *Pelargonium sidoides* is a potent anti-adhesive agent against *Helicobacter pylori* and could therefore be a useful choice to avoid the first step of a bacterial infection.

P 240

Impact of fertilization on the accumulation of leaf salicylates in four field-grown dark-leaved willow (Salix myrsinifolia Salisb.) clones

Paunonen R[1], Julkunen-Tiitto R[1], Tegelberg R[1], Rousi M[2]
[1]University of Joensuu, Department of Biology, PO Box 111, FIN-80101, Joensuu, Finland; [2]The Finnish Forest Research Institute, Finlandiantie 18, FIN-58450, Punkaharju, Finland

Due to their anti-inflammatory and analgesic properties, salicylates are medically interesting phenolic compounds [1]. In some patients, asperin, a synthetic derivative of salicin (β-D-glucoside of 2-hydroxybenzyl alcohol), induces more side effects than salicin [1]. Thus, herbal drugs could be excellent alternatives to asperin. The leaves of dark-leaved willow (*Salix myrsinifolia*) contain salicylates (salicin and its derivatives) [2], and are therefore a promising source of herbal drugs. The aim of the present study was to clarify the impact of fertilization on leaf biomass and salicylates in four dark-leaved willow clones. Willows were established from cuttings in May 2002 and were grown on plastic mulch in field trials in Eastern Finland. Two fertilization treatments were used: 0 and 150 kg (N)/ha. In August 2004, leaves were collected and air-dried. Soluble phenolics were extracted with methanol and quantified using HPLC/DAD [3]. Results showed that fertilization increased significantly leaf biomass (g plant^{-1}, dw) ($F = 6.458$, DF = 1, $P < 0.05$) and concentrations (mg/g, dw) of leaf salicortin (a derivative of salicin) ($F = 6.098$, DF = 1, $P < 0.05$) and total salicylates ($F = 4.424$, DF = 1, $P < 0.05$). The increased production of other phenolics (chlorogenic acid and quercetin-3-galactoside) did not limit the salicylate accumulation. However, most salicylates varied quantitatively among clones, and clones also responded differently to fertilization ($P < 0.05$). Thus, the selection of clones for cultivation should be made with care. *Acknowledgements*: The Academy of Finland (project no. 64308), University of Joensuu. **References:** 1. Pierpoint, W.S. (1994), Adv. Bot. Res. 20: 163 – 235. 2. Julkunen-Tiitto, R., Meier, B. (1992), Planta Med, 58: 77 – 80. 3. Julkunen-Tiitto, R. *et al.* (1996), Trees-Struct. Funct 11: 16 – 22.

P 241

Pharmacological in vivo test to evaluate the bioavailability of some St. John's wort innovative oral preparations

Bilia AR[1], Bergonzi MC[1], Isacchi B[1], Galeotti N[2], Ghelardini C[2], Vincieri FF[1]
[1]Department of Pharmaceutical Sceinces, University of Florence, 50019 Sesto Fiorentino, Florence, Italy; [2]Department of Preclinical & Clinical Pharmacology, University of Florence, 50139 Florence, Italy

Preparations based on extracts of St. John's wort are widely marketed for treating mild to moderately severe depressive disorders and other health conditions such as anxiety and sleep disorders [1]. Active principles are not yet discovered and flavonols, based on quercetin aglycone, naphthodianthrones (hypericin and pseudohypericin) and phloroglucinols such as hyperforin, adhyperforin seems to be related to this action. Thus, flavonols and naphthodianthrones are polyphenols, quite polar derivatives but their water solubility is

very scarce; phloroglucinols are lipophilic and completely not water-soluble constituents. In addition, hypericins and hyperforins are not stable with regard to heat and light [2]. In this study the optimisation of technological and pharmaceutical aspects of dried commercial extract of St. John's wort were evaluated by the *in vivo* "Porsolt test". Solid dosage forms containing β-cyclodextrin and micellear systems (SDS, ASC-8) were compared in the "Porsolt test" with the extract alone. The extract showed the antidepressant activity in the mice after 60 minutes and with the dosage of 100 mg/kg. The same antidepressant activity appeared in 30 min with a micellar solution of SDS 40mM containing the same quantity of extract (100 mg/kg), while with micelles of ASC-8 40 mM the effect appeared at 15 min and with a dosage of 30 mg/kg. In the case of colyophilized with β-cyclodextrin the best results were obtained at 30 min, administering 60 mg/kg of the extract. *Acknowledgements:* The financial support of MIUR (PRIN 2004) and Ente Cassa di Risparmio di Firenze is gratefully acknowledged for financial support. **References**: 1. Chatterjee, S.S. *et al.* (1998), Pharmacopsychiatry 31: 7 – 15. 2 Bilia A.R. *et al.* (2001), Int. J. Pharm. 213: 199 – 208.

P 242

Composition of the Essential Oils from Three Species from Labiatae from Iran

Mojab F, Nickavar B
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shahid Beheshty University of Medical Sciences, Tehran, P.O. Box 14155 – 6153, Iran

The aerial parts of three species from Labiatae (*Thymus carmanicus* Jalas, *Salvia hypoleuca* Benth. and *Teucrium Stocksianum* Boiss.) from Iran were obtained by hydrodistillation in a clevenger-type apparatus for 3 h (2 %, 0.3 % and 0.5 %, respectively). The species were collected from Karkas-Kuh (Natanz area, Province Isfahan), Ab-ali (North of Tehran) and Siahoo, North of Bandar-Abbas, Province Hormozegan), respectively. This oil has been examined by GC and GC/MS. The components of the oil were identified by comparison of their fragmentation patterns of mass spectra and retention indices with those published in the literature and presented in the MS computer library. In the oil of *T. carmanicus*, monoterpenes predominated over sesquiterpenes. Thymol and carvacrol were the major components of the oil, 20.8 and 52.8 %, respectively. Other components were η-terpinene (5.4 %), *p*-cymene (4.1 %) and borneol (1.5 %). In the oil of *T. Stocksianum* α-pinene (24.5 %) and α-copaene (3.4 %) were major compounds; and in the oil of *S. hypoleuca*, bicyclogermacrene (15.3 %), β-caryophyllene (14.6 %), viridiflorol (13.3 %), spathulenol (12.5 %) δ-elemene (7.7 %), β-pinene (7.2 %) and α-pinene (5.9 %) were major compounds.

P 243

Artemisinin and flavonoids yield from aqueous extracts and tinctures of Artemisia annua L

Bilia AR[1], Gabriele C[1], Bergonzi MC[1], Melillo de Malgalhaes P[2], Vincieri FF[1]
[1]Department of Pharmaceutical Sceinces, University of Florence, 50019 Sesto Fiorentino, Florence, Italy; [2]Divisão de Agrotecnologia, CPQBA-UNICAMP, C.P. 6171, 13.081.970 Campinas, SP, Brasil

Malaria morbidity and mortality continue to increase across the entire world [1]. This is largely as a result of the continued use of chloroquine and sulfadoxine-pyrimethamine, despite widespread resistance. Artemisinin is an interesting molecule to treat multi-drug-resistant *Plasmodium falciparum* malaria. It is extracted from the plant *qinghao* (*Artemisia annua* L. or sweet wormwood) [2]. After the discovery of the active principle artemisinin almost all the clinical evaluations have focused on pure, isolated artemisinin and its derivatives. Clinical trials with patients using teas or decoctions have appeared in the most recent literature [3 – 5], after development of high artemisinin-yielding plants (> 0.5 % per dried weight). The principal aim of such investigations is related to the possibility for populations in endemic areas to cultivate selected breedings of *A. annua* and prepare teas or decoctions with a positive effect in the treatment of malaria. In this study the qualitative profile and content of artemisinin and polymethoxyflavones is investigated on infusions and decoctions prepared with different methods and on 40 % w/v and 60 % w/v tinctures using a hybrid form of *A. annua* successfully cultivated in Brasil. The aerial parts of the plant contained 0.52 % artemisinin per dry weight, and approximately 27 – 40 % of this artemisinin could be extracted by simple tea preparation methods or decoctions and the best extraction is obtained with a short decoction (5 min) followed by an infusion of 9 g herbal drug in 1 L water. Tinctures 40 % w/v extracted about 26 % artemisinin while tincture 60 % w/v about 40 %. The content of total polymethoxylated flavonoids in the plant was about 2.6 % mainly represented by chrisoplenetin plus casticin (1.4 %), eupatin (0.8 %) and artemetin (0.4 %). The total flavonoid content extracted in the infusions ranged 30 – 60 % and that of tinctures was less than 40 %. Tinctures showed also the presence of chrisoplenol. *Acknowledgements:* The financial support of MIUR (PRIN 2004) and Ente Cassa di Risparmio di Firenze is gratefully acknowledged for financial support. **References**: 1. World Health Organization, (2000), Trans. R. Soc. Trop. Med. Hyg. 94: 1 – 90. 2. O'Neill, P. *et al.* (2004), J. Med. Chem. 47: 2945 – 2964. 3 Mueller, M.S. *et al.* (2000), J. Ethnopharmacol. 73: 487 – 493. 4 Mueller, M.S. *et al.* (2004), Trans. Royal Soc. Trop. Med. Hyg. 98: 318 – 321. 5 Räth, K. *et al.* (2004), Am. J. Tropical Med. Hyg. 70: 128 – 132.

P 244

Yields in phenylpropanoids and antioxidant properties of different aqueous extracts of lemon verbena (Lippia citriodora K.)

Bilia AR, Giomi M, Innocenti M, Vincieri FF
Department of Pharmaceutical Sciences, Via Ugo Schiff,6 50019, Sesto Fiorentino, Italy

Lemon verbena *(Lippia citriodora* K.) contains several flavonoids and phenylpropanoids mainly represented by verbascoside [1]. In order to develop a rich phytocomplex of such constituents' different aqueous extracts (*i. e.* a decoction obtained 5 min. boiling, one obtained after 20 min. boiling and a tea) were prepared from the dried plant and liophylised. Quantification of constituents in the dried extracts was carried out by HPLC-DAD-MS and the chromatographic conditions were the following: a binary system H_2O (pH 3.2 by HCOOH) and CH_3CN using a multi-step linear solvent gradient elution method. Total time of analysis was 28 min and flow rate was 0.8 mL/min. The column was a Varian Polaris ™ C18-E (250 x 4.6 mm i.d., 5 μm) mantained at 26 °C with a pre-column of the same phase. By UV and MS spectral data were identified the constituents of the 3 extracts. The qualitative profiles of the three extracts was quite similar, 9 constituents were identified, mostly of them represented by verbascoside and its analogues, besides luteolin and apigenin derivatives and one iridoid, verbenalin. However, the yield of such constituents and in particular phenylpropanoids was very different being in the lyophilized infusion 8.3 % with respect to the decoctions, 1.7 or 4.4 %. The lowest content of phenylpropanoids in the liophilised decoctions was probably due to the heat instability of verbascosides and analogs. In view of the pharmacological interest of verbascoside and analogs as antioxidants, the DPPH test was also carried out according to Son and coworkers [2]. *Acknowledgments:* The financial support of MIUR (PRIN 2004) is gratefully acknowledged. **References**: 1. Valentao, P. *et al.* (2002), Biol. Pharm. Bull. 2: 1324 – 1327. 2. Son, S., Lewis, B.A. (2002), J. Agric. Food Chem. 50: 468 – 472.

P 245

Fatty Acid Patterns of the Various Parts of Turkish Pistacia vera L. Tree

Aslan M, Orhan I
Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey

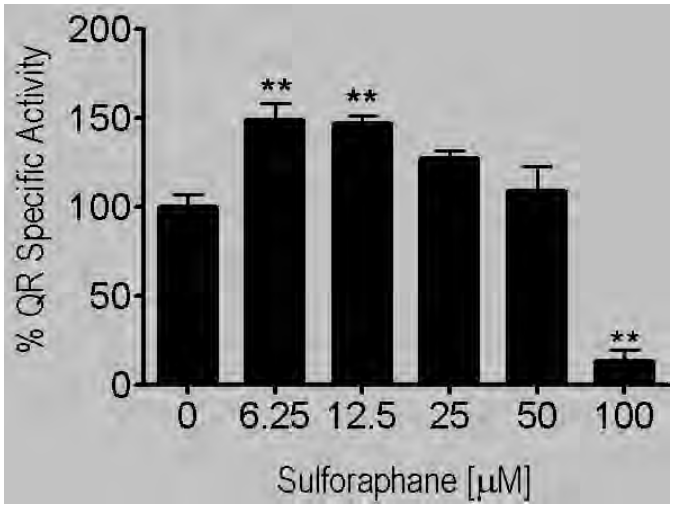
Pistacia vera L. (Anacardiaceae) is a small tree grown in southern Europe and Asia minor, being the only species within the 11 species belonging to the genus *Pistacia* that produces edible nuts. Pistachio is a nut of the tree having an edible green kernel enclosed in a woody shell. *P. vera* is widely cultivated in southern Anatolia for its nuts and has a significant contribution to the major agricultural exports of Turkey. Extensive researches have exerted that pistachio nuts are a rich source of fatty acids [1 – 6]. A survey for fatty acid composition was made for the waste products of *P. vera* (pistachio tree) grown in Turkey. The waste products and various parts of the tree were classified as fresh leaves (FL), dried leaves (DL), stem (ST), branches (BR), fresh skin of natural-woody shell (non-processed) (FSN), fresh kernel (FK), and skin of processed-woody shell (SP). In this study, gas chromatography-mass spectrometry data revealed that FSN, FL, DL, and, ST could be evaluated to be rich sources for fatty acids. In particular, FL contains a remarkable amount of linolenic acid (30.4 ± 3.28 %). **References**: 1. Agar, It., Sarmiento, C. *et al.* (1995), Acta Horticult. 419: 405 – 410. 2. Agar, It., Kaska, N., Kafkas, S. (1995), Acta Horticult. 419: 417 – 422. 3. Aslan, M., Orhan, I., Sener, B. (2002), Int. J. Food Sci. Technol. 37: 333 – 335. 4. Garcia, Jm., Agar, It., Streif, J. (1992), Gartenbauwissenschaft 57: 130 – 133. 5. Kucukoner, E., Yurt, B. (2003), Eur. Food Res. Technol. 217: 308 – 310. 6. Satil, F., Azcan, N., Baser, K.H.C. (2003), Chem. Nat. Compds. 39: 322 – 325.

P 246

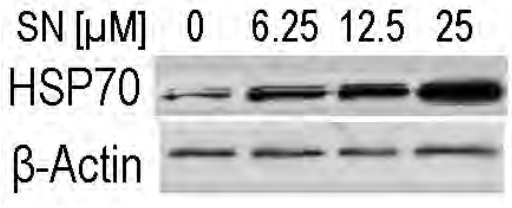
Induction of Cytoprotective Mechanisms by the Chemopreventive Isothiocyanate Sulforaphane in Rat and Murine Hepatoma Cell Lines

Hamed A, Fry J
School of Biomedical Sciences, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK

Sulforaphane [1-isothiocyanato-4(methylsulfinyl) butane, SN] is a well known cruciferous chemopreventive phytochemical that induces the anticarcinogenic enzyme quinone reductase (QR, NQO1, EC 1.6.99.2) and other phase II detoxification enzymes [1]. The present study aimed to investigate the induction of QR activity and heat shock protein 70 (HSP70) expression in the rat hepatoma (FGC4) and murine hepatoma (hepa1c1c7) cells by SN.



(A)



(B)

Figure (1): Responses of FGC4 cells to SN treatment; induction of QR (A) and HSP70 (B).

Following 24 hours exposure to non-toxic concentrations of SN (as assessed by neutral red uptake), FGC4 cells were less sensitive to QR activity induction (Figure 1A) as compared to hepa1c1c7 (significant induction of 150 %± 16 vs. 440 %± 44, respectively at 6.25 μM SN, $p < 0.01$) which may be due to the high basal QR activity of FGC4 cells. In a preliminary experiment, SN had no effect on HSP70 expression in hepa1c1c7 (data not shown). Interestingly, SN significantly induced HSP70 expression in FGC4 (Figure 1B) as revealed by western blotting and densitometric analysis (250 %± 49 at 25 μM SN, $p < 0.01$). These data represent the first report of the induction of HSP70 expression *in vitro* by SN as a cytoprotective mechanism induced by this promising chemopreventive compound. In support of these data, Hu et al. [2] have very recently reported the induction of HSP70 genes in livers of SN- treated mice. *Acknowledgements*: Egyptian Government, School of Biomedical Sciences (Nottingham, UK). **References**: 1. Zhang, Y. *et al.* (1994), PNAS 91: 3147 – 3150. 2. Hu, R. *et al.* (2006), Cancer Lett. In press.

P 247

Essential oils from leaves, stems and ripened seed capsules of Hypericum undulatum

Guedes AP, Fernandes-Ferreira M
Departament of Biology, University of Minho, Campus de Gualtar, 4710 – 057 BRAGA, Portugal

Hypericum undulatum Willd. (Guttiferae) is a common herb in Portugal, growing in wet places and in the riverside edges. The phenolic extracts of leaves and aerial parts of this species have already been studied, showing the presence of hypericin, quercetin, quercetin sulphate, rutin, mangiferin, chlorogenic acid [1; 2]. However studies of its volatile component are scarce. The essential oils were obtained by hydrodistillation of a small amount of fresh leaves, stems and ripened seed capsules harvested in September. More than 40 compounds were detected in the leaves and ripened seed capsules, while in the stems 20 compounds were detected. The compounds were identified by GC-MS and quantified by GC. Excepting for the stems, in which there wasn't any oxygenated compound, the identified compounds in the essential oils from all samples distributed by monoterpene hydrocarbons (MH), oxygenated monoterpenes (MO), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (SO) and alkanes. The most complex essential oils were those obtained from leaves. In both leaves and ripened seed capsules the major compound group was the sesquiterpene hydrocarbons. However, in both essential oils, the major compound was an *n*-alkane (*n*-nonane). Caryophyllene oxide and globulol were the two major oxygenated-sesquiterpenes in those two samples. *n*-Nonane was also the most represented in the stem essential oils, the major group of compounds being *n*-alkanes. β-Pinene, a monoterpene hydrocarbon, was also well represented in the three samples. **References***:* 1. Seabra, R.M. *et al.* (1991), Rev. Port. Farm. 12: 16 – 18. 2. Seabra, R.M. *et al.* (1992), Fitoterapia 68: 473 – 474.

P 248

Analysis of Phenolic Acids from Actaea spec. by Capillary Electrophoresis

Prinza S, Singhubera J, Zhub M, Koppa B
[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; [2]Medical Institute POB 76, Beijing University of Chinese Medicine and Pharmacology, Bei San Huan Dong Lu No. 11, Beijing 100029, PR China

The rhizome of *Actaea racemosa* L. (syn. *Cimicifuga racemosa* L.), Ranunculaceae, is used for the treatment of menopausal disorders. Regarded as well accepted alternative to standard hormone therapy, the increasing demand of black cohosh leads to overharvesting of the wildcrafted plant in the US. In Asia related *Actaea* spec. are cultivated and pharmaceutically used in TCM (*e.g. A. dahurica*, *A. foetida*, *A. heracleifolia*, *A. simplex*). Problems in sourcing of *A. racemosa* plant material and adulterations with those *Actaea* spec. used in TCM are the consequence, requiring a sound analytical system for quality control. Whereas the pattern of triterpene glycosides is not eligible for a qualitative fingerprint, the phenolic acids (caffeic acid, ferulic acid, isoferulic acid, fukinolic acid and the cimicifugic acids A, B, D, E and F) provide a convincing tool in sample identification to distinguish between different *Actaea* spec. [1]. The latter cimicifugic acids from the rhizomes of *A. racemosa* were fractionated and identified according to [2] and [3]. A rapid method for the qualitative analysis of the phenolic acid fingerprint of methanolic extracts of the above mentioned *Actaea* spec. has been established on capillary electrophoresis (CE). Baseline separation of all phenolic acids was achieved on a 50 µm capillary (70 cm, 60 cm to detector) with a 25mM borate buffer (pH 9.0) at 25 kV within 25 minutes, representing a time and solvent saving method for quality control and a sound alternative to HPLC. **References**: 1. Kusano, G. (2001), Yakugaku Zasshi 121: 497 – 521. 2. Stromeier, S. *et al.* (2005), Planta Med. 71: 495 – 500. 3. Kruse, S.O. *et al.* (1999), Planta Med. 65: 763 – 764.

P 249

Sesquiterpenoids and phenolics from roots of Cichorium endivia var. crispum

Kisiel W, Michalska K
Department of Phytochemistry, Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna Str, Pl-31 – 343 Krakow, Poland

Endive (*Cichorium endivia* L.) and chicory (*C.intybus* L.) are the most popular species of the genus *Cichorium* (Asteraceae, Lactuceae). Leaves of commercial varieties are used as a salad additive or a vegetable and roots are considered wastes. Chicory is also known as a traditional herbal remedy which improve digestive and metabolic functions. A single report [1] on sesquiterpene lactones of *C. endivia* roots revealed the presence of germacranolides, eudesmanolides and lactucin-like guaianolides. We have undertaken an investigation of roots of *C. endivia* L. var. *crispum* Lam. The dried roots were extracted with ethanol and the extract, after sequential fractionation on silica gel followed by semipreparative RP HPLC, gave 13 sesquiterpene lactones, including 11 guaianolides, and three phenolics. The guaianolides 8-deoxylactucin and lactucopicrin appeared to be major sesquiterpene lactone constituents, and the guaianolides hieracin II and macrocliniside G were found in *Cichorium* species for the first time. Moreover, a new natural product was isolated and characterized as 10β-methoxy-1α (10), 11β(13)-tetrahydrolactucin. In addition, the roots yielded methyl- and ethyl *p*-hydroxyphenylacetates, and ethyl *trans*-caffeate, the latter in a relatively high amount. The formation of the ethyl esters is likely to occur during the extraction procedure. All the compounds were characterized by spectral methods. 8-Deoxylactucin, also known as the major sesquiterpenoid of chicory roots [2], was reported to inhibit DNA binding of the transcription factor NFκB [3] and cyclooxygenase-2 protein expression [4]. **References**: 1. Seto, M. *et al.* (1988), Chem. Pharm. Bull. 36: 2423. 2. Kisiel, W., Zielinska, K. (2001), Phytochemistry 57: 523. 3. Siedle, B. *et al.* (2004), J. Med. Chem. 47: 6042. 4. Cavin, C. *et al.* (2005), Biochem. Biophys. Res. Commun. 327: 742.

P 250

A convenient TLC method for the quality control of turmeric

Sriphong L[1], Phattanawasin P[1], Sotanaphun U[2]
[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University; Nakhon Pathom, 73000 Thailand; [2]Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, 73000 Thailand

Turmeric, the dried rhizome of Curcuma longa Linn., is well documented for its medicinal properties and widely used for the treatment of several diseases. The biological effects of turmeric have been attributed to its constituent curcumin that has been studied for its anti-inflammatory, anti-angiogenic, antioxidant, wound healing and anticancer effects [1, 2]. For quality control, the content of curcuminoids in turmeric was to be determined by the spectrometric method at 420 nm according to Thai Herbal Pharmacopoeia (THP) Vol. 1 [3]. In this study, a simple and rapid thin layer chromatography (TLC) method for the determination of three curcuminoids, curcumin (CUR), desmethoxycurcumin (DES) and bisdesmethoxycurcumin (BIS) in turmeric was developed and validated. The method was performed on pre-coated silica gel TLC plates and the mobile phase consisting of chloroform-hexane-methanol (1:1:0.1, v/v/v). Quantification of each curcuminoid was carried out by image analysis technique using Photoshop software. The amount of curcuminoids in ten turmeric samples assayed by the proposed method was compared to those assayed by the official method in THP vol. 1. The statistical test showed no significant differences ($p > 0.05$) between the methods, indicating that this TLC method is acceptable and convenient to assess the quality of turmeric as an alternative method for routine analysis. *Acknowledgements:* The authors are thankful to Institute of Research and Development, Silpakorn University for providing funds for the research project. **References**: 1. WHO Monographs on Selected Medicinal Plants Part 1 Vol. 1 (1999), World Health Organization, Geneva, Switzerland. 2. Radha, K. *et al.* (2006), Life Sciences, 78: 2081 – 2087. 3. Thai Herbal Pharmacopoeia Vol. 1 (1995), Prachachon Co., Ltd., Bangkok.

P 251

Achillea millefolium L. s.l. – is the antiphlogistic activity mediated by protease inhibition?

Benedek B[1], Melzig MF[2], Kopp B[1]
[1]Department of Pharmacognosy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria; [2]Institut für Pharmazie, Freie Universität Berlin, Königin-Luise-Straße 2+4, D-14195 Berlin, Germany

Achillea millefolium L. s.l. is traditionally used not only in the treatment of gastro-intestinal and hepato-biliary disorders, but also as an antiphlogistic drug. As various proteases, for instance human neutrophil elastase (HNE) and matrix metalloproteinases (MMP-2 and -9), are associated with the inflammatory process, the aim of this study was to test a crude plant extract in different protease inhibition assays for understanding the mechanisms of antiinflammatory action. Furthermore, two fractions enriched in phenolic compounds – flavonoids and dicaffeoylquinic acids (DCCAs), respectively – were also tested in order to evaluate the contribution of those substances to the antiphlogistic activity of the drug. Briefly, various concentrations of the extract and the two fractions were incubated with the respective proteases and a chromogenous substrate. After measuring the absorbance against a blank sample, the percentage of protease inhibition was determined and allowed calculation of the IC_{50} values. The extract and the flavonoid fraction inhibited HNE showing IC_{50} values of 20 µg/mL, whereas the DCCA fraction was less active (IC_{50}= 65 µg/mL). The inhibitory activity on MMP-2 and -9 was observed at IC_{50} values from 600 to 800 µg/mL, whereas the DCCA fraction showed stronger effects than the flavo-

noid fraction and the crude extract. In conclusion, the antiphlogistic activity of *Achillea millefolium* L. s.l. is at least partly mediated by inhibition of human neutrophil elastase and matrix metalloproteinase-2 and -9, whereas the extract was equally or even stronger effective than the two fractions which is consistent with the holistic approach of phytotherapy.

P 252

Antiproliferative and apoptotic effects of garlic on chronic myeloid leukemia cell line

Sunguroglu A[1], Akay GG[1], Ozkal P[1], Varol N[1], Akcora D[1], Altinok B[2], Gokmen D[3], Avci A[4], Ergüder IB[4], Devrim E[4], Durak I[4]
[1]Department of Medical Biology, Ankara University School of Medicine, ANKARA; [2]Institute of Biotechnology, Ankara University, ANKARA; [3]Department of Medical Statistics, Ankara University School of Medicine, ANKARA; [4]Department of Biochemistry, Ankara University School of Medicine, ANKARA

INTRODUCTION: Garlic is a plant commonly used for seasoning food in many different cultures of the world, and its medicinal properties have been known since ancient times. Epidemiological studies have shown that enhanced garlic consumption is closely related with reduced cancer incidence. In vitro studies indicate that garlic has antiproliferative and apoptotic effects on different cancer cell lines including HL-60 (human acute myeloid leukemia cell line). However, there are no reports on whether or not it affects CML (chronic myeloid leukemia) cell lines *in vitro*. CML is a myeloproliferative disorder that is characterized by Philedelphia (Ph) chromosome. This chromosome is caused by resiprocal translocation t(9;22)(q34;q11.2) which results in BCR-ABL fusion gene produces a fusion tyrosine kinase (FTKs). The fusion tyrosine kinases create bipartite proteins in which the kinase is hyperactivated by an adjoining oligomerization domain. Oncogenic tyrosine kinases are thought to induce either directly or indirectly a critical repertoire of transforming events, namely uncontrolled cell growth, genomic instability and protection of DNA–damaged cells from apoptosis. We hypothesized that garlic could cause apoptosis in CML cells. Therefore, in this study, it is aimed to investigate possible antiproliferative and apoptotic effects of garlic on 32Dp210 (BCR-ABL fusion gene (+) mouse CML cell line) and 32D (wild type mouse myeloid cell line) cell lines. **MATERIALS and METHODS:** Cells were grown at 37^0C under a humidified, 5 % CO_2 atmosphere in RPMI 1640 medium supplemented with 20 % fetal calf serum. Cells were incubated with garlic extract at final concentrations of 1 % (w/v) and 0.4 % (w/v) for 0, 24, 48 and 72 hours. Cell viability was detected by MTT assay and apoptosis was determined morphologically. **RESULTS:** It is demonstrated that garlic has antiproliferative and apoptotic effects on both of the cell lines. All of the concentrations were found to be statistically different ($p < 0.001$) in respect to their antiproliferative and apoptotic effects. The most effective apoptotic and antiproliferative concentration was found 0.4 % (w/v). It has been calculated that at this concentration the death risk of 32Dp210 was 2.08 times higher than 32D. Our results indicate that garlic could be used as a potential chemopreventive agent in CML.

P 253

Anxiolytic effects of Lavender (Lavandula angustifolia) odour on the mongolian gerbil (Meriones unguiculatus) elevated plus-maze

Hornby BF[1], Starkey NJ[2], Brown SL[1], Lea RW[3]
[1]Department of Psychology, University of Central Lancashire, Preston. Lancashire. UK. PR1 2HE. [2]Department of Psychology University of Waikato.Private Bag 3105. Hamilton. New Zealand. [3]Department of Biological Sciences, University of Central Lancashire PR1 2HE

The prolonged effects of (*Lavandula angustifolia* L.*)* lavender odour inhalation were examined in gerbils on the elevated plus maze. Mature male and female gerbils were exposed to lavender odour over two week or 24 hour periods, and compared to a no-lavender condition. This pattern of results was compared with the effects of diazepam (1 mg/kg) ***i.p.*** after two week administration. The Jonckheere- Terpstra test for ordered alternatives was used, with the Mann Whitney U test to examine group differences within significant trends. Traditional measures of open entries showed an increasing trend over the two weeks exposure, (chronic lavender odour vs. no odour control $U = 166$, $p < 0.05$). Whereas, stretch-attend frequency, an ethological measure indicative of anxiety, decreased after exposure to lavender odour (acute vs. control $U = 71$, $p < 0.001$ and chronic vs. control $U = 25$, $p < 0.001$). Likewise, exploratory behaviour, total head-dip frequency, increased after lavender exposure (acute $U = 35$, $p < 0.001$ and chronic exposure $U = 28$, P, 0.001). These results are comparable with chronic diazepam administration. There were sex differences in protected head-dip, an ethological indicator of anxiety: after two weeks exposure females showed a significant decrease in protected head-dips compared to both males ($U = 39$, $p < 0.05$) and to female controls ($U = 10$, $p < 0.01$). In conclusion, exposure to lavender odour may have an anxiolytic profile in gerbils similar to that of the anxiolytic diazepam. In addition, prolonged, two week lavender odour exposure increased exploratory behaviour in females indicating a further decrease in anxiety in this sex.

P 254

Structural characterization of two galactofuranomannan isolated from the lichen Thamnolia vermicularis var. subuliformis

Omarsdottir S[1], Petersen BO[2], Paulsen BS[3], Togola A[3], Duus JØ[2], Olafsdottir ES[1]
[1]University of Iceland, Faculty of Pharmacy, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland; [2]Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark; [3]University of Oslo, Institute of Pharmacy, Department of Pharmacognosy, P. B. box 1068, N-0316 Oslo, Norway

Lichens are symbiotic organisms consisting of a fungus and an algae and/or cyanobacterium. Of 13, 500 lichen species growing worldwide, less than 100 species have been investigated for polysaccharide content. Lichen polysaccharides are mainly of three different structural types: β-glucans, α-glucans and galactomannans [1]. The aim of the study was to isolate and structurally characterize two galactofuranomannans, Ths-4 and Ths-5 from the lichen ***Thamnolia vermicularis*** (Sw.) Schaer. var. ***subuliformis*** (Ehrh.) Schaer. using ethanol fractionation, anion-exchange and size exclusion chromatography. The average molecular weight of Ths-4 and Ths-5 was estimated to be 19 and 200 kDa, respectively. Structural characterization of Ths-4 and Ths-5 and their partially hydrolysed derivatives was performed by methanolysis and methylation analysis. The intact and partially hydrolysed Ths-4, was further analysed using NMR-spectroscopy (1D, COSY, NOESY, TOCSY, HSQC and HMBC). According to the data obtained, the heteroglycans Ths-4 and Ths-5 have similar structures, but have large difference in molecular weight. The structure is composed of 3-*O*-linked and 5-*O*-linked galactofuranosyl-chains linked to a mannan core. The mannan core consists of a main chain of α-(1→6)-linked mannopyranosyl residues, substituted at O-2 with either a single α-mannopyranosyl unit or an α-Man*p*-(1→2)-α-Man*p*-(1→2)-α-Man*p* group in the ratio of approximately 1:3, respectively. *Acknowledgements*: Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules, Icelandic Council of Science, University of Iceland Research Fund, The Icelandic Research Fund for Graduate Students, Nordic Council of Ministers, The Bergthoru and Thorsteins Scheving Thorsteinsson, Finn Tønnesen, NUFU project PRO22/2002. **Reference**: 1. Olafsdottir, E.S., Ingólfsdóttir, K. (2001), Planta Med 67: 199 – 208.

P 255

Aqueous Rooibos extract: development of a new functional food ingredient based on a botanical extract

Villar A, Alaouia S, Buchwald-Werner S
Cognis Iberia s.l, Pol. Ind. San Vicente, E-08755, Castellbisbal, Barcelona, Spain; Cognis Deutschland GmbH & Co KG, Rheinpromenade 1, D-40789, Monheim am Rhein, Germany

Functional food can be defined as food consumed as part of the normal diet that provides additional health benefits beyond the traditional nutrients it contains; that has demonstrated physiological benefits; and/or that reduces the risk of nutrition-related diseases [1]. This idea that food can be health-promoting beyond its traditional nutritional value is gaining acceptance among consumers and health professionals who see functional foods as an attractive, convenient and tasty way of receiving health benefits via whole food. Within this new trend, botanical extracts are attracting increasing interest as ingredients which can confer functionality to traditional foods and beverages. This new use of botanical extracts has created the need for product development so that products are able to satisfy the various requirements of the food industry. This poster outlines the procedure followed in developing a functional food ingredient based on an extract of the plant *Aspalathus linearis* (Bum.f.) R. Dahlgren (Rooibos). Rooibos is a South African plant traditionally associated with health benefits and appreciated for its mild, sweet taste and lack of caffeine [2, 3]. The different issues involved in the development of this functional ingredient are described – from the initial study defining market requirements to delivery of the final product to the food manufacturer. The development stages include: studying the traditional use of the plant, selecting the right raw material, designing a production process that meets food industry requirements, developing analytical methods to determine quality, stability and specifications, establishing food regulatory status, providing scientific evidence that supports the product's activity, defining the product application in food and beverage matrices, developing analytical methods to detect the ingredient in the food product and initiating marketing tools and claims that respond to food market trends. **References**: 1. Position of the American Dietetic Association: functional foods (1999), J. Am. Diet. Assoc. 99(10): 1278 – 1285. 2. Erickson, L. (2003), Herbalgram 59: 34 – 45. 3. Joubert, E. *et al.* (1995), Proceedings of Recent Development of Technologies on Fundamental Foods for Health. Korean Society of Food, Science and Technology. Seoul, Korea.

P 256

Effects of aqueous garlic extract on oxidant/antioxidant status in 32 D and 32 Dp cell lines

Durak I[1], Sunguroglu A[2], Avci A[1], Devrim E[1], Ergüder IB[1], Akay GG[2], Ozkal P[2], Varol N[2], Akcora D[2], Altinok B[3], Gokmen D[4]
[1]Ankara University School of Medicine, Department of Biochemistry, Sihhiye 06100 Ankara – Turkey; [2]Ankara University School of Medicine, Department of Medical Biology, Sihhiye 06100 Ankara – Turkey; [3]Ankara University Institute of Biotechnology, Ankara – Turkey; [4]Ankara University School of Medicine, Department of Medical Statistics, Sihhiye 06100 Ankara – Turkey

It was aimed to investigate possible effects of aqueous garlic extract on oxidant/antioxidant status in 32 D (wild type mouse myeloid cell) and 32 Dp210 (BCR-ABL fusion gene (+) mouse myeloid cell) cell lines. Chronic myeloid leukemia (CML) is a myeloproliferative disorder that is characterized by Philedelphia (Ph) chromosome. This chromosome is caused by resiprocal translocation t(9;22)(q34;q11.2) which results in BCR-ABL fusion gene. We hypothesized that garlic could cause apoptosis in CML cells. Therefore, in this study, it is aimed to investigate possible antiproliferative and apoptotic effects of garlic on 32Dp210 (BCR-ABL fusion gene (+) mouse CML cell line) and 32D (wild type mouse myeloid cell line) cell lines. For this aim, aqueous garlic extract (10% w/v) was added into the cell line media at 2 different final concentrations (0.4 and 1%). At the 0 time and 24, 48 and 72 hours later, oxidant (malondialdehyde-MDA level and xanthine oxidase-XO activity) and antioxidant (superoxide dismutase-SOD, glutathione peroxidase-GSH-Px and catalase-CAT activities) parameters were measured in the cell lines. It was observed that the garlic extract caused no change in XO and antioxidant enzyme activities but increased MDA level in the 32 D cell line. However, in the 32 Dp210 cell line treated by the garlic extract, significant increases in MDA level (1.63 nmol/million cells at 0 time vs. 4.05 nmol/million cells at the 72nd hour), XO and antioxidant enzyme activities were found. In conclusion, it has been suggested that garlic directly causes oxidant stress in 32 D cell line owing to its own oxidant ingredients and, that the oxidant stress created in 32 Dp210 cell line owing to garlic treatment might occur through increased XO activity and/or its own oxidant ingredients. Although antioxidant enzyme activities were found to increase in the 32 Dp210 cell line, it seemed that this compensatory change could not prevent the oxidant stress created. The oxidant potential of garlic extract might play part in the possible anticancer property of the garlic which was supposed by several investigators.

P 257

Immunomodulating effects of lichen-derived polysaccharides on monocyte-derived dendritic cells

Omarsdottir S[1], Olafsdottir ES[1], Freysdottir J[2]
[1]Faculty of Pharmacy, University of Iceland, Hagi, Hofsvallagata 53, IS-107 Reykjavik, Iceland; [2]NaturImm Ltd/Centre for Rheumatology Research, Landspitali-University Hospital, IS-101 Reykjavik, Iceland

Dendritic cells (DCs) belong to the innate immune system and play an important role as a bridge between the innate and the adaptive immune response. In this study the effects of eleven different chromatographically purified and well-characterised lichen polysaccharides on the maturation of DCs were tested by analysing the secretion of IL-12p40 and IL-10 by human monocyte-derived dendritic cells *in vitro*. Eight of the polysaccharides upregulated IL-10 secretion by the DCs, as compared with unstimulated cells, with the IL-10 secretion induced by the β-glucans lichenan and Ths-2 (1) and the heteroglycans Pc-4 (2) and thamnolan (3) reaching significant levels. IL-12p40 secretion was significantly upregulated by the β-glucan lichenan and the heteroglcyans Pc-2 (2), Pc-4, thamnolan and Ths-4 (4), while the mature dendritic cells stimulated with the heteroglycan Pc-1 secreted significantly less IL-12p40 than the unstimulated cells. Proportional index (PI) was used to determine the relationship between the IL-12p40 and IL-10 secretion. The PI of all the β-glucans, *i.e.* lichenan, pustulan and Ths-2, and the heteroglycan thamnolan, was significantly lower than the PI observed for the unstimulated cells, which was mainly due to increased IL-10 secretion. Therefore, these polysaccharides could be considered suitable candidates in tolerance and anti-inflammatory studies, as IL-10 is one of the major cytokines involved in tolerance and anti-inflammatory responses. *Acknowledgements*: Icelandic Council of Science **References**: 1. Olafsdottir, E.S. *et al.* (2003), Phytomedicine 10: 318 – 324. 2 Omarsdottir, S. *et al.* (2005), Phytomedicine 12: 461 – 467. 3. Olafsdottir, E.S. *et al.* (1999), Phytomedicine 6: 273 – 279 4. Omarsdottir, S. unpublished results.

P 258

Analgesic and anti-inflammatory activities of the aqueous extracts of Maytenus senegalensis, Stereospermum kunthianum and Trichilia emetica used in the treatment of dysmenorrhoea in Mali

Sanogo R[1,2], Diallo D[1,2], Maiga A[1,2], De Tommasi N[3], De Pasquale R[4]
[1]Faculty of Medicine, Pharmacy, Odontostomatology, University of Bamako – Mali; [2]Département médecine Traditionnelle, B.P. 1746 Bamako – Mali; [3]Dipartimento di Scienze Farmaceutiche, Università di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy; [4]Dipartimento Farmaco-Biologico, Università di Messina, Vill. SS. Annunziata, 98168, Messina, Italy

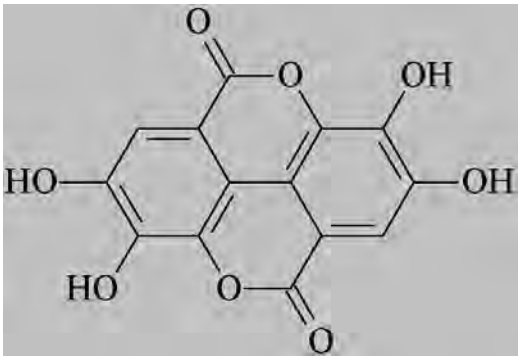
The use of traditional herbal remedies is commonly encountered in the rural and urban areas in Mali. Traditional medicine is one of the surest means to achieve total health care coverage of the Africa's population. In Mali, more than 80 percent of the population depends upon traditional medicine and medicinal plants for primary health care. Our project is a contribution to the pharmacological and toxicological studies of three medicinal plants used in the treatment of dysmenorrhoea in Mali. The principal goal of the project is to propose improved traditional prescription developed with extracts of the three plants for the health care of the Malian population, especially women. Our previous report presented the ethnobotanical information on the three plants: *Maytenus senegalensis* Lam. (Celastraceae), *Stereospermum kunthianum* Cham. (Bignoniaceae) and *Trichilia emetica* Vahl. (Meliaceae) [1]. Preliminary phytochemical analysis of the aqueous extracts revealed the presence of coumarins, tannins, polysaccharides, leucoanthocyans, saponins glycosides etc. Here we studied the analgesic and anti-inflammatory activities of aqueous extracts of leaves, bark and roots of these plants. Investigations were carried out on acetic acid-induced writhing (pain) and hind paw oedema in mice. Results showed the decoctions 10% to possess significant anti-nociceptive and anti-inflammatory activities at the dose of 25mL/kg administrated orally in mice compared to control group ($P < 0.05$, test *t*-Student). The best analgesic activity was found with the leaves of *M. senegalensis*, *S. kunthianum* and *T. emetica*, respectively 72, 85 and 75% of protection against pain. These data corroborate the traditional use of these three plants in the treatment of dysmenorrhoea. *Acknowledgements.* This project is supported by grants International Foundation for Science (IFS) N° F/3771 – 1 (Dr. Rokia Sanogo) **Reference**: 1. Sanogo, R., Diallo, D. (2005), Study of three plants traditionally used in Mali in the treatment of dysmenorrhoea (I): Ethnobotanical information on *Maytenus senegalensis*, *Stereospermum kunthianum* and *Trichilia emetica* (Poster N°437, GA conference, Florence, August 2005).

P 259

Low Molecular Weight Polyphenols in insect infected leaves of Quercus ilex L. (Fagaceae)

Skaltsa H[1], Karioti A[1], Karabourniotis G[2], Bilia AR[3]
[1]Department of Pharmacognosy & Chemistry of Natural Products, School of Pharmacy, Panepistimiopolis-Zografou, 15771 Athens, Greece; [2]Laboratory of Plant Physiology and Morphology, Department of Agricultural University of Athens, Iera Odos 75, 11855 Botanikos, Athens, Greece; [3]Department of Pharmaceutical Sciences, University of Florence, Ugo Schiff 6, Polo Scientifico, Sesto Fiorentino, 50019, Florence, Italy

Plant-insect interactions in Mediterranean ecosystems have not hitherto sufficiently studied in terms of Chemical Ecology. *Quercus ilex* is an excellent model for this type of study, as its leaves present impressive alterations after insect attacks. In this study, low molecular weight polyphenols were studied by HPLC in samples of insect infected leaves. The leaves were extracted with solvents of increasing polarity and the methanol extract was further submitted to chromatographic separations, mainly column chromatography and HPLC and afforded gallic, protocatechuic, vanillic, caffeic, ferulic, and ellagic acids. Comparative HPLC study of healthy and infected leaves showed an increase in the phenolic content. The structures of the isolated compounds were established by means of 1D & 2D NMR.



P 260

The Use of Near Infrared Spectroscopy to discriminate between THC-rich and hemp forms of Cannabis

Wilson N, Heinrich M
Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29 – 39 Brunswick Square, London, WC1N 1AX, UK

The main psychoactive component of 'drug type' cannabis, Tetrahydrocannabinol (THC), is present at only very low levels in hemp, which is often used in the food and textile industry. Near Infrared (NIR) spectroscopy is ideally suited to the identification and quality control of plant material. This work illustrates of potential of NIR spectroscopy to differentiate between 'drug type' cannabis and hemp. The different plant materials were scanned on a FOSS NIR-Systems 6500 spectrophotometer with the Rapid Content Sampler module and Vision® software. A spectral library containing samples of THC-rich cannabis or hemp in the form of dried flowering tops or leaf was constructed and samples were assigned as either 'high THC' or 'low THC'. The use of spectral correlation methods allowed for the correct identification of all samples in the library. Principal Component Analysis (PCA) (The Unscrambler® software) was also carried out on the spectral library and the scores plot discriminated between the 'high' and the 'low' THC content samples. The first Principal Component loading correlated with the NIR spectrum of THC, further supporting the evidence that the differences seen between the two sets of samples were due to the THC content. The library was 'interrogated' with further samples, which included material with the cannabinoids removed by solvent extraction and old samples of 'drug type' cannabis. The scores plots obtained were consistent with their THC content. This demonstrated the robustness of the analytical models used to discriminate between the THC-rich and hemp forms of Cannabis.

P 261

The allergenic potential of sesquiterpene lactones in phytomedicines from Arnica – an immunologic revision

Lass C[1,2], Vocanson M[3], Schempp C[1], Nicolas JF[3], Martin SF[1#], Merfort I[2#]*
[1]Clinical Research Group Allergology, Department of Dermatology; [2]Department of Pharmaceutical Biology and Biotechnology, University of Freiburg, Stefan-Meier-Str. 19 D-79104 Freiburg, Germany; [3]Institut National de la Sante et de la Recherche Medicale (INSERM) U503, Lyon Cedex, France.# joint senior authors

Preparations of *Arnica montana* L. flowers have been used in traditional medicine since a long time to treat a variety of inflammatory diseases. The secondary metabolites that mediate the anti-inflammatory effects are sesquiterpene lactones (SLs) of the 10α-methylpseudoguaianolide type like helenalin and 11α,13-dihyrohelenalin, and their ester derivatives. Several studies have shown that SLs

exert this effect in part by inhibiting activation of the transcription factor NF-κB. Despite the proven anti-inflammatory effects, Arnica preparations are often considered as strong contact sensitizers and inducers of allergic contact dermatitis. This bad reputation is based on results from a guinea pig model where different preparations from *Arnica montana* and their isolated SLs turned out to be strong inducers of skin erythema and on case reports in the literature. In contrast to these findings, we had no success in causing contact hypersensitivity (CHS) to the same SLs and preparations in a well accepted mouse model, on the contrary, we observed an anti-inflammatory effect of Arnica tinctures in an allergic ear swelling reaction caused by the strong contact sensitizer TNCB. Further studies were undertaken to find out if $CD4^+CD25^+$ regulatory T cells (Treg) actively prevent CHS to Arnica tinctures. Although we failed causing CHS in $CD4^+CD25^+$ T cell-depleted mice, our preliminary studies using MHC $II^{0/0}$ mice indicate that CHS to Arnica can be induced. As in CHS to TNCB and other allergens, $CD8^+$ T cells are the effector cells. Our results show that immunosuppressive mechanisms such as the action of Treg cells prevent CHS to Arnica. According to these findings, SLs and tinctures from Arnica have to be classified as weak contact sensitizers. *Acknowlegement*: We gratefully acknowledge financial support from Kneipp company.

P 262

Composition and antimicrobial activity of the essential oil of six Hypericum species from Serbia

Skaltsa H[1], Saroglou V[1], Marin PD[2], Rančić A[3], Veljić M[2]
[1]Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, 157 71, Athens, Greece; [2]Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia; [3]Institute for Biological Research "Siniša Stanković", Despota Stefana 142, 11000 Belgrade, Serbia & Montenegro

The essential oils of six *Hypericum* sp. growing in Serbia were analyzed by GC and GC-MS [1]. The main constituents were revealed as follows: *H. alpinum*: Waldst. et Kit. non Vill. (-)-β-pinene, γ-terpinene, (-)-(*E*)-caryophyllene; *H barbatum*: Jacq. (-)-α-pinene, (-)-β-pinene, (-)-limonene, (-)-(*E*)-caryophyllene, (-)-caryophyllene oxide; *H. rumeliacum*: Boiss. (-)-α-pinene, (-)-β-pinene, (-)-limonene, *H. hirsutum* L.: *nonane*, undecane, (-)-(*E*)-caryophyllene, (-)-caryophyllene oxide; *H. maculatum* L.: spathulenol, globulol; *H. perforatum* L.: (-)-α-pinene, (Z)-β-farnesene, germacrene D; Monoterpene hydrocarbons were shown to be the main group of the taxa belonging to the section Drosocarpium, while the taxa of section Hypericum were more rich in sesquiterpene hydrocarbons. The essential oils were screened for their antimicrobial activity [Table 1], using the microdilution method [2]. *H. barbatum* essential oil was proven the most active against all tested bacteria.

Table 1. Minimum Inhibitory Concentrations (MICs) of essential oils (μg/mL).

MIC	alpi	barb	rume	mac	perf	hirs	Control*
Bacillus cereus	12.5	6.25	12.5	12.5	12.5	12.5	50
Micrococcus luteus	12.5	6.25	12.5	12.5	12.5	25	50
Sarcina lutea	12.5	6.25	6.25	12.5	12.5	12.5	50
Staphylococcus aureus	12.5	6.25	6.25	12.5	12.5	25	50
Agrobacterium tumefaciens	25	25	25	25	25	50	100
Escherichia coli	50	25	25	25	25	50	100
Proteus mirabilis	-	50	50	50	50	-	200
Pseudomonas aeruginosa	-	50	25	25	50	-	-
Pseudomonas tolaasii	50	25	25	25	25	50	200
Salmonella enteritidis	50	25	25	25	25	50	200
Candida albicans	-	25	25	50	50	-	200

* Control: Streptomycin for bacteria; Bifonazole for *Candida albicans*

References: 1. Adams, R. (2001), Identification of Essential oil components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publishing Corporation, Carol Stream, Illinois, USA. 2. Daouk, K.D. *et al.* (1995), J. Food Prot. 58: 1147 – 1149.

P 263

Phytochemical and Biopharmaceutical Analysis of Willow Bark

Krauze-Baranowska M[1], Sznitowska M[2], Pobłocka-Olech L[1], Głód D[1]
[1]Department of Pharmacognosy and [2]Department of Pharmaceutical Technology, Medical University of Gdańsk, Gen. J. Hallera 107, 80 – 416 Gdańsk, Poland

Willow bark is the herbal remedy used for centuries as antiphlogistic and analgesic. The 2 D HPLC system was developed to determine the chemical composition of *S. purpurea* L., *S. daphnoides* Will. and *S. acutifolia* Willd. bark and dried extracts obtained from this plant material. In all analysed species the presence of salicin, salicortin, naringenin 5-O- and 7-O-glucosides, naringenin and isosalipurposide, 6"-p-coumarylisosalipurposide, catechin and pyrocatechin was confirmed. Pyrocatechin was isolated from the bark of *S. purpurea* for the first time (the structure elucided by NMR and MS). The content of pyrocatechin varied from 0.94 % in dried extract of *S. purpurea* to 0.05 % in the bark of *S. daphnoides*. The biopharmaceutical evaluation of the material was performed by a pharmacopoeial dissolution test. Ph.Eur. The test was carried out for the extract (1500 mg) or pulverised *Salix* bark (3000 mg). Fast (within 30 min) and practically complete dissolution of salicin, salicortin, pyrocatechin, naringenin 5-O and 7-O glucoside was observed from the extract. For isosalipurposide and p-coumarylisosalipurposide dissolution process was slower. Unlike for the extract dissolution of the active compounds from the pulverised cortex was incomplete – irrespective of the compound, with the exception of p-coumarylisosalipurposide (13 – 20 % dissolved), 60 – 83 % of the substance was dissolved after 30 min and up to 4 h maximum 10 % was additionally released. Practically no difference was noticed between dissolution rate in water and in HCl solution. Such profiles indicate that due to the pulverisation of the bark (passed through 0.315 sieve) a large portion of the active substances is easily available for dissolution and absorption. *Acknowledgments*: The work was financially supported by the Polish State Committee for Scientific Research (KBN) Grant No PBZ-KBN-092

P 264

Effect of calcium on enzyme activities and phenolic accumulation in Hypericum androsaemum cell cultures

Paranhos A
Faculty of Pharmacy, University of Coimbra, Rua do Norte, 3000 – 295 Coimbra, Portugal

The aerial parts of *Hypericum androsaemum* L. have been used in folk medicine for its diuretic and hepatoprotective properties [1], which are attributed to the several flavonoids and phenolic acids found in the plant. Suspension cultures were established from hypocotyl-derived callus using MS medium supplemented with 2,4-D (1 mg/L) and BA (0.5 mg/L). The total flavonoid and total hydroxycinnamic acid contents of cells were evaluted according to [2] and [3], respectively. Levels of these compounds exhibited a similar pattern of changes over the cell cycle, reaching a minimum on day 7 and a maximum during the stationary phase (day 14). Culture of cells for 7 and 14 days in nutrient media containing high concentrations of $CaCl_2$ (15 or 18 mM) induced a substantial increase in the accumulation of flavonoids (up to 2-fold) and a small raise (20 – 30 %) in the levels of hydroxycinnamic acids, with the most pronounced effects being observed at the longer incubation period. Catalase activity in 7-day treated cultures was 2 to 3-fold higher than in control cultures, while that of superoxide dismutase was 30 % lower. By contrast, the levels of both enzyme activities showed no significant alterations in 14-day treated cells possessing the highest amount of phenols. These results are in agreement with an increased production of H_2O_2 in treated cultures and suggest that phenolic compounds may play a role in protecting cultured cells against oxidative stress. *Acknowledgements*: Center of Pharmaceutical Studies **References**: 1. Novais, M. *et al.* (2004), J. Ethnopharma-

col. 93: 183 – 195. 2. Lamaison, J., Carnat, A. (1990), Pharm. Acta Helv. 65: 315 – 320. 3. Lamaison, J. *et al.* (1991), Pharm. Acta Helv. 66: 185 – 188.

P 265

Oral treatment with the Crataegus special extract WS® 1442 inhibits cardiac hypertrophy in rats with DOCA-salt or aortic banding induced hypertension

Koch E, Spörl-Aich G
Preclinical Reseach, Dr. Willmar Schwabe GmbH & Co. KG, Willmar-Schwabe-Str. 4, 76227 Karlsruhe, Germany

Cardiac hypertrophy (CH) is an adaptive enlargement of the myocardium in response to diverse pathophysiological stimuli. Whereas this process is generally a beneficial response that temporarily augments cardiac output, sustained hypertrophy often becomes maladaptive and is a leading cause for the development of heart failure. Activation of the protein phosphatase calcineurin is discussed as a major intracellular signaling pathway that contributes to the growth of cardiomyocytes. We have previously observed that WS® 1442, a special extract from leaves with flowers of Crataegus ssp., inhibits the enzymatic activity of calcineurin. Thus, it was the aim of the present study to evaluate if WS® 1442 affects the development of CH in animal models of hypertension. Hypertension and subsequent CH was induced in rats by aortic bending (AB) or administration of deoxycorticosterone (DOCA) in combination with NaCl/KCl-substituted drinking water. Animals were treated orally for a period of 14 (AB) or 28 days (DOCA-salt) with vehicle (0.2 % agar suspension) or WS® 1442 (100 and 300 mg/kg/day). On the final day, animals were anaesthetized and blood pressure (BP) and heart rate were measured following cannulation of the carotic artery. After euthanization, the heart was removed and the weights of the entire heart and the left ventricle were obtained. In both experimental models a marked increase of BP as well as enlargement of the heart and the left ventricle were observed. Treatment with WS® 1442 dose-dependently lowered the pathologically increased BP but had no effect on the BP in normal control animals. In parallel with the reduction of the BP development of cardiac hypertrophy was inhibited. The present study demonstrates that oral treatment of rats with WS® 1442 prevents development of CH induced by primary or secondary hypertension and thus supports its therapeutic use in the treatment of mild forms of heart failure.

P 266

Absolute configuration and conformation of 3 tetralone derivatives from Ammannia baccifera

Techatanawat I[1,2], Houghton PJ[2], Hylands PJ[2]
[1]Pharmacognosy Research Laboratories, King's College London, 150 Stamford Street, London SE1 9NH, United Kingdom; [2]Government Pharmaceutical Organisation, 75/1 Rama VI Road, Ratchatewi, Bangkok 10400, Thailand

The ethanol extract of *Ammannia baccifera* L. (Lythraceae) was subjected to vacuum liquid column chromatography over silica gel eluted with *n*-hexane: dichloromethane: methanol (step gradient) followed by column chromatography (silica gel eluted with dichloromethane: methanol) and multi-preparative thin-layer chromatography. (-)-(4*R*)-Hydroxy-1-tetralone, (-)-(4*S*)-acetoxy-1-tetralone, (-)-(4*S*)-hydroxy-1-tetralone-4-*O*-β-*D*-glucoside, β-sitosterol and β-sitosterol-β-*D*-glucoside have been purified and identified by the joint application of UV spectroscopy, mass spectrometry, NMR spectroscopy, specific rotation and circular dichroism spectroscopy. Further light on the precise nature of the shape of the molecule was provided by a more detailed analysis of the ^{1}H NMR spectrum. Since the cyclohexanone ring was flexible, the conformation of the substituted group at C-4 of 3 tetralone derivatives was an average of the pseudoaxial and pseudoequatorial orientation [1]. The absolute configuration and conformation responsible for the NMR measurement of (-)-(4*R*)-hydroxy-1-tetralone, (-)-(4*S*)-acetoxy-1-tetralone and (-)-(4*S*)-hydroxy-1-tetralone-4-*O*-β-*D*-glucoside were β-equatorial, α-axial and α-axial orientation respectively. This is the first report of the presence of (-)-(4*R*)-hydroxy-1-tetralone, (-)-(4*S*)-acetoxy-1-tetralone and (-)-(4*S*)-hydroxy-1-tetralone-4-*O*-β-*D*-glucoside in the ethanol extract of *A. baccifera*. *Acknowledgements:* I. Techatanawat thanks the Thai Government Pharmaceutical Organisation for financial support **Reference**: 1. Talapatra, S. K. *et al.* (1988), Phytochemistry 27: 3929 – 3932.

P 267

Effect of an extract from red grapes and perilla oil (TUIM® arteria) on experimental atherosclerosis in mice

Veveris M[1], Koch E[2]
[1]Department of Medicinal Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Preclinical Reseach, Dr. Willmar Schwabe GmbH & Co. KG, Willmar-Schwabe-Str. 4, 76227 Karlsruhe, Germany

A number of epidemiological studies have demonstrated that moderate consumption of red wine is associated with a reduced mortality from cardiovascular diseases. Although ethanol may contribute to the health benefits of red wine, there is strong evidence that these effects are mainly due to the anti-oxidative action of polyphenols. Similarly, it is increasingly recognized that (n-3) polyunsaturated fatty acids [(n-3)-PUFA] have positive effects on risk factors for coronary heart disease. A reason for the insufficient intake of (n-3)-PUFA is low consumption of oily fish, the richest source of these FA. An alternative source of n3-PUFA is α-linolenic acid which is contained in high concentrations in Perilla oil derived from the seeds of the plant *Perilla frutescens* (L.) Britt.. Based on these observations, TUIM® arteria, a combination of an extract from red grapes (100 mg/capsule) and perilla oil (450 mg/capsule) has been developed as a dietary food for special medical purposes, ***i. e.*** for patients with metabolic disorders such as hypercholesterolemia or diabetes. It was the aim of the present study to examine if the proposed beneficial effects can be demonstrated in animal models of atherosclerosis: 1) experimental atherosclerosis in genetically susceptible C57BL/6J mice and 2) mice with experimental endothelial dysfunction and fed an atherogenic diet. Animals were fed daily for up to 6 months with 250 or 750 mg/kg TUIM® arteria by gavage which is equivalent to the consumption recommendation in humans. Administration of TUIM® arteria significantly decreased the serum concentrations of malondialdehyde as well as total and LDL-cholesterol in both animal models. In addition, the development of atherosclerotic lesions in the aorta was reduced. The results demonstrate that consumption of TUIM® arteria induces favorable changes in the lipoprotein profile and exerts antioxidative activity which correlate with risk reduction for the development of fibro-fatty atherosclerotic lesions in the aorta.

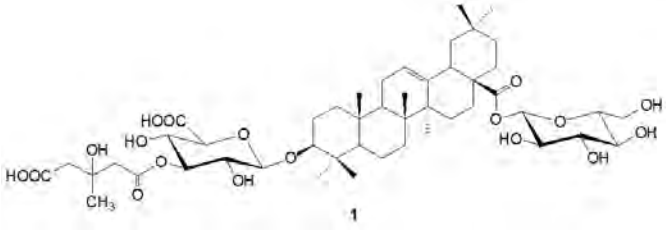
P 268

Triterpene saponins from Calendula arvensis

Kirmizibekmez H[1,2], Bassarello C[3], Pizza C[3], Calis I[2]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, TR-34755 Erenkoy, Istanbul, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey; [3]Department of Pharmaceutical Sciences, University of Salerno, Via Ponte Don Melillo 84084 Fisciano-Salerno, Italy

Calendula species especially *C. officinalis* L. (Marigold) are widely used in European and western Asian traditional medicines for skin complaints, wounds, burn, dysmenorrhoea and duodenal ulcers [1]. As a part of our studies on the Turkish medicinal plants, we investigated the secondary metabolites of *C. arvensis* L., which is used as sudorific and for the treatment of menstrual irregularities in Anatolian folk medicine [2]. The aerial parts were extracted with MeOH. The MeOH extract was suspended in water and partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH. Extensive chro-

matographic studies on the *n*-BuOH soluble fraction led to the isolation of a new triterpene saponin, arvensoside C (**1**) in addition to four known saponins, arvensosides A and B, glycoside C and calenduloside D. Three known flavonol glycosides, isorhamnetin 3-*O*-β-D-glucopyranoside, quercetin 3-*O*-β-D-glucopyranoside and quercetin 3-*O*-β-D-galactopyranoside were also obtained and characterized from the EtOAc fraction. The structures of the isolates were elucidated by 1D and 2D NMR and MS experiments.



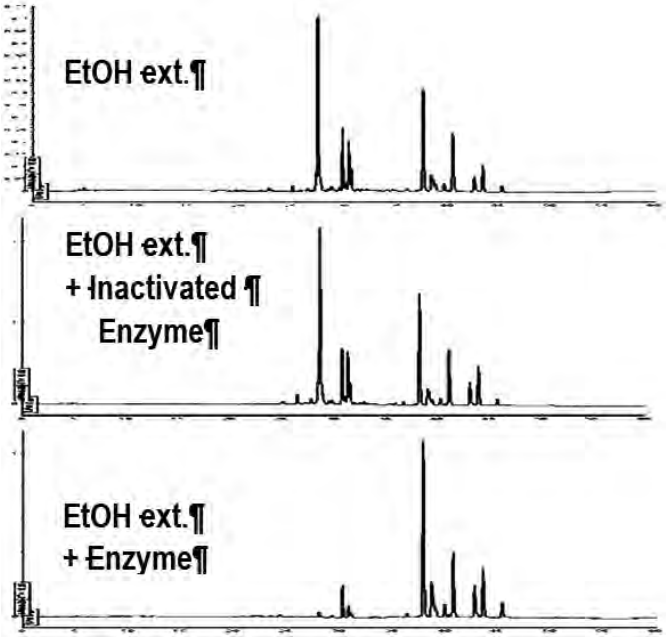
References: 1. Yoshikawa, M. *et al.* (2001), Chem. Pharm. Bull. 49: 863 – 870. 2. Baytop, T. (1999), Therapy with Medicinal Plants in Turkey (Past and Present), Nobel Tip Kitapevleri. Istanbul, p. 371.

P 269

Increase of Aurantio-Obtusin Content in Cassiae Semen by the Treatment of Crude Enzyme Extract from Aspergillus kawachii

Kwon SH[1], So JH[1], Yang EJ[1], Choi SH[1], Jeong HH[1], Hur JM[1], Jeon M[1], Lee YY[2], Suh DY[2], Rhee IK[1], Song KS[1]
[1]College of Agriculture and Life Sciences, Kyungpook National University, Daegu 702 – 701, Korea; [2]Yeongnam Agricultural Research Institute, NICS, RDA, Milyang, Kyungnam 627 – 803, Korea

"Suchi", a kind of processing technique, have been used for reducing toxicity or changing medicinal efficacy of oriental crude drugs. The techniques, however, are rarely used nowadays due to their complexity and lack of scientific backgrounds. On the other hand, food processing techniques such as heating, extrusion, and enzyme treatment (fermentation) might be applied to oriental crude drugs in order to increase the contents and/or to change the chemical structure of biologically active compounds.



The effect of processing on chemical compositions of fifty commonly used oriental crude drugs was investigated. As a result, an increased peak was found in HPLC analysis of Cassiae Semen which was treated with enzyme solution from *Aspergillus kawachii*. The increased peak was isolated by column chromatography and identified by spectroscopic analysis as an anthraquinone, aurantio-obtusin. At 37°, the obtusin reached its maximal level at 50 min after on set of the crude enzyme treatment (before treatment: 24.55 ± 2.06 mg/g, after treatment: 72.31 ± 1.58 mg/g). The crude enzyme extract from *A. kawachii* has been known to have a strong glycosidase activity, therefore aurantio-obtusin appeared to be produced by the cleavage of its corresponding glycosides. Obtusin has been known to have ant-mutagenic, anti-phytopathogenic activity. Above results suggested that the simple processing might be useful for increasing the contents of biologically active substances in oriental drugs.

P 270

Alkamides from Echinacea angustifolia roots inhibit Cyclooxygenase-2-dependent Prostaglandin synthesis in Human Neuroglioma Cells

Woelkart K[1], Bauer R[1], Hinz B[2]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitätsplatz 4/I, A-8010 Graz, Austria; [2]Department of Experimental and Clinical Pharmacology and Toxicology, Friedrich Alexander University Erlangen-Nürnberg, Fahrstrasse 17, 91054 Erlangen, Germany

During past years inhibition of the cyclooxygenase-2 (COX-2) enzyme has been proven as an effective strategy to suppress pain and inflammation. Based on this and other mechanistic findings, interest has also renewed in the molecular pathways underlying the anti-inflammatory effects of herbal drugs. The present study addressed this issue and investigated the impact of several polyunsaturated alkamides isolated from a CO_2 extract of the roots of *Echinacea angustifolia* DC. with both activity and expression of COX-2. Experiments were performed using the human neuroglioma cell line H4, which has been established as a suitable model for studying molecular mechanisms and pathways involved in COX-2 expression [1, 2]. A 48-h treatment of H4 human neuroglioma cells with the CO_2 extract led to a significant downregulation of prostaglandin E_2 formation. Analysis of 8 different alkamides revealed a contribution of undeca 2*Z*-ene-8,10-diynoic acid isobutylamide (A5), dodeca-2*E*-ene-8,10-diynoic acid isobutylamide (A7) and dodeca-2*E*,4*Z*-diene-8,10-diynoic acid 2-methylbutylamide (A8) to this response. Using an established short-term COX-2 activity assay all three alkamides were shown to interfere with COX-2 activity. In contrast, none of the COX-2-suppressing nor any other tested alkamide was found to inhibit COX-2 expression at the transcriptional and translational level. Overall, our results suggest that certain alkamides derived from *Echinacea angustifolia* roots may contribute to the pharmacological action of the herbal extract by inhibiting COX-2-dependent prostaglandin E_2 formation at sites of inflammation. **References**: 1. Ramer, *et al.* (2003), Mol. Pharmacol. 324: 621 – 626. 2. Hinz, B. *et al.* (2004), Mol. Pharmacol. 64:1189 – 1198.

P 271

The identification of new aromatic cytokinins in Arabidopsis thaliana by hybrid Q-Tof mass spectrometry

Hauserova E, Dolezal K, Novak O, Strnad M
Laboratory of Growth Regulators, Palacky University and Institute of Experimental Botany ASCR, Slechtitelu 11, 783 71, Olomouc, Czech Republic

Cytokinins [1] are plant hormones that affect a wide array of biological processes. They are involved in the growth and development of plants. Some of the cytokinin-derived compounds are also specific inhibitors of cyclin-dependent kinases and exhibit an interesting therapeutical effect against various types of diseases. This work is focused on the isolation of new di- and tri-substituted aromatic cytokinins in *Arabidopsis thaliana* (L.) Heynh. and their identifica-

tion by mass spectrometry. In general, preparation of cytokinin samples constitutes of 3 individual steps – extraction of target analytes, solid phase extraction and immunoaffinity purification step. An efficient batch immunoaffinity extraction (IAE) method [2] was developed and optimized for the purification of new cytokinins and their corresponding ribosides. The combination of simple C18 solid phase extraction with batch IAE provides fast, easy to use and cost-effective technique for routine samples processing. A general screening for new cytokinins was performed on Acquity Ultra Performance Liquid Chromatography (UPLC) linked to a Quattro *micro* API mass spectrometer equipped with an electrospray interface and photodiode array detector (Waters). The daughter ion spectra in positive ion mode were obtained for all tested standard compounds and their monitoring was based on multiple reactions monitoring (MRM). The high-resolution measurement was done on a hybrid mass analyser Q-Tof *micro* with electrospray ionisation technique, connected to capLC instrument (Waters). The accurate masses of the parent ions and its fragments were calculated and used for the determination of the elementary composition and the structure confirmation. Using this technique we were able to identify new aromatic cytokinin ribosides. **References**: 1. Mok, D.W.S., Mok, M.C. (2001), Ann. Rev. Plant Physiol. Plant Mol. Biol. 52: 89 – 118. 2. Hauserova, E. *et al.* (2005), J. Chromatography A 1100: 116 – 125.

P 272

Flavan-3-ols and procyanidins from the bark of Salix purpurea

Nahrstedt A[2], Jürgenliemk G[1], Petereit F[2]
[1]Institute of Pharmaceutical Biology, Universitätsstr. 31, D-93053 Regensburg, Germany; [2]Institute of Pharmaceutical Biology and Phytochemistry, Hittorfstr. 56, D-48149 Münster, Germany

From a hydroethanolic extract obtained from the bark of *Salix purpurea* L. (Salicaceae) the flavan-3-ols catechin, epicatechin, gallocatechin, catechin-3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester [1], the dimeric procyanidins B1, B3 [2] and the trimeric procyanidins epicatechin-(4ß8)-catechin-(48)-catechin [3] and epicatechin-(4ß8)-epicatechin-(4ß8)-catechin [4] were isolated. Their structures were elucidated by 1H-, 13C-NMR inlcuding COSY, HSCQ and HMBC methods and CD. The 13C-NMR spectral data of a fraction containing higher oligomeric procyanidins indicate an average degree of oligomerization of 4 to 5 flavan-3-ol units with dihydroxylated B-rings and predominance of the relative 2,3-*cis*-stereochemistry; B-ring trihydroxylated units were not detected. *Acknowledgements*: Bionorica AG, Neumarkt (Germany), for financial support and the extract material. **References**: 1. Hsu, F.L. *et al.* (1985), Phytochemistry 24: 2089 – 2092. 2. Kolodziej, H. (1986), Phytochemistry 25: 1209 – 1215. 3. Foo, L.Y., Karchesy, J.J. (1989), Phytochemistry 28: 1743 – 1747. 4. Shoji, T. *et al.* (2003), J. Agric. Food Chem. 51: 3806 – 3813.

P 273

Development of an HPLC – method for the analysis of mixture of natural ingredients

Alaoui S[1], Gomeza A[1], Buchwald-Werner S[2], Fornera P[1], Villar A[1]
[1]Cognis Iberia s.l, Pol. Ind. San Vicente, E-08755, Castellbisbal, Barcelona, Spain; [2]Cognis Deutschland GmbH & Co KG, Rheinpromenade 1, D-40789 Monheim am Rhein, Germany

Herbal ingredients are increasingly used in nutritional supplements and functional foods, products which can often claim health benefits in terms of reducing risk of disease and which are part of the overall well-being trend. Analytically, these natural ingredients are sometimes very complex due to their unknown matrix effect. In some cases, a mixture of different herbal extracts is needed in order to achieve an adequate level of activity either by a synergistic effect or by acting at different areas in the body to ensure a satisfactory effect. In these cases, the analytical complexity increases with the number of ingredients, their concentration, their polarity, their different responses and their level of interaction. To ensure the quality of any complex formulation and to fulfill legal requirements, complete analytical control is necessary. High performance chromatography is the most popular method for carrying out analysis of non-volatile natural ingredients. A suitable sample preparation is important to reduce the complexity and ensure a reliable result. An analytical method for a mixture containing five different natural ingredients – lutein esters, natural vitamin E, bilberry extract, passion flower extract and green tea extract – was successfully developed. The recovery of each ingredient is more than 90 % except for passion flower that is lower (more than 70 %). This method is suitable for routine quality control of the final product to ensure that the active ingredients are used at the right dosage, and for product stability tests and bioavailability studies.

P 274

Antimicrobial, antioxidant and cytotoxic activities of selected medicinal plants from Yemen

Al-Fatimi M[1], Wurster M[3], Schröder G[2], Lindequist U[3]
[1]Faculty of Medicine and Health Sciences, Pharmacy Section, Department of Pharmacognosy, Aden University Aden,Yemen; [2]Institute of Medical Microbiology, Ernst-Moritz-Arndt-University Greifswald, Germany; [3]Institute of Pharmacy, Dep. Pharmaceutical Biology, Ernst-Moritz-Arndt-University Greifswald, Germany

Ninety crude extracts, including dichloromethanic, methanolic and aqueous extracts from 30 plants used in Yemeni ethnomedicine to treat common infections, were screened in vitro for antibacterial, antifungal, antioxidant and cytotoxic activities. Three Gram-positive bacteria and two Gram-negative bacteria, *Candida maltosa* and 5 opportunistic human pathogenic fungi (2 yeasts, 3 hyphomycetes) have been used as test organisms. Extracts of *Acacia nilotica* (L.) Del., *A. tortilis* (Forsk.) Hayne, *Commiphora foliacea* Dhotar W, *Ficus vasta* Forsk., *Ocimum forskolei* Benth., *Plicosephalus curviflorus, Salvadora persica, Sansevieria aff. ehrenbergii, Solanum nigrum* L. and *Tamarindus indica* L. showed antibacterial activities against at least four bacterial strains with methanolic extract of *Tamarindus indica* flowers being the most active, followed by the methanolic extract of the fruits from *Ficus vasta*. Of the 30 plants tested, thirteen showed antifungal activity. Methanolic extracts of *Azima tetracantha* Lam. and *Solanum incanum* L. inhibited the growth of all tested pathogenic fungi. In the DPPH assay extracts from 10 plants showed activities comparable to those of ascorbic acid. The highest antioxidative activities could be found in the methanolic extracts of *Acacia nilotica* leaves and *Tamarindus indica* fruits. The extracts of 5 plants, *e.g. Plicosephalus curviflorus* and *Commiphora kua*, exhibited remarkable cytotoxic activities against cultivated FL cells. The results confirm the great potential of ethnomedicinal plants from the Arabian region and are useful for rationalizing the use of medicinal plants in primary health care in Yemen. *Acknowledgements:* The authors would like to thank Deutscher Akademischer Austauschdienst (DAAD) for a grant enabling the stay of Dr. Al-Fatimi at Ernst-Moritz–Arndt University Greifswald, that was used to carry out this research.

P 275

Quality caracterisation of propolis tinctures by pharmacopoeial parameters and wax content

Cvek J[1], Zubčić S[1], Vitali D[2], Vedrina-Dragojević I[2], Tomić S[1], Medić-Šarić M[2]
[1]Agency for medicinal products and medical devices, Ksaverska cesta 4, 10000 Zagreb, Croatia; [2]Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

The increasing use of propolis preparations nowadays requires setting of clear criteria for their quality control. For that purpose, ten tinctures of propolis samples from different Croatian regions were subjected to analysis of general pharmacopoeial parameters which

are fundamental for the creation of quality specification. These are relative density (determined using instrument Mettler Toledo DE40 Density Meter), dry residue of extract (determined according to Ph. Eur. 5.0 method), and content of ethanol and its possible impurities – methanol and isopropanol (developed and validated gas chromatography method for their simultaneous analysis was applied). Additionally, by the method of Woisky and Salatino we determined the content of waxes as the main inactive constituents in order to determine the level of their migration from crude propolis samples to the prepared tinctures (extraction solvent: 80 % V/V ethanol; drug – preparation ratio = 1:10) [1]. Relative density values increased along with the increase of dry residue of extract (e. g. the lowest values were determined in propolis tincture from South Dalmatian Islands: d_{20} = 0.8688, $RSD_{(n=2)}$ = 0.42 % and dry residue = 4.40 % w/w, $RSD_{(n=2)}$ = 0.80 % while the highest values were obtained for propolis tincture from central Croatia: d_{20} = 0.8841, $RSD_{(n=2)}$ = 0.01 % and dry residue = 7.62 % w/w, $RSD_{(n=2)}$ = 0.28 %). Investigated validation parameters for GC method met the acceptance criteria (correlation coefficient ≥ 0.999; precision: $RSD_{(n=21)}$ = 0.60 %; average $recovery_{(n=21)}$: 99.08 %; DL = 0.001 % for methanol and 0.002 % for isopropanol; QL = 0.003 % for methanol and 0.006 % for isopropanol). Ethanol content was in range from 73.98 to 77.74 % V/V ($RSD_{(n=3)}$ = 0.06 – 2.43 %) which is in accordance with USP 29 requirement for alcohol content in herbal extracts (90 – 110 % of declared amount). Contents of methanol and isopropanol were below detection limits. Presence of waxes was not observed in propolis tinctures indicating the suitability of applied extraction method. **Reference**: 1. Woiski, R.G., Salatino, A. (1998), J. Apic. Res. 37(2): 99 – 105.

P 276

Pharmacological Effects of a Decolorised St. Johns Wort Extract Designed for Topical Application

Erdelmeier CAJ, Koch E
Preclinical Research Department, Dr. Willmar Schwabe Pharmaceuticals, Willmar-Schwabe-Str. 4, 76227, Karlsruhe, Germany

St. John's Wort extract (=*Hypericum perforatum* L. extract) is a very well-established phytotherapeutic drug for the treatment of nervous disorders. Its beneficial effects against mild to moderate depression have been proven in several clinical studies. On the other hand, in the form of its oil, St.John's Wort was also externally used for the treatment of sores, ulcers, burns, myalgia, and bruises [1]. More recently, beneficial dermatological effects were demonstrated for a lipophilic preparation of St. Johns Wort [2]. As for several ingredients of St. John's Wort, anti-viral, anti-bacterial, anti-proliferative and anti-inflammatory properties have been reported, its extracts may be effective against respective diseases as well, when applied topically. Normally, St. Johns Wort extract are deeply coloured due to presence of chlorophyll and proanthocyanidin pigments. In order to provide for an extract, acceptable for use in a topical preparation, we have developed a procedure to remove pigments from a crude extract. The method employed a selective solid phase extraction with Diaion HP-20 (polystyrene resin) to obtain an extract largely free from green (chlorophylls) and brownish (proanthocyanidins) pigments. This extract and its individual components were tested for activity against *Herpes simplex* Virus (HSV-1) and gram-positive bacteria including methicillin-resistent *Staphylococcus aureus* (MRSA). Antiphlogistic properties were examined using the croton oil ear-oedema model. Furthermore, the extract was tested for inhibition of human keratinocyte proliferation (psoriasis), and for inhibition of 5a-reductase (acne). The extract displayed significant activity in these test models, with a variable contribution of the individual ingredients. **References**: 1. Maisenbacher, P. (1991), Ph.D. Thesis, Tübingen. 2. Schempp, Ch. *et al.* (2003), Phytomedicine 10: 31 – 37.

P 277

Apoptosis inducing activity of an extract from saw palmetto (Serenoa repens) berries towards human cancer cells

Hostanska K[1], Suter A[2], Melzer J[1], Saller R[1]
[1]University Hospital Zürich, Dept. of Internal Medicine, Institute for complementary medicine; Rämistrasse 100, 8091 Zürich, Switzerland; [2]A. Vogel Bioforce AG, 9325 Roggwil, Switzerland

Phytotherapeutic formulations based on Saw palmetto (*Serenoa repens* (Bartr.) Small) berry extract (SRE) have tradionally been used for treating prostate-related problems. This study was aimed to evaluate the cytotoxicity and mode of cell death caused by commonly used ethanolic SRE (Prostasan®; DER 9 – 12:1; 96 % v/v ethanol) on some human cancer cells. We investigated the antiproliferative and apoptosis inducing activity of SRE on breast MCF-7 (ER^+) and MDA MB231 (ER^-), prostate LNCaP (AR+) and DU 145 (AR-), colon HT29, lung A549, renal Caki-1 and bladder J82 cells. The growth of all 8 human cancer cells after 48 h established by WST-1 assay was inhibited by SRE dose dependently with GI_{50} values between 107 and 327ug/mL. ER^+ MCF-7 and AR^+ LNCaP cells responded with highest sensitivity to SRE (GI_{50}: 107 and 127.7 ug/mL. The viability of cells was higher as 80 %.Vehicle treated cells (0.5 % v/v ethanol) were always included. This concentration did not affect the viability, proliferation or apoptosis of cells. The killing and growth inhibiton of 7 cell lines were partially apoptosis-related. Apoptosis induction was confirmed by Annexin V adherence using flow cytometry in all cell lines at GI_{50} which exerted low toxicity. The amount of apoptotic cells at their GI_{50} concentrations lay between 22.5 – 36.3 %. Apoptosis induction was comparable to genistein and quercetin (5×10^{-5}M) used as controls. SRE did not induce apoptosis in only A549 cells. Results of this study provide evidence that SRE exerted antiproliferative effect is triggered by induction of apoptosis. The results also suggest that patients taking SRE on longer term may profit also from a chemopreventive effect.

P 278

Effects of extracts from Valeriana officinalis L. in pharmacological studies

Hattesohl M[1], Feistel B[2], Sievers H[3], Lehnfeld R[3], Winterhoff H[1]
[1]Institute of Pharmacology and Toxicology, Domagkstraße 12, 48149 Muenster, Germany; [2]Finzelberg GmbH & Co. KG, Koblenzer Straße 48 – 56, 56626 Andernach, Germany; [3]PhytoLab GmbH & Co. KG, Dutendorfer Straße 5 – 7, 91487 Vestenbergsgreuth, Germany

Though several clinical studies revealed sleep improving properties of aqueous and ethanolic extracts from *Valeriana officinalis* L., neither the mode of action is known nor the active constituents are identified. A sedative effect, which predominantly is assumed to be responsible for efficacy, could not be demonstrated in most clinical studies or pharmacological investigations. The therapeutic indication given in the ESCOP monograph [1] "relief of temporary mild nervous tension and/or difficulty in falling asleep" may not only indicate a sedative but rather a tranquillizing effect of such extracts. Thus it was the aim of this study to test extracts and fractions from *Valeriana officinalis* L. for sedation (narcotica induced sleeping time, locomotor activity) and anxiolysis (elevated plus maze) following acute administration to female NMRI mice. Dosages up to 1000 mg/kg bw no sedation was observed, neither motility was reduced nor sleeping time increased by aqueous as well as by alcoholic extracts. On the contrary a methanolic, an ethanolic extract and a fraction derived from the latter extract clearly increased the test parameters of the elevated plus maze (percentage of time on the open arms, percentage of open arm entries) indicating an anxiolytic effect. These results suggest that the efficacy of extracts from *Valeriana officinalis* L. can be ascribed to their anxiolytic activity, an effect which is in accordance with the indication of the monograph. **Reference**: 1. ESCOP (2003) ESCOP Monographs – The Scientific Foundation for Herbal Medicinal Products, ESCOP, Exter.

P 279

Essential oils from Anethum graveolens, Levisticum officinale and Pimpinella anisum hairy root cultures: composition, antibacterial and antioxidant activities

Costa MM[1], Bounatirou S[2], Miguel MG[3], Faleiro ML[3], Figueiredo AC[1], Barroso JG[1], Pedro LG[1], Deans SG[4], Scheffer JJC[5]
[1]Universidade de Lisboa, Faculdade de Ciências de Lisboa, DBV, Centro de Biotecnologia Vegetal, C2, Campo Grande, 1749 – 016Lisbon, Portugal; [2]Faculté des Sciences de Tunis, Université Tunis el Manar, Campus Universitaire, 2092 Tunis, Tunisia; [3]Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Campus de Gambelas, 8005 – 139 Faro, Portugal; [4]Dept. of Pharmaceutical Sciences, University of Strathclyde, Glasgow G4 0NR, Scotland, UK; [5]LACDR, Leiden University, Gorlaeus Laboratories, PO Box 9502, 2300 RA Leiden, The Netherlands

Anethum graveolens L., *Levisticum officinale* D.W.J.Koch and *Pimpinella anisum* L. hairy root cultures were maintained at 24 °C and 80r.p.m. in five different culture media, under darkness or 16 h light photoperiod conditions. The essential oils isolated by distillation-extraction from these hairy root cultures were studied for their chemical composition, and their antibacterial and antioxidant activities. The oil composition was analysed by GC and GC-MS; the antibacterial activity was tested by the disc diffusion technique-against a *Salmonella* spp., *Bacillus cereus* (C1060), *Staphylococcus aureus* (ATCC6538) and *Listeria innocua* (CLF2/95). The antibiotic chloramphenicol was used as control. The antioxidant activity was determined by TBARS, scavenging capacity (DPPH) and reductive potential. Results were submitted to analysis of variance by ANOVA procedures (SPSS12.0 for Windows). Significant differences between means were determined by Tukey's Post Hoc tests; $p \leq 0.05$ was regarded as significant. Phenylpropanoids were the major components of the oil isolated from the *A. graveolens* hairy roots, while sesquiterpenes dominated the *P. anisum* hairy root oils. Depending on the light conditions and culture media tested, *L. officinale* hairy root oils were dominated by either monoterpenes, polyacetylenes or other components. *L.officinale* and *P.anisum* hairy root oils showed antibacterial activity against some of the tested strains, with inhibition zones smaller than that of the antibiotic. *A. graveolens* hairy root oils showed antioxidant capacity similar to BHT with the TBARS assay. Although lower than BHA, *P. anisum* hairy root oils showed best results with the reductive potential evaluation and DPPH test. *Acknowledgments*: This study was partially funded by FCT, under research contract POCTI/AGG/42961/2001

P 280

Effects of STW 5 (Iberogast®) on prostaglandin$F_{2\alpha}$ –induced contractions of ileum of mice in vitro

Hagelauer D[1], Kelber O[2], Weiser D[2], Heinle H[1]
[1]Institute of Physiology, University of Tuebingen, Gmelinstrasse 5, 72076 Tuebingen, Germany; [2]Steigerwald Arzneimittelwerk GmbH, Scientific Department, Havelstr. 5, 64295 Darmstadt, Germany

STW 5 (Iberogast®) is a phytotherapeutic combination of nine herbal extracts and used in the treatment of functional gastrointestinal diseases *i.e.* motility disturbances. Prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$), a pro-inflammatory mediator, has been shown to play an important role in modulating intestinal motility under physiological as well as under pathophysiological conditions [1]. The aim of this study is to determine the effects of STW 5 and its herbal components on the $PGF_{2\alpha}$-induced contractile activity in mouse ileum. Smooth muscle rings of the ileum are mounted in a perfused organ bath and longitudinal spontaneous peristaltic activity and tonus are recorded. First the effect of $PGF_{2\alpha}$ [10^{-6} M] on the ileum is measured thus obtaining the control response, then the application is repeated to the same sample pretreated with different herbal extracts and STW 5 (diluted 1:100), respectively. Each tissue specimen is used for only one extract. The preparations show a very stable spontaneous peristaltic contractility (mean amplitude 5.8 ± 2 mN; mean frequency 25 ± 5/minute). $PGF_{2\alpha}$ induces a transient tonic contraction with a low increase of frequency of the spontaneous peristaltic activity. The plant extracts influence $PGF_{2\alpha}$-induced contractility in different ways: peppermint, chamomile, angelica root and milk thistle inhibit the $PGF_{2\alpha}$-induced tone as well as the amplitude of spontaneous contractility, whereas liquorice root and melissa show a minor effect on these measurement parameter. The combination STW 5 inhibits mainly the $PGF_{2\alpha}$-induced tonic contraction. STW 5 (Iberogast®) and its components can inhibit the $PGF_{2\alpha}$-induced contraction. These findings suggest a spasmolytic activity of STW 5 in irritable bowel syndrome and inflammation induced increased motility. *Acknowledgement:* Supported by Alfred Teufel-Stiftung, Nagold, Germany **References**: 1. Frantzides, C.T. *et al.* (1992), Am. J. Physiol. 262(3 Pt 1): G488 – 97.

P 281

Hypothermic effects of hops could be antagonized with the competitive melatonin receptor antagonist luzindole

Grundmann O[1], Brattström A[2], Koetter U[2], Butterweck V[1]
[1]College of Pharmacy, Department of Pharmaceutics, University of Florida, Gainesville, POBox 100494, 32610, USA; [2]Zeller Medical AG, 8590 Romanshorn, Switzerland

Flowers of *Humulus lupulus* L. (Cannabinaceae), commonly known as hops, are traditionally used to relief insomnia, anxiety, excitability and restlessness associated with tension headache and gastrointestinal spasms. However, little information is available about the underlying sleep inducing mechanism of hops. It has been shown previously that a combination of valerian and hops interacts with serotoninergic 5-HT_6 and melatoninergic ML_1 receptors [1]. Melatonin is known to have both hypnotic and hypothermic effects at physiological levels. Indeed, the hypnotic effect may be mediated via the hypothermic action [2]. The above considerations and the traditional use of hops as a sleep inducer prompted us to evaluate the hypothermic activity of hops extract (HE) in mice. In a dosage of 250 mg/kg HE significantly decreased body temperature in male BL6/C57 J mice (ΔT -1.1 °C) 2 h after oral administration. The effects of the plant extract were comparable to melatonin (50 mg/kg; ΔT -0.8 °C; 2 h after *i.p.* injection). The hypothermic effects of both, melatonin and HE could be antagonized with the competitive melatonin receptor antagonist luzindole. Thus, these data suggest that the hypothermic effects of HE are mediated through activation of melatonin receptors. **References**: 1. Abourashed, E.A. *et al.* (2004), Phytomedicine 11: 633 – 638. 2. Zemlan, F.P. (2005), J. Clin. Psychiatry 66: 384 – 390.

P 282

Improvement of learning in rats by desoxypeganine

Jalali MS[1], Moormann J[2], Winterhoff H[1]
[1]Institute of Pharmacology and Toxicology, Domagkstr. 12, 48149 Muenster, Germany; [2]HF Arzneimittelforschung GmbH, St. Johannes 5, 59355 Werne, Germany

Desoxypeganine (DOP), an alkaloid from *Peganum harmala* L., inhibits acetyl- and buturylcholinesterase as well as monoaminooxidase A *in vitro*. This drug was tested for an improvement of learning in rats. Shuttle box apparatus (active avoidance) was used to assess cognitive performance. Unimpaired rats proved to learn rather quick and showed an enormous long-term memory. Such animals are not suited for such tests as an improvement of learning by a cholinesterase-inhibitor can be only shown in animals with a memory deficit or under impaired conditions for learning. Therefore test conditions were modified in order to affect learning efficiency: In contrast to previous studies animals were not habituated to the shuttle box and training was performed only on separate test days not continuously. DOP was investigated in three different shuttle box tasks: 1) with young male Wistar rats, 2) with young rats which were treated with ethanol *i.p.* 46 days before testing, 3) with old AA rats, since more than one year on 10 % alcohol in a two bottle free choice

paradigm. No improvement by DOP was seen in condition 1, only a slight effect in condition 2, whereas a pronounced and long lasting effect could be shown under condition 3. These animals showed a very bad learning under control conditions. Thus these results underline the importance of a learning deficit for detecting effects of cholinesterase inhibitors on learning.

P 283

Inhibitory effects of Willow bark extracts on proinflammatory processes in LPS activated human monocytes

Kelber O[2], Bonaterra GA[1], Kinscherf R[1], Weiser D[2], Metz J[1]
[1]Institute of Anatomy and Cell Biology III, University of Heidelberg, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany; [2]Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295, Darmstadt, Germany

Willow bark (*Salix daphnoides*, purpurea, and fragilis) is successfully applied in the treatment of painful and pro-inflammatory diseases. Aim of the present study was to study the effects of five fractions (A-E) of a standardized willow bark extract, differing in polyphenol and salicylate content, on the regulation of inflammatory processes in activated human monocytes. Monocytes from buffy coats of healthy subjects were isolated by Histopaque-density gradient centrifugation and adhesion. The monocytes were pre-incubated for 90 min with 5 – 30 μg/mL willow bark extract and their fractions A-E, 30 μg/mL diclofenac or aspirin. Thereafter they were incubated in serum-free RPMI 1640 medium with interferon-gamma (INF-γ; 50 U/mL; 45 min) and lipopolysaccharide (LPS; 1 μg/mL) for 5 to 48 hours. Apoptosis of monocytes (YO-PRO-1®-staining), intracellular NO-concentration (DAF-FM-diacetate), gene (real-time PCR) and protein (Cell Elisa) expressions of caspase-3, cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha, and nitrite concentration in the supernatant (Griess–reagens) were analyzed. The willow bark extract and all fractions (A-E) inhibited the pro-inflammatory increase of survival rate of monocytes by IFN-gamma/LPS significantly. The increased gene and protein expressions of COX-2 and tumor necrosis factor-alpha, and the inhibitory effects on nitrite relase and NO-concentration of LPS activated monocytes were significantly reduced by willow bark extract and to varying extents by its fractions. The anti-inflammatory effects of the plant extracts were compared to the NSAID diclofenac and to aspirin, which were used as reference. Fractions of a standardized willow bark extract differ in their inhibitory effects on inflammatory processes suggest a predominant role of the polyphenol content.

P 284

Flavonoid comparative analysis of GM/wt wheat

Ioset JR[1], Urbaniak B[2], Ndjoko K[1], Martin F[1], Gruissem W[2], Sautter C[2], Hostettmann K[1]
[1]Laboratoire de Pharmacognosie et Phytochimie, Ecole de Pharmacie Genève-Lausanne, Université de Genève; Quai Ansermet 30, 1211 Genève 4, Switzerland; [2]Institute of Plant Sciences, Swiss Federal Institute of Technology, Universitaetstrasse 2, CH-8092 Zürich, Switzerland

A rapid, non-sophisticated and reproducible analytical HPLC/UV method was developed to qualitatively and quantitatively compare the flavonoid content of wild and transgenic type Swiss spring wheat plants. Well characterized homozygotic, T4 generations of transgenic Frisal, Golin and Greina wheat varieties were obtained by transformation with antifungal genes of broad spectrum effect like chitinase and glucanase, ribosome-inactivating protein (RIP), as well as with a transgene of a specific effect – KP4 against smuts and bunts. HPLC/UV profiles of wheat GM varieties were compared with the respective wild type plant. This comparative profiling was coupled with computerized tools enabling a statistical processing of the data including cluster analyses. The structures of the major detected flavonoid derivatives were elucidated on-line including LC/DAD-UV analysis with post-column addition of UV shift reagents and LC/MS^n experiments. Different lines of transgenic wheat with increased antifungal resistance showed only small differences to their respective wild type, whereas differences between varieties were remarkable. These results indicated that the insertion of those specific resistance genes did not interfere with the biosynthetic pathways of flavonoids and provide a simple, robust and reliable methodology for the comparative profiling of flavonoid-containing plants. **Reference**: Clausen, M. *et al.* (2000), Nat. Biotechnol. 18: 446 – 449.

P 285

Effects of Saw palmetto extract in vitro on receptors and enzymes which are relevant in incontinence

Suter A, Bommer S
A. Vogel Bioforce AG, 9325-Roggwil, Switzerland

Preparations of saw palmetto (*Sabal serrulata* Roem. & Schult., *Serenoa repens* (Bartn) Small.) are widely used in the treatment of benign prostate hyperplasia BPH. Clinical trials have shown that the efficacy of saw palmetto is superior to placebo and as good as 5-alpha-reductase-inhibitors and alpha-blockers. So far several mechanism of action has been postulated which lead to the decrease of symptoms of BPH. This includes inhibition of 5-alpha-reductase, cyclooxygenase, lipoxygenase and $alpha_1$-receptors. One of the main symptoms connected to BPH is incontinence. At the time only scarce data is available how saw palmetto extract may affect receptors and enzymes which are known to play an important role in incontinence. Thus we investigated with radioligand binding the influence of an ethanolic saw palmetto extract (Prostasan®; DER 9 – 12:1; 96 % V/V ethanol) on the alpha-1-adrenoreceptor, the muscarnic receptors M1, M2 and M3, the nicotinic acetylcholine receptor, and the serotonin and norepinephrine transporter. The extract showed only a marginal binding affinity to the alpha-1-adrenoreceptor and to the muscarinic receptors M1 and M2 and almost no inhibition of the nicotinic acetylcholine receptor. Interestingly, the extract seemed to inhibit at pharmacologically relevant concentrations the muscarinic receptor M3 and had a high binding affinity to the serotonin transporter. Further experiments are warranted to prove these findings which may contribute to a broader understanding of the mechanism of action of saw palmetto in the treatment of BPH.

P 286

Natural COX-2 inhibitors and effects on colon cancer cells

Pettersson J[1], Huss U[1], Karlsson PC[2], Choi YH[3], Verpoorte R[3], Rafter JJ[2], Bohlin L[1]
[1]Division of Pharmacognosy, Department of Medicinal Chemistry, Uppsala University, BMC Box 574, SE-751 23 Uppsala, Sweden; [2]Division of Medical Nutrition, Department of Biosciences and nutrition, Karolinska Institute, Novum, SE-141 57 Huddinge, Sweden; [3]Division of Pharmacognosy, Section metabolomics, Institute of Biology, Leiden University, 2300 RA Leiden, The Netherlands

The cyclooxygenase-2 enzyme (COX-2) is involved in prostaglandin biosynthesis and thus plays a significant role in the process of inflammation. Different types of natural compounds can affect the COX-2 enzymatic activity [1]. In recent years it has also been suggested that COX-2 is involved in cancer development [2]. As many natural compounds are present in the human diet this study has focused on discovery of natural COX-2 inhibitors present in food. A number of human fecal water samples (the aqueous phase of feces) from vegetarians were collected and assessed for effects on COX-2 enzymatic activity and also for effects on protein expression level in human colon cancer cells (HT-29). Of 14 samples analyzed, 13 decreased COX-2 protein levels in the cells (19 – 63 % inhibition) [3]. Most samples also significantly decreased prostaglandin (PGE2) production in HT-29 cells [3]. Solid phase extraction was performed to trace the activity of the fecal water samples. The resulting water

fraction was found to be responsible for the inhibitory activity of the samples, suggesting that the active compounds are most likely polar in nature. The chemical content of fecal water was analyzed using chromatographic techniques. Gas chromatography-mass spectrometry was used to study the content of the fecal water, with an emphasis on finding phenolic compounds, whereas nuclear magnetic resonance (NMR) was used to obtain an overview of the total fecal water content. The NMR metabolite profiles of vegetarian fecal water samples were very similar, suggesting that the colonic content of the individuals in the study population were similar in composition. A variety of compounds were identified including several phenolic compounds, amino acids and fatty acids. To the best of our knowledge, the use of NMR as a tool in studying colonic contents represents a new approach that would potentially be very useful in colon cancer research. **References**: 1. Perera, P. *et al.* (2000), Bioactive compounds from natural sources, Tringali, C., Ed., Taylor & Francis: London, pp. 433 – 472. 2. Soslow, R.A. *et al.* (2000), Cancer 89: 2637 – 2645. 3. Karlsson, P.C. *et al.* (2005), J. Nutr. 135: 2343 – 2349.

P 287

The drug-extract-ratio of aqueous/ethanolic Harpagophyti radix extracts has to be revised

Spriano D[1], Krasniqi B[2], Strompen T[2], Tobler M[2], Meier B[1]
[1]University of Applied Sciences, Grüental, CH-8820 Wädenswil, Switzerland; [2]Bioforce AG, CH- Roggwil, Switzerland

The ratio of the herbal substance to the herbal preparation (drug-extract ratio = DER) is one of the most important criteria to approve the therapeutic equivalence of different herbal preparations. The approval itself is very important in simplified registration procedures. The herbal guideline of SWISSMEDIC [1] is an example thereof: DER is placed on second position of totally nine criteria. Dingermann [2] called for a DER-declaration in its natural range. Actually, there is a discrepancy between the published DER and the DER in its natural range of ethanolic Devil's Claw [*Harpagophytum progumbens* (Burch.) DC ex Meissn.] root extracts. A DER of 4.4 – 5:1 is declared for most of the products in Germany. This is not the DER of the native extract in its natural range. A study with ethanol/water mixtures of 0 to 95 % of ethanol showed a low DER for all mixtures up to 70 %. The results are reproducible. DER has therefore to be reduced to 1.5 – 2.5:1 as it is correctly done for water extracts. A native ethanolic (60 %) extract has been analytically compared with a commercially available extract of a declared DER 4.4 – 5:1. No phytochemical differences could be detected: Iridoid glycosides, saccharides, phenylethyl-derivatives [3, 4] and plant acids have been analysed by HPLC quantitatively; TLC-fingerprint has been adapted additionally to the lipophilic fraction. A percolation of the cutted, dried root showed no differences in the chemical composition of the three main fractions. In all the extracts and fractions, a high amount of stachyose (ca 45 %) has been detected. We conclude that the declared DER of most of the ethanolic Devil's Claw products is not in accordance with the natural range. A special extraction procedure can be excluded due to phytochemical equivalence. A revision is necessary. As a consequence, the ESCOP [5] (and other) recommendations for the dosage of Devil's Claw roots have to be corrected from 2 – 5 g of the drug per day down to 1.5 – 3 g/day for painful osteoarthritis or to 1000 mg/day for extracts. **References**: 1. Swissmedic (2004), Anleitung zum Einreichen von Zulassungsgutachten für pflanzliche Arzneimittel der Humanmedizin (Phyto-Anleitung), Paragraph IV A1). 2. Dingermann, T. (2000), Transparenzkriterien für pflanzliche, homöopathische und anthroposophische Arzneimittel. Karger-Verlag, Freiburg und Basel. ISBN 3 – 8055 – 7045 – 7. 3. Boje, K. *et al.* (2003), Planta Med. 69: 820 – 825. 4. Munkombwe, N.M. (2003), Phytochem. 62: 1231 – 1234. 5. The Scientific Foundation for Herbal Medicinal Products (2003), ESCOP-Monographs, Second Edition, Harpagophyti radix, Thieme, Stuttgart, New York, pp. 233 – 240.

P 288

Effects of various flavonoids on xanthine oxidase activities in vitro and on plasma uric acid levels in oxonate-induced rats

Sarawek S[1], Butterweck V[1]
[1]College of Pharmacy, Department of Pharmaceutics, University of Florida, Gainesville, POBox 100494, 32610, USA

Xanthine oxidase (XO) is the key enzyme that catalyzes the oxidation of xanthine and hypoxanthine into uric acid, and plays an important role in producing hyperuricemia and gout [1]. It has been shown in the literature that flavonoids showed potent XO inhibitory activities *in vitro* [2]. However, less information about the *in vivo* hypouricemic activities of flavonoids are available. In the present study it was therefore of interest to investigate if the *in vitro* XO inhibitory activities of various flavonoids can be correlated with hypouricemic effects *in vivo*. Eight flavonoids including flavones, flavonols, and flavanones were used to inhibit the XO activity *in vitro*. The aglycones luteolin, apigenin, kaempferol and quercetin were shown to have potent xanthine oxidase inhibitory activities with IC_{50} values less than 5 μM. Glycosides such as luteolin-7-*O*-glucoside and rutin showed weaker activities with IC_{50} value greater than 20 μM. Eriodictyol and naringenin showed low activities with IC_{50} value greater than 50 μM. All compounds were administered orally to oxonate-induced hyperuricemic rats at doses of 50 and 100 mg/kg. None of the tested flavonoids elicited any hypouricemia *in vivo* after oral administration. This lack of effect might be due to the low intestinal absorption of the flavonoids or the first pass effect through the liver. **References**: 1. Donald voet JGV. (2004), Biochemistry. 3 ed., Von Hoffmann Corporation. p 1096. 2. Nguyen, M.T.T. *et al.* (2006), Planta Med. 72: 46 – 51.

P 289

Anti diarrheal activity of root extracts of Elephantopus scaber L

Vrushabendra Swamy BM[1], Jayaveera KN[2], Kumar GS[1], Ashok Kumar CK[3], Sreedhar C[1]
[1]Rural college of Pharmacy, P.B No 10, D.S Road, Devanahalli 562110, Bangalore, Karnataka, India; [2]JNTU, 515 001, Anatapur, Andhra Pradesh, India; [3]Sri Lakshmi Narasimha college of Pharmacy, sf.316, Chittor, 517132, Andhra Pradesh, India

Elephantopus scaber L. (Asteraceae) is a small herb, which grows in the wild throughout the hotter parts of India. The plant has been used in the Indian System of Medicine as an analgesic, diuretic, astringent, antidiarrhea and anti-emetic [1]. In the present study, we evaluated the antidiarrheal activity of roots of *E.scaber* against several experimental models of diarrhea in rats. The antidiarrheal activity of ethanol and aqueous extracts of *E. scaber* was evaluated using castor oil-induced diarrheal model in rats [2]. Further, we evaluated the effect of ethanol and aqueous extracts on gastrointestinal tract motility after charcoal meal administration [2], and PGE_2 induced intestinal fluid accumulation (enteropooling) [3]. The plant extracts showed significant ($p < 0.05$) inhibitory activity against castor oil induced diarrhea (Table 1) and PGE_2 induced enteropooling in rats when tested at 200 mg/kg (Table 2). Both extracts also showed significant ($p < 0.001$) reduction in gastrointestinal motility in charcoal meal test in rats (Table 3). The results point out the possible antidiarrheal effect of the plant extracts and substantiate the use of this herbal remedy as a non – specific treatment for diarrhea in folk medicine. *Tables edited.* *Acknowledgements:* All authors are grateful to our honorable secretary Mr. C. Basavaraj. Rural college of Pharmacy, Devanahalli, Bangalore, Karnataka, India for financial assistance. **References**: 1. Avani, K., Neeta, S. (2005), Ind. J. Pharmacol. 37:126 – 127. 2. Venkatesan, N. *et al.* (2005), J. Pharm. Pharmaceut. Sci. 8: 39 – 45. 2. Gunakkurna, A. *et al.* (2005), J. Ethnopharmacol. 98: 241 – 244.

P 290

Echinacea and its alkamides – an assessment of potential CYP-P450 enzyme inhibition?

Modarai M[1], Gertsch J[2], Suter A[3], Kortenkamp A[1], Heinrich M[1]
[1]The School of Pharmacy University of London29/39 Brunswick Square London WC1N 1AX United Kingdom; [2]Department of Chemistry and Applied Biosciences, Office HCI H494.4, Wolfgang-Pauli-Str. 10, ETH Hönggerberg CH-8093 Zürich Switzerland; [3]Bioforce AG, 9325 Roggwil, Switzerland

Echinacea is a top selling HMP, yet there is still limited and unclear information regarding possible interactions between Echinacea and other concurrent medicines. The objective was to analyse the inhibitory potential of the standardised Echinacea extract (Echinaforce®) and two alkamides: dodeca 2*E*,4*E*,8*Z*,10*E*/*Z* tetraenoic acid isobutylamide (TAI) and dodeca 2*E*,4*E*-dienoic acid isobutylamide (DAI) on single baculovirus expressed Cytochrome P450 isoforms – CYP1A2, CYP2C19, CYP2D6 and CYP3A4 as stipulated by the German regulatory authority BfArM (Bundesinstitut fur Arzneimittel und Medizinprodukte) [1]. In a modified fluorometric 96-well plate assay enzyme activity was measured by detecting the fluorescent metabolite produced from the reaction of the substrate with the CYPs [2]. The substrates used were 7-BFC (CYP3A4), CEC (CYP1A2, CYP2C19) and AMMC (CYP2D6). Control reactions were also set up to account for intrinsic fluorescence of the extract and the effect of ethanol on the enzyme. The extract and its alkamides showed moderate inhibitory activity against CYP enzymes, but these effects are unlikely at the doses of Echinaforce® normally encountered in clinical setting (Table). The lowest IC50 value recorded in our study was 1.96 µg/mL for TAI. Based upon a recent bioavailability study, these values would be 4900 folds higher than the anticipated maximal concentration in hepatocytes, assuming that there are no potential losses of the alkamide via distribution, uptake etc [3]. With these IC_{50} values it is unlikely that inhibitory concentrations will be reached within the liver.

Table: Median inhibitory concentrations (IC_{50}) and the upper and lower 95 % confidence limits (depicted in brackets) for Echinaforce® and alkamides against CYP isoforms.

	CYP1A2	CYP2C19	CYP2D6	CYP3A4
Echinaforce®(µg/mL)	26.54 (23.81 – 29.57)	53.47 (32.30 – 88.79)	60.97 (50.29 – 73.91)	19.49 (18.80 – 20.20)
DAI (µg/mL)	No inhibition	23.35 (17.37 – 31.40)	10.10 (7.209 – 14.151)	5.17 (4.11 – 6.52)
TAI (µg/mL)	No inhibition	18.91 (14.86 – 24.05)	6.76 (5.21 – 8.77)	1.91 (1.74 – 2.09)

Acknowledgements: Bioforce UK for funding this project. **References**: 1. http://www.bfarm.de/cln_042/nn_424630/DE/Arzneimittel/besTherap/amPflanz/ ampflanz-node.html. 2. Crespi, C.L. *et al.* (1997) Analytical Biochemistry 248: 188 – 190. 3. Bauer, R. *et al.* (2005) Presentation, GA conference, Florence.

P 291

Antinociceptive effect of the essential oil of Lippia sidoides on mice

Marçal RM[1], Ptak DM[1], Krempser RR[1], Krempser MR[1], Cardoso GC[2], Santos RB[3], Blank AF[3], Alves PB[2]
[1]Physiology Department, [2]Chemistry Department; [3]Agronomy Engeneering Department, Federal University of Sergipe, Av. Marechal Rondon, s/n; CEP 49.100 – 000, São Cristóvão, SE, Brazil

Lippia sidoides Cham. (Verbenaceae), an aromatic medicinal shrub, is used in Brazilian folk medicine to combat bacterial infections, inflammation and pain [1]. The aim of this study was to investigate the possible analgesic effect of *Lippia sidoides* essential oil on two pain models in mice, namely acetic acid-induced writhing [2] and hot-plate [3] tests. Leaves of *L. sidoides* were collected in São Cristóvão county (10¤56'S, 37¤05'W), Brazil. Essential oil was obtained by steam distillation in a Clevenger-type apparatus (4.1 % yield) and analyzed by gas chromatography/mass spectroscopy. p-Cymene (26.8 %), thymol (21.9 %) e myrcene (12.8 %) are identified as the major constituents. A dose-related antinociceptive effect was obtained in the acetic acid-induced writhing test at doses of 100, 200, and 400 mg/kg (s.c.; $p > 0.5$; $p < 0.05$; $p < 0.01$, respectively). In the hot-plate test, the essential oil (25 – 200 mg/kg; s.c.) significantly increased the latency at doses of 100 ($p < 0.05$) and 200 mg/kg ($p < 0.01$). The essential oil-induced antinociception in hot-plate test (200 mg/kg; s.c.) was antagonized by naloxone (3 mg/kg; ***i.p.***). In conclusion, the essential oil of *Lippia sidoides* showed antinociceptive effect in chemical and thermal models of nociception in mice. The activation of opioidergic system appears to play a crucial role in the observed antinociceptive effect. *Acknowledgments*: CNPq. **References**: 1. Girão, V.C.C. *et al.* (2003), Preventive Veterinary Medicine 59: 95. 2. Koster, R. *et al.* (1959), Fed. Proc. 18: 412. 3. Ankier, S.I. (1974), Eur. J. Pharmacol. 27: 1.

P 292

Possible involvement of muscarinic mechanisms in contractile response of guinea pig ileum by Erythrina velutina

Marçal RM[1], Carvalho ACS[1], Almeida DS[1], Mello ICM[1], Antoniolli AR[1]
[1]Physiology Department, Federal University of Sergipe, Av. Marechal Rondon, s/n; CEP 49.100 – 000, SE, Brazil

The plant *Erythrina velutina* Willd (Fabaceae) is popularly used to combat pain and anxiety in Brazil [1]. We have recently reported that *E. velutina* (AEEV) produces an opioid-like antinociceptive effect in mice [2]. In the present study, we investigated the mechanism of action of AEEV in the guinea pig ileum. *E. velutina* leaves, collected in Brazil (10¤56'S, 37¤05'W), were infused and lyophilized (8.32 %). Terminal segments of guinea pig ileum ($n = 5 – 8$) were mounted in an organ bath and isotonic contractions were recorded. AEEV (0.05 – 2.5 mg/mL) contracted the guinea pig ileum (0.86 ± 0.31 g – 1.45 ± 0.16 g) and increased neurogenic contractions (0.1 Hz; 0.5 ms; 40 V) by a maximum of 57.7 ± 9.1 % ($p < 0.01$). Tetrodotoxine (1 µM; $p < 0.01$; 38.2 ± 2.5 %), a neuronal sodium channel blocker, and the muscarinic receptor antagonist atropine (10 µM; $p < 0.01$; 38.7 ± 13.2 %) reduced contractile response induced by aqueous extract (1.5 mg/mL). Verapamil (10 nM; 52.4 ± 9.8 %; $p < 0.01$), an L-type Ca^{+2} channel blocker, or low Ca^{+2} concentration (76.0 ± 6.1 %; $p < 0.05$) also reduced the contractile response to AEEV. Atropine (10 µM) along with verapamil (10 nM) abolished AEEV-induced contractile response ($p < 0.001$; 98.1 ± 1.4 %). In conclusion, an opioid-like response could not be detected in the guinea pig ileum. Indeed, these results suggest that the contractile response of the aqueous extract of *Erythrina velutina* involves a neurotransmitter release, possibly acetylcholine, muscarinic receptor activation, augmentation of Ca^{+2} entry through L-type calcium channels and calcium release from the intracellular stores. *Acknowledgments*: CNPq. **References**: 1. Dantas, M.C. *et al.* (2004), J. Ethnopharmacol. 94: 12. 2. Marchioro, M. *et al.* (2005), Fitoterapia 76: 637.

P 293

Growth Inhibitor of insect larvae from Paulownia tomentosa

Kunugi A, Yun YS, Kobayashi A
School of Life Science, Tokyo University of Pharmacy and Life Sciences, 1432 – 1 Horinouchi, Hachioji, 192 – 0392, Tokyo, Japan

Paulownia (*Paulownia tomentosa* (Thumb.) Sieb. & Zucc. ex Steud.) has been used as a traditional medicine for stomach disease in China. In Japan, it is called 'Kiri', which is often used as the material of chests of drawers. When paulownia is used industrially, a lot of sawdust is generated. From sawdust of paulownia, we isolated the paulownin as a major compound and some paulownin derivatives, and examined the growth inhibition of insect larvae with MeOH extract and paulownin. First, one with MeOH extract treatment (2.5, 5.0, and 10.0 mg/mL), one with paulownin treatment (2.5, 5.0, and 10.0 mg/mL), and a control group were prepared. We measured the instars of larvae every week for 3 weeks after MeOH extract and paulownin were added in nutrient medium, and the head width of each larva was measured after three weeks. The results showed

instars of larvae were delayed and head diameters of larvae were smaller for MeOH extract and paulownin treatment groups in comparison with control. In conclusion, we proved that sawdust of paulownia has a protecting effect against insects.

P 294

Monitoring of distributed Schizandra chinensis (Turcz.) Baill in Korea

Jun KH, Yeong LA, Won LH, Byungkil C, JinMi C, JiHyun C, Seol J, Kyoung KH
Quality Control of Herbal Medicine Department, Korea Institute of Oriental Medicine, 461 – 24, JeonminDong, YuseongGu, 305 – 811, Daejeon, Korea

This study was investigated that quality inspection of distributed *Schizandra chinensis* (Turcz.) Baill in Korea. To evaluate the quality of these herbal medicines, we carried out TLC pattern analysis, foreign matter in purity, loss on drying, ash, acid-insoluble ash, oil content, dilute ethanol-soluble, water-soluble, ether-soluble extracts and HPLC analysis. As a result, TLC pattern analysis of gomisin A and shizandrin was showed R_f value 0.64 and 0.74, respectively. Foreign matter in purity, loss on drying, ash and acid-insoluble ash were measured by average 1.30 % (± 1.08), 12.59 % (± 1.65), 4.08 % (± 0.67) and 0.53 % (± 0.15), respectively. Average oil content, dilute ethanol-soluble extract, water-soluble extract and ether-soluble extract were observed by 0.70(± 0.07)%, 38.53(± 5.92)%, 39.72(± 4.91)% and 12.00(± 1.65)%, respectively. To measured contents of schizandrin and gomisin A, we were quantitatively analyzed using HPLC. The average contents of schizandrin and gomisin A were detected by 0.60 % (± 0.02) and 0.12 % (± 0.004), respectively. As a result of this study, we could suggest quality standard of each item of *Schizandra chinensis* (Turcz.) Baill.

P 295

Separation and quantitative analysis of anthraquinones in Morinda officinalis How. by HPLC

Kyoung KH, Byungkil C, Mi CJ, Yeong LA, Won LH
Quality Control of Herbal Medicine Department, Korea Institute of Oriental Medicine, 461 – 24, JeonminDong, YuseongGu, 305 – 811, Daejeon, Korea

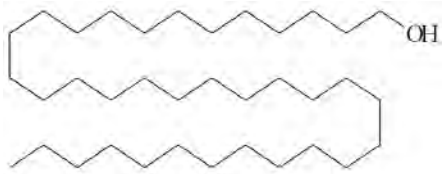
Morinda officinalis How.(Rubiaceae) is used in folk medicine as a tonic, warming, sex impulse and anti-inflammatory agent in Asia. From *n*-hexane and ethylacetate extracts of the root of *Morinda officinalis* How., we have isolated two known anthraquinones compounds and their structures were identified rubiadin(I) and rubiadin-1-methyl ether(II) by NMR. Our object was to determine the rubiadin-1-methyl ether content in root of *M. officinalis by* HPLC-PDA. Chromatography was performed using a reversed-phase system with Luna C_{18} column, flow rate 1.0 mL/min, UV 280nm and acetonitrile-water (50:50, v/v), as the mobile phase. The rubiadin-1-methyl ether content of *M. officinalis* from four different districts in Korea were determined to be 0.013 %.

P 296

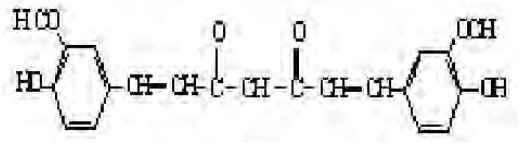
Preharvest Combined Application of Triacontanol and Kinetin Could Ameliorate the Growth, Yield and Curcumin Content of Turmeric (Curcuma longa L.)

Masroor M, Khan A, Singh M, Naeem M, Nasir S
Plant Physiology Laboratory, Botany Department, Aligarh Muslim University, Aligarh-202002. India

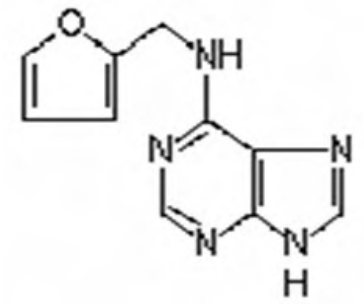
A simple randomized pot experiment was conducted at the Department of Botany, A.M.U., Aligarh during the year 2004 – 2005 to study the influence of foliar application of two potent plant growth regulators (PGRs), namely, Triacontanol (TRIA) and Kinetin (KN) on turmeric (*Curcuma longa* L.). The five treatments applied four times at fortnightly intervals comprised Control (T0), 10^{-6} M TRIA + 1.0×10^{-6} M KN (T1), 10^{-6} M TRIA + 5.0×10^{-6} M KN (T2), 10^{-6} M TRIA + 1.0×10^{-5} M KN (T3) and 10^{-6} M TRIA + 5.0×10^{-5} M KN (T4). Various selected attributes viz. plant height, fresh mass, number of leaves, number of tillers per plant, total carbohydrate, protein, nitrogen, phosphorus, potassium and curcumin contents in the rhizome and total chlorophyll content in leaf were recorded. T3 was found to be most effective for most parameters. Correlation analysis revealed that rhizome yield was contributed by increased plant height (R = 0.985), fresh weight of shoot (R = 0.997), number of leaves (R = 0.983), leaf-N content (R = 0.999) and total chlorophyll content (R = 0.997). Curcumin yield was significantly contributepd due to leaf-N content (R = 0.999) and total chlorophyll content (R = 0.992). T3 enhanced the rhizome yield by 25.7 % and curcumin by 39.9 % over their respective controls. Thus, the combined application of the two PGRs in the form of foliar spray may be successfully adopted for improved productivity and quality of turmeric.



Triacontanol



Curcumin



Kinetin

P 297

Antiasthmatic potential of aqueous extract of Cassia occidentalis

Vadnere G[1], Somani R[2], Singhai AK[3]
[1]Smt.S. S.Patil College of Pharmacy, Chopda-425 127 (MS), India; [2]Sinhgad College of Pharmacy, Pune-411 041(MS), India; [3]Dept. of Pharm. Sci, Dr. HS Gour University, Sagar-470 003 (MP), India

Asthma is a chronic inflammatory disease of the airways characterized by the fibrosis of the airways, hyperplasia and hypertrophy of smooth muscle cells and mucus secretary cells due to infiltration of activated eosinophils and activation of mast cells and lymphocytes. Various traditional medicinal plants have been used in folk medicine to treat a wide range of physical ailments such as asthma and bronchitis. *Cassia occidentalis* L. (Caesalpiniaceae) leaf juice is traditionally used for the treatment of the respiratory tract ailments and is well-known drug in Ayurvedic and Unani system of medicine. This plant is abundant in the region of North Maharashtra commonly known as *Kasmardan*. Since no scientific studies have been carried out on the leaf the present study evaluates the antiasthmatic activity an aqueous extract of *C. occidentalis* (COAE) on *in vitro* and *in vivo* animal models. *In vitro* studies carried out on histamine-induced contraction in isolated goat tracheal chain and *in vivo* studies on milk- induced eosinophilia, mast cell degranulation and ca-

pillary permeability in mice (n = 5). The results showed that aqueous extract of *C. occidentalis* inhibited the contractile effect of histamine ($P < 0.05$). A dose dependent contraction of goat tracheal chain is observed. Treatment with COAE (200 mg/ kg, *i.p.*) decreased eosinophilia by 71 % while mast cells were protected 66 % from degranulation as compared to control group. Also, COAE decreased capillary permeability by 69 % in mice was evident from its effect on optical density of the dye. Thus, COAE showed antihistaminic, mast cell stabilizing and decreasing capillary permeability effect and hence possesses potential role in the treatment of asthma.

P 298

Ruscus aculeatus Trade in Turkey: Is It Sustainable?

Coşkun M, Güvenç A, Kılıç CS, Arıhan O
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100 Tandoan, ANKARA, TURKEY

Due to Turkey's rich floral diversity, plants in Turkey have been subjected to studies for many years. In addition, it has been known that trade in medicinal plants mainly exist by harvesting directly from nature. If this is done in great quantities, then it will surely disturb the balance of the nature. *Ruscus* species belong to the family Liliaceae. They are rhizomatous evergreen shrubs or perennial herbs, usually dioecious. They have small scale-like stem leaves; leaf-like cladodes, unisexual flowers opening one at a time in the axil of a membranous or leaf-like scale on the adaxial (upper) or abaxial (lower) surface of cladodes. Fruit is a large berry with 1 – 4 seeds. Four species and 2 varieties, a total of 5 taxa grow naturally in Turkey (*R. aculeatus* L. var. *aculeatus*, *R. aculeatus* L. var. *angustifolius* Boiss., *R. hypoglossum* L. *R. colchicus* P. F. Yeo and *R. hypophyllum* L.)[1]. Underground parts of *R. aculeatus* are used in the treatment of diseases due to its ruscogenin content which is used as a starting compound in hormone synthesis. Roots *of R. aculeatus* have been exported for the last 3 decades. Although quantities vary from year to year, an annual of 900 tones of dried and cleaned roots are exported. This equals 4500 tones of fresh roots. Collection of such high quantities will effect the natural population negatively. In Terme, *Ruscus* population is destroyed due to extensive collection between 1982 and 1990. A similar case is observed in Adapazarı, Karasu town. Since harvesting was done via tractors, its population in sandy areas is destroyed and can not be regenerated. In other areas such as Balıkesir, Çanakkale, Bursa, Aydın, Osmaniye, Hatay, Hendek, Düzce and Bafra, harvesting was done by collecting by hand with pick axes and hoes so a decrease in the population was observed but it was not so severe and a partial regeneration has occurred. Currently, no big destruction in these areas is observed. **Reference**: Davis, P.H. (1984), Flora of Turkey and East Aegean Islands, Volume 8, Edinburgh University Press, Edinburgh, UK.

P 299

Destenotil – a combination of Troxerutin and Aescin to treat inner ear perfusion disturbances

Siegers CP[1], Tegtmeier M[2]
[1]Institute of Experimental and Clinical Pharmacology and Toxicology, University of Luebeck, Ratzeburger Allee 160,D-23538 Luebeck; [2]Schaper & Bruemmer GmbH&Co.KG, Bahnhofstr. 35, D-38259 Salzgitter, Germany

Destenotil is a fixed combination of troxerutin (450 mg) and aescin (25 mg) per capsule. Indications for this combination are inner ear perfusion problems of different aetiology. The efficacy of Destenotil (n = 34) versus pentoxyfyllin (n = 34 patients) was tested in a randomized clinical study as group comparison; end point was the improvement of hearing after 40 – 44 days of treatment. Hearing was measured by threshold audiometry; a difference of > 10dB was judged as a significant improvement. **Results:** After Destenotil treatment hearing was significantly improved, in 23 of 34 patients the threshold was changed more than 10dB (sign-test, $p < 0.05$). With pentoxyfyllin hearing was also improved, albeit to a lesser degree. Both drugs were well tolerated, major adverse drug effects were not observed with either treatment.

P 300

Unusual chemical transformations of natural flavonoids

Zenkevich IG[1], Eschenko AY[2], Makarova SV[3], Makarov VG[2]
[1]St. Petersburg State University, Chemical Research Institute, Universitetsky pr, 26, St. Petersburg 109504, Russia; [2]Interregional Center "Adaptation", Piskarevsky pr, 47/5, St. Petersburg 333333, Russia; [3]St. Petersburg Chemical Pharmaceutical Academy, Prof. Popov str. 14, St. Petersburg 444444, Russia

Any new data on chemical properties of natural flavonoids seem to be interesting in many aspects. They are, for instance, i. activity of carbonyl group in flavanonole molecules (like taxifolin) in reactions of nucleophylic substitution, ii. the tautomerization of flavanonoles that leads to endiol forms, and iii. the sensitivity of flavonoles (like quercetin) to nucleophylic attack on C2-atom of ring B, including bases catalyzed hydrolysis. First group of reactions (i) includes the formation of MO-TMS derivatives prior their GC analysis that was recommended for some dihydroflavones (*e.g.*, naringenin [1]), that means the possibility of similar reactions with other NH_2-compounds. The tautomerization (ii) was proposed first for the explanation of formation of alphitonin from taxifolin at hydrothermolysis [2]. However, no direct evidences of endiol existence are known up to present, namely its fixing in the form of TMS-derivatives like it is known for isoflavones [3]. The irreversible instability of quercetin in basic media is confirmed by the results of its stepwise UV-spectrophotometric titration. The registration of UV spectra of this compounds in the pH range 4→10 indicates the natural changes; a part of them can be explained by ionization ArOH ArO^- [pKa ≈ 7.1 (7-OH)]. Surprisingly, the following restoration of initial pH-value 4 gives not H-form of quercetin, but another compound (λ_{max} 294 nm, no absorbance at 350 – 400 nm). Possible explanations of this phenomenon are discussed. *Acknowledgements:* DIOD Co., Moscow, Russia. **References**: 1. http://www.upsc.se/RTI.pdf. 2. Ohmura, W.O. *et al.* (2002), Holzforshung 56:493 – 497. 3. Joannou, G.E. (2000), Tetrahedron Lett. 41: 7925 – 7928.

P 301

Improvement of Culture of Black caraway (Nigella sativa L.) in Kermanshah (Iran)

Abdolhamid P[1], Sohbat B[2]
[1]Azad Islamic University, 6,Kermanshah, Iran;and Razi university,67155,Kermanshah, Iran; [2]Razi University,67155 Kermanshah, Iran

Black caraway (*Nigella sativa)* from the *Ranunculaceae* family, is one of the most useful medicinal plants which grows wild in some regions of Iran, such as Kermanshah. In India and Middle East countries, the seeds of Black caraway, are used as spice and seasoning, and also used as an additive in bread. *N. sativa* contains l*inoleic, oleic,* and *palmetic acid* [1]. Because of substances such as thymoquinone and di-thymoquinone in their seeds [2], it is also used as an anticancer, antidiabetic, antimicrobial, and antialergic agent. Regarding the importance of this medicinal plant, a research study was conducted in determining to phenological stages, and to find the best density culture, in the Agricultural College of Kermanshah (Iran) at Razi University, in 2000. In this study, four row spacing *i.e.* 40, 50, 60 and 70 cm were studied by using randomized complete block design (RCBD) with four replications. Various characteristics including height of plant, the number of follicles per plant, the number of seeds in each follicle, weight of one thousand seeds, biological yield, grain yield, harvest index, percentage of oil, and essential oil were used. According to experiment results, the comparison of mean grain yield, by using Duncan test, revealed that when the seeds were planted in 40 centimeters spaced rows, the grain yield was highest, and equal to 660 kg per hectare. Having compared the means with this treatment and other treatments showed a significant difference between the grain yields. The oil

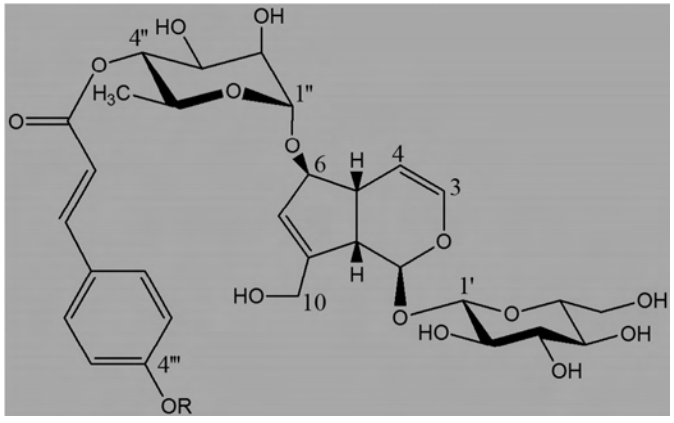
and essence of produced seeds in this study were 28 % and 0.148 % respectively. **References**: 1. Atta, M.B. (2003), Food Chemistry 83: 63 – 68. 2. Ghosheh, A.O., Houdi, A.A., Crooks, A.P. (1999), J. Pharm. Biomed. Anal. 19: 757 – 762.

P 302

Acylated iridoid glycosides from the flowers of Verbascum lasianthum Boiss. ex Bentham

Tatli II[1], Khan IA[2], Akdemir ZS[3]
[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University, Sihhiye, 06100, Ankara, Turkey; [2]National Center For Natural Products Research Institute of Pharmaceutical Sciences, University of Mississippi, University, 38677, Mississippi, USA; [3]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Sihhiye, 06100, Ankara, Turkey

Verbascum, commonly known as "Mullein", is represented by 228 species in the flora of Turkey [1]. During our field expeditions on Turkish folk medicine, we have recorded that the flowers of *Verbascum lasianthum* Boiss. ex Bentham are used for hemorrhoids in southwest Anatolia [2]. *Verbascum* species have been known to be rich in iridoid glycosides. *Verbascum* is well known for its variety of iridoids being of value for taxonomic evaluation of this genus. In previous studies, we described the isolation of nine iridoid glycosides and two phenylethanoid glycosides from the roots of *V. lasianthum* [3, 4]. In a continuation of the studies on *Verbascum lasianthum*, chromatographic studies (VLC, HPLC and CC) on the water soluble parts of the methanolic extract resulted in the isolation two new iridoid glycosides, 6-*O*-(4'''-*O*-*trans*-*p*-coumaroyl)-α-L-rhamnopyranosylaucubin (**1**), 6-*O*-(4'''-*O*-*trans*-*p*-methoxycinnamoyl)-α-L-rhamnopyranosylaucubin (**2**), and five known iridoid glycosides, sinuatol (**3**), aucubin (**4**), geniposidic acid (**5**), catalpol (**6**), ajugol (**7**) as well as a known saponin, ilwensisaponin A (**8**) from the flowers of *Verbascum lasianthum*. Their structures were determined by spectroscopic methods (UV, IR, 1D, 2D NMR and MS).



1: R = H; **2**: R = CH^3

References: 1. Huber-Morath, A. in P. H. Davis (ed) (1978), Flora of Turkey and the East Aegean Islands, University Press, Edinburgh, Vol. 6, pp. 461 – 603. 2. Tuzlaci, E., Erol, M.K. (1999), Fitoterapia 70: 593 – 610. 3. Akdemir, Z.S. *et al.* (2004) Turk. J. Chem. 28: 101 – 110. 4. Akdemir, Z.S. *et al.* (2004) Turk. J. Chem. 28: 227 – 234.

P 303

Anti-inflammatory and antinociceptive activities of Verbascum lasianthum Boiss. ex Bentham

Tatli II[1], Kupeli E[2], Yesilada E[2], Akdemir ZS[3]
[1]Department of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University, Sihhiye, 06100, Ankara, Turkey; [2]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Etiler, 06330, Ankara, Turkey; [3]Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Sihhiye, 06100, Ankara, Turkey

Infusions of *Verbascum lasianthum* Boiss ex. Bentham flowers have been used for hemorrhoids in Turkish folk medicine [1]. In order to evaluate this information, MeOH and H_2O extracts prepared from *Verbascum lasianthum* flowers were investigated for ***in vivo*** anti-inflammatory activity using carrageenan-induced hind paw edema model [2] and for antinociceptive activity using the *p*-benzoquinone-induced writhing model in mice [3]. The H_2O extract showed a weak inhibitory effect, while the MeOH extract was significantly active (in a dose of 250 mg/kg). Hence, bioassay-guided fractionation procedures were conducted with this extract. Chromatography techniques (VLC, HPLC and CC) have led to the isolation of seven iridoid glycosides as well as a saponin and their structures were elucidated by spectral techniques (NMR and MS). All isolated compounds were separately administered to the both models. Aucubin and ilwensisaponin A were found to possess significant anti-inflammatory activities, per os without inducing any apparent acute toxicity as well as gastric damage, ranging between 25.0 – 33.3 % at 125.1 mg/kg and 29.3 – 38.2 % at 387.3 mg/kg doses*, respectively. Indomethacin (36.3 – 45.9 % at 10 mg/kg*) was used as reference drug. These compounds were also found to display significant antinociceptive activity as compared to ASA. Results of the present study support the utilization of the plant in Turkish folk medicine. *($p < 0.001 – 0.05$ Significant from control) **References**: 1. Tuzlaci, E., Erol, M.K. (1999), Fitoterapia 70: 593 – 610. 2. Yesilada, E., Küpeli, E., (2002), J. Ethnopharmacol. 79: 237 – 248. 3. Okun, R. *et al.* (1963), J. Pharm. Exp. Ther. 139: 107 – 109.

P 304

Effect of elicitation and feeding on the precursors for the production of taxanes in Taxus baccata L. suspension culture

Landa P, Marsik P, Pribylova M, Vanek T
Department of Plant Tissue Cultures, Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo n. 2,. 166 10 Prague 6, Czech Republic

Cell cultures of *Taxus* species are considered as a promising alternative source for the anticancer drug taxol (paclitaxel) and related taxanes [1, 2]. In this work we studied effect of jasmonic acid (JA) as an elicitor and 10-deacetylbaccatin III (10-DAB) and N-benzoyl-3-phenyl-isoserin (BPI) as precursors on taxanes production in suspension cultures of *T. baccata* L.. Baccatin III, taxol and 7-epitaxol content was measured in the medium and cells by HPLC with UV/VIS detector. The production of taxanes increased significantly after the addition of the precursors. The combination of all three treatments gave the highest yield of taxanes (45.23 mg/L of media) with 10 % taxol. Baccatin III content in the medium increased 292 times, taxol 24 times and 7-epitaxol 23 times compared with control cultures. In cultures fed with precursors JA, baccatin III as well as taxol and 7-epitaxol production was affected positively. In control cultures most taxanes were retained in cells (89 %) and only negligible amounts were measured in the medium. When the production reached maximum, 81 % of total taxanes were found in the medium. Taxol was distributed almost equally between the medium (53 %) and the cells (47 %). In our experiment 10-DAB was essential for the increasing of taxanes production. We presume that JA acts upon the taxol biosynthetic path between baccatin III and taxol in *T. baccata* suspension culture because of the relatively highest enhancement of taxol when elicited and none-elicited cultures are compared. However, the content of baccatin III was also increased after the treatment with JA [3]. *Acknowledgements*: This work was supported by

S4055301 project of ASCR. **References**: 1.Yukimune, Y. *et al.* (1996), Nat. Biotechnol. 14: 1129 – 1132. 2. Srinivasan, V. *et al.* (1997), Plant. Cell Rep. 16: 600 – 604. 3. Czech Patent 19 – 05.

P 305

Medicinal plants and conservation efforts in the buffer zone of Kure Mountains National Park (Bartin – Turkey), particularly in Ulus region

Ozkan AM
Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 06100, Tandogan-Ankara,Turkey

The Kure Mountains National Park lies between Kastamonu and Bartin, in the Western Black Sea Region of Turkey. It is a place of beauty and magnificent wildlife and has a particular importance related to the age and size of its forests, biodiversity, and variety of its endemic wildlife. Hence, The World Wide Fund for Nature (WWF) has listed the area as one of a hundred forest hot spots in Europe deserving priority conservation. It was declared a National Park in 2000 due to its natural and cultural richness. The core area which covers an area of over 50 000 hectares is surrounded with a buffer zone, in which local inhabitants, mostly living with low income will be able to play an active role in the conservation of the area. The aim of the present study is to understand the overall picture of medicinal plant knowledge, use and commercialization in the Ulus region of the buffer zone. Additionally, to develop a public education program to encourage the preservation of local knowledge of medicinal plants, forest viability, plant survival and healthy communities. The main results demonstrate that; medicinal plants play a major role in local people's health care; certain species of medicinal plants are commercialized at a large scale and become locally rare and sustainable, controlled harvesting may be beneficial both for the local economy and the habitat conservation. The results also reveal that; a successful approach recognizes the power of local communities and conservation of medicinal plants cannot be effective without the support and involvement of local people.

P 306

Rapid TLC analysis of Ranunculus bulbosus L. homeopathic tincture

Gehrmann B[1], Melzig MF[2]
[1]Einhorn-Rats-Apotheke, Markt 10 – 12, 25813 Husum, Germany; [2]Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str. 2+4, 14195 Berlin, Germany

Ranunculus bulbosus L., Bulbous Buttercup or Crowfoot (Ranunculacea) is a perennial plant native to the Northern parts of Europe and to the North eastern parts of the United States. Ethanolic tinctures of Ranunculi bulbosi herba cum radice are used in homoeopathy for skin diseases, rheumatism, gout, (zoster) neuralgia, influenza, and meningitis [1, 2]. *Ranunculus bulbosus* L. is monographed in the HAB 2005 [3]; however, the described TLC procedure is a general comparative method. Thus, we propose a rapid and simple TLC analysis using the DESAGA H-separating chamber and different solvent systems containing e. g. ethyl acetate, methanol, water at different proportions as mobile phase and diverse silica gel plates (*e. g.* Si 60, HPTLC-, RP-material) as stationary phase. The optimised TLC conditions are performed on different samples of homoeopathic Bulbous Buttercup tinctures and provide chromatograms showing satisfying distributions of characteristic zones in the range of R_f values from about 0.2 to 0.8. The described TLC methods are time saving (running time ca. 3 min) and only need a small amount of solvents (mobile phase 1 – 2 mL/performance), sorbens (plates 5 x 5 cm), and homoeopathic tincture (application of 10 – 20 µL/performance). The TLC method may be proposed for an improved homoeopathic pharmacopoeia monograph of *Ranunculus bulbosus* L. **References**: 1. Brendler, Th., Grünwald, J., Jänicke, Chr., Editors. (2003), Herbal Remedies, CD-ROM, medpharm, Scientific Publishers, Stuttgart. 2. Homöopathisches Repetitorium (2003), Deutsche Homöopathie-Union, Karlsruhe. 3. Deutsches Homöopathisches Arzneibuch (2005), Monograph Ranunculus bulbosus (Edition 2000).

P 307

Antipyretic activity of the aqueous leaf extract of Byrsocarpus coccineus

Akindele AJ, Adeyemi OO
Department of Pharmacology, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria

The aqueous leaf extract of *Byrsocarpus coccineus* Schum and Thonn. (Connaraceae), ABC, was investigated for antipyretic activity in rats and rabbits using yeast [1], amphetamine [2] and lipopolysaccharide [3] induced pyrexia models. In control rats, yeast (10 mL/kg, *s.c.*) caused elevation of rectal temperature of 1.7°C 19 h after administration. The extract (100, 200 and 400 mg/kg, *p. o.*) produced a significant ($p < 0.05$) dose dependent inhibition of temperature elevation. Peak inhibitory effect was observed at 1 h post therapy (42.1, 47.2 and 63.6 % inhibition, respectively for ABC at 100, 200 and 400 mg/kg). The effect at 400 mg/kg was greater than that of acetylsalicylic acid, ASA (100 mg/kg, *p. o.*; 43.2 %). An elevation in rectal temperature of 1.9°C was provoked in control rats by amphetamine (10 mg/kg, *i.p.*) 0.5 h after administration while in control rabbits, lipopolysaccharide from *E. coli* (0.2 µg/kg, *i. v.*) elicited an elevation of 1.1°C, 1.5 h post challenge. In both models, ABC produced a significant ($p < 0.05$) dose and time dependent direct reduction of elevated temperature with peak effect observed at 3.5 h post therapy. Percent reduction of fever values were 50.2, 61.1 and 84.3, respectively for ABC at 100, 200 and 400 mg/kg (*p. o.*) in respect of the amphetamine test. The effect at 400 mg/kg was about the same as that of ASA (85.3 %) in this case, but it was lower (44.9 %) compared to the standard drug (96.6 %) in the lipopolysaccharide test. The results obtained in this study suggest that the extract possesses antipyretic activity. **References**. 1 Mukherjee, K. *et al.* (2002), Phytother. Res. 16: 686 – 688. 2 Berkan, T. *et al.* (1991), Planta Med. 57: 34 – 37. 3 Vogel, H.G., Vogel, W.H. (1997), Drug Discovery and Evaluation. Springer–Verlag Berlin Heidelberg, New York.

P 308

Antioxidant oligomeric proanthocyanidins from Cistus salvifolius

Qa'dan F[1], Petereit F[2], Mansour K[3], Nahrstedt A[2]
[1]Faculty of Pharmacy, The University of Petra, P.O.Box: 961343 Amman-Jordan; [2]Institut fuer Pharmazeutische Biologie und Phytochemie, Hittorfstrasse 56, 48149 Muenster, Germany; [3]School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL, UK

The purified proanthocyanidin oligomers of *Cistus salvifolius* herb extract accounted for 78 % of the total proanthocyanidins and 73 % of the total antioxidant activity of this extract [1]. To elucidate the structure of the oligomer, it was depolymerized by acid catalysis in the presence of phloroglucinol [2]. The structures of the resulting flavan-3-ols and phloroglucinol adducts were determined on the basis of 1D-and reverse 2D-NMR (HSQC, HMBC) experiments of their peracetylated derivatives, MALDI-TOF-MS and CD- spectroscopy [3]. These observations resulting from the degradation with phloroglucinol were confirmed by ^{13}C-NMR spectroscopy of the oligomer (4). The mean molecular weight of the higher oligomeric fraction was estimated to be 5 – 6 flavan-3-ol-units. *Acknowledgments*: F Qa'dan would like to acknowedge gratefully the DAAD and the Deanship of Research at the University of Petra for funds and grants (Grant No.1/5/2002). We wish to acknowlege also the help of Dr.H.Lahl, Ms.M.Heim (Inst.f. Pharmazeutische Chemie, Münster) and Dr. Brian Lockwood (School of Pharmacy, Manchester) for the NMR-spectra. **References**: 1. Al-Khalil, S. (1995), Int. J. Pharmacognosy 33(4): 317 – 323. 2. Kennedy, J.A., Graham, P.J. (2001), J.

Agric. Food Chem. 49:1740 – 1746. 3. Balas, L., Vercauteren, J. (1994), Magn. Reson. Chem. 32: 386 – 393. 4. Eberhardt, T., Young, R.A. (1994), J. Agric. Food Chem. 42: 1704 – 1708.

P 309

Analgesic, Antipyretic and Anti-Inflammatory Properties of Mezoneuron Benthamianum Baill (Caesalpiniaceae)

Mbagwu HOC[1,2], Anene RA[1], Adeyemi OO[1]
[1]Department of Pharmacology, College of Medicine, University of Lagos, Idi-Araba, P. M. B. 12003, Lagos Nigeria; [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, P. M. B. 1017, Uyo, Nigeria

The analgesic, antipyretic and anti-inflammatory effects of the aqueous extract of *Mezoneuron benthamianum* (MB) were evaluated in mice, rats and rabbits using the mouse writhing, tail flick, hot plate and formalin-induced pain tests; 2,4-Di-nitrophenol (DNP), D-Amphetamine and *Escherichia coli* Lipopolysaccharide-induced pyrexia and carrageenan, egg albumin and xylene- induced oedema [1,2]. The extract (400 – 1600 mg/kg) and acetylsalicylic acid (ASA), (100 mg/kg) produced a significant ($P < 0.05$) inhibition of the second phase response in the formalin pain model, while only the highest dose (1600 mg/kg) of the extract showed a comparable anti-nociceptive effect in the first phase. The extract also showed a dose-dependent inhibition of acetic acid induced abdominal writhing. The tail flick latency and the hot plate pain threshold were dose dependently enhanced by the extract but these were significantly lower than that produced by morphine (2 mg/kg). The 2,4-DNP and D-Amphetamine (10 and 5 mg/kg, *i.p.*respectively) increased the rectal temperatures of rats within 30 minutes of their administration. The extract at doses of 400, 800 and 1600 mg/kg produced significant lowering of the elevated body temperature in rats. The extract (800 mg/kg) administered orally to rabbits passages with *E. coli* lipopolysacharride was able to relieve the pyrogen induced fever. The antipyretic effect produced by the extract was comparable to a standard antipyretic drug, ASA. The extract (400 – 1600 mg/kg) administered 1 h after carrageenan-induced paw swelling did not inhibit the oedema. No inhibitions were observed with the egg albumin and xylene induced oedema models. Phytochemical analysis revealed the presence of flavonoids, tannins, cardiac glycosides, anthraquinones, and saponins in the extract. Administration of the extract up to 2 g/kg (orally) did not produce any toxic effect in the acute toxicity studies in mice. The LD_{50} of the extract when administered intraperitoneally was 1021.31 mg/kg. The data obtained show that MB extract possesses analgesic and antipyretic activities but lacks an anti-inflammatory property. **References**: 1. Koster, R. *et al.* (1959), Fed. Proc. 18: 418 – 420. 2. Winter, C. *et al.* (1962), Fed. Proc. 46: 118 – 126.

P 310

Effects of Phenolic Compounds from Hypericum perforatum L. on the solubility and permeation of Hypericin in vitro

Sieger R, Nahrstedt A
University of Münster. Institute of Pharmaceutical Biologie and Phytochemistry, Hittorfstraße 56, D-48149 Münster, Germany

The naphthodianthrone hypericin is one of the active constituents in extracts of *Hypericum perforatum* L., widely used in the therapy of mild to moderate depression. Former studies have shown that phenolic compounds such as procyanidin B1 and some flavonoids are essential for hypericin's activity in the forced swimming test [1] in that they improve hypericin's low water solubility [1, 2] and increase its plasma levels in rats [3]. First part of our studies was to investigate the influence of *Hypericum* phenolic compounds on the octanol/buffer partition coefficient of hypericin. Addition of polyphenols (mainly flavonoids) to the system resulted in a decreased octanol/buffer partition coefficient (LogD) of hypericin and a considerably higher concentration of it in the aqueous phase: Without such coeffector, hypericin's LogD-value was 4.73 ± 0.05; but when the most active quercetin-3-O-glucuronide was present the LogD-value was 2.52 ± 0.001. We then undertook an *in vitro* pharmacokinetic study, using the Caco2 cell line as a model for intestinal absorption of hypericin without and in the presence of coeffectors. The apparent permeability (P_{app}) was measured in both directions, apical to basolateral and basolateral to apical. The results for hypericin without coeffector showed a 300-fold higher P_{eff}-value from basolateral to apical ($P_{eff\ (baso.\ to\ api.)}$ $12.89 \cdot 10^{-6}$ cm/s) than in the reverse direction ($P_{eff\ (api.\ to\ baso.)}$ $0.36 \cdot 10^{-6}$ cm/s). In the presence of *Hypericum* polyphenols the P_{eff}-values for both directions became nearly equal; this effect was comparable to that of the selective synthetic MRP1/2-inhibitor MK-571. Thus certain polyphenols not only cause higher plasma levels of hypericin by its increased solubility, but also by its decreased apical efflux by ABC transporters. **References**: 1. Butterweck, V. *et al.* (1998), Planta Med. 64:291 – 294. 2. Jürgenliemk, G., Nahrstedt, A. (2003), Pharmazie 58:200 – 203. 3. Butterweck, V. *et al.* (2003), Planta Med. 69:189 – 192.

P 311

Effect of development stage at harvest on the content of flavonoids and phenolic acids in aerial parts of Greek oregano (Origanum vulgare L. ssp. hirtum (Link.))

Grevsen K[1], Fretté XC[2], Nørbæk R[2], Christensen LP[2]
[1]Department of Horticulture, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark; [2]Department of Food Science, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

Aromatic plants are used for their culinary qualities as well as for their preservative and medicinal properties. Greek oregano is known for its flavour, which is mainly due to volatile terpenes. In addition, oregano and related herbs possess anti-microbial, anti-viral and antioxidant activities [1]. The antioxidant properties of oregano are mainly due to flavonoids and phenolic acids. The content of these compounds in plants depend on a number of factors, such as growing conditions, development stage at harvest and genotype. The aim of the present study was to investigate how the concentration of flavonoids and phenolic acids in oregano, depends on the development stage of the plant during the growing season.
Plants were cultivated in 2003 and 2004 and the aerial parts were harvested at five different development stages during the growing season (from beginning of July to late August) and samples stored at -24 ¤C until analysis. Several flavonoids and phenolic acids were identified from methanol extracts by LC-MS and NMR spectroscopy, and quantified in extracts by RP-HPLC. The major phenolics were apigenin 6,8-di-*C*-glucopyranosyl, luteolin 7,4'-di-*O*-glucuronide, luteolin 7-*O*-glucuronide, lithospermic acid B and rosmarinic acid. Flavonoids varied from 2.8 – 5.2 mg/g dry matter (DM) (2003) and 5.3 – 6.8 mg/g DM (2004), and phenolic acids from 1.4 – 9.9 mg/g DM (2003) and 24 – 53 mg/g DM (2004). The highest content of flavonoids were obtained at the 3rd harvest late in July (near full flowering stage) in both years and for phenolic acids the highest content were obtained at the 2nd harvest (early flowering stage) in the beginning of July also in both years. The conclusion of the present study is that the development stage has a significant impact on the content of flavonoids and phenolic acids in Greek oregano, and that an optimal harvest time of this type of oregano depends on the flavonoids or phenolic acids of interest. **Reference**: 1. Dorman, H.J.D. *et al.* (2004), J. Agric. Food Chem. 48: 2576 – 2581.

P 312

Effect of essential oil of Citrus cinensis cv new hall – Citrus aurantium (indigenous in Greece) upon growth of Yarrowia lipolytica

Gortzi O[1], Papanikolaou S[2], Lalas S[1], Galiotou-Panayotou M[2], Mitliaga P[3]
[1]Department of Food Technology, Technological Educational Institute of Larissa (Karditsa Branch), Terma N. Temponera str, GR-43100, Karditsa, Greece; [2]Laboratory of Food Microbiology and Biotechnology, Department of Food Science and Technology, Agricultural University of Athens 75 Iera Odos str, GR-11855, Athens, Greece; [3]Department of Agricultural Products Marketing and Quality Control, Technological Educational Institute of Western Macedonia (Florina Branch), Terma Kontopoulou str, GR-53100, Florina, Greece

The effect of essential oil from *Citrus cinensis* cv *new hall* – *Citrus aurantium* (indigenous in Greece) upon growth of the dimorphic non-conventional yeast *Yarrowia lipolytica* strain ACA-DC 50109 was studied. The microorganism was aerobically cultivated in batch mode in carbon-limited media. The essential oil was added into the culture medium in different quantities while the control experiment was carried out without addition. The essential oil caused a relatively important decrease of the highest concentration of biomass produced. Additionally, biomass yield on glucose consumed was significantly decreased with the addition of the oil on the cultivation medium. Moreover, the addition of the essential oil considerably increased the lag time of the culture. In all trials, a remarkable drop the pH value of the medium was observed due to the biosynthesis of small amounts of organic acids. Given that one principal component of this membrane is the one of cellular lipids, it was assumed that the extraction and the analysis of cellular lipids could provide information about the microbial behaviour. Total lipids were extracted, methanolyzed and analyzed with the aid of G.L.C. In the control experiment, the culture conditions did not favour accumulation of storage lipid inside the yeast cells and, hence, lipid produced corresponded to 5 – 9 % (wt/wt) in dry cell mass. Similar concentrations of cellular lipids were produced when essential oil was added in various amounts. When essential oils were added, an increase of lower aliphatic chain saturated fatty acids was observed, suggesting an alteration in the membrane function. *Acknowledgements*: This study has been co-funded by 75 % from E.E. and 25 % from the Greek Government under the framework of the Education and Initial Vocational Training Program – Archimedes II. **References**: 1. Aggelis, G., Komaitis, M. (1999), Biotechnol. Lett. 21: 747 – 749 2. Aggelis, G. *et al.* (1998), Anton. Leeuw. Int. J. G. 73: 195 – 198.

P 313

Simultaneous determination of ginsenosides and polyacetylenes in American ginseng (Panax quinquefolium L.) using high-performance liquid chromatography

Christensen LP[1], Jensen M[2], Kidmose U[1]
[1]Department of Food Science, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark; [2]Department of Horticulture, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, DK-5792 Aarslev, Denmark

Panax quinquefolium L. (American ginseng) is native to North America, and is one of the most widely used medicinal herbs in the world together with other ginseng species. The alcoholic extract of ginseng roots has been widely used as a tonic against cancer, diabetes, cardiovascular disorders, and immune functions [1]. The active principles of ginseng roots appear to be polyacetylenes and dammarane saponins (ginsenosides), which are normally determined by different analytical methods. The aim of this study was to develop a method for simultaneous determination of both ginsenosides and polyacetylenes of *P. quinquefolium* roots. A high-performance liquid chromatography (HPLC) method was developed for simultaneous determination of ginsenosides and polyacetylenes from ginseng extracts. Polyacetylenes and ginsenosides were extracted from fresh ginseng roots with 100 % methanol followed by extraction with 80 % methanol, which ensured a complete extraction of both types of bioactive compounds. The combined methanol extracts were subjected to HPLC analysis on a reversed-phase (RP) C18 column using a gradient consisting of acetonitrile and water. The major polyacetylenes were identified as falcarinol and panaxydol by 1D- and 2D-NMR spectroscopy and the major ginsenosides as R_{b1}, R_{b2}, R_c, R_d, R_e and R_{g1} by comparison with authentic standards on HPLC. The HPLC method was validated and used to quantify the content of polyacetylenes and ginsenosides in root hairs, lateral roots and main roots of 5-year old American ginseng. The total mean concentration of polyacetylenes and ginsenosides was approximately 4.5 and 2 times higher in root hairs, respectively, compared to the main roots, indicating possibilities for production of differentiated ginseng preparations. The developed HPLC method can also be used as a quality control of fresh ginseng roots as well as dried root material and various ginseng preparations. **Reference**: 1. Sticher, O. (1998), Chemtech. 28: 26 – 32.

P 314

Determination of flavonoids in extracts of Epilobii angustifolii herba by HPTLC-densitometry

Bazylko A, Kiss AK, Kowalski J
Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Warsaw 1 Banach St, 02 – 097 Warsaw, Poland

Epilobium angustifolium L., *Oenotheraceae* is used in folk medicine. The herb is rich in polyphenolic compounds such as flavonol-3-*O*-glycosides, phenolic acids and tannins [1]. Flavonoids analyses have shown that quercetin glycosides are predominant in *Epilobium angustifolium* [2]. Several studies suggest that at least flavonoids are partly responsible for the biological action of the herb [3, 4]. The separation and quantitative determination of quercetin glycosides in methanolic and aqueous extracts of *Epilobii angustifolii herba* by HPTLC-densitometry was established. Ethyl acetate/formic acid/water 68:2.5:03 was used as a mobile phase and silica gel as a stationary phase. The flavonoids were more abundant in the aqueous extract than in the methanolic one. In both extracts quercetin glucuronide was the dominating compound, 2.12 % and 1.78 % respectively. Our method is fast, easy and selective particularly for quercetin glucuronide determination in Epilobium extracts. **References**: 1. PDR for Herbal Medicines (1998), Medical Economics Company, Montvale, New Jersey 2. Ducrey, B. *et al.* (1995), Phytochemistry 38: 129. 3. Tita, B. *et al.* (2001), Farmaco 56: 341. 4. Kiss, A. *et al.* (2006), Pharmazie 61: 66.

P 315

Effect of herbal formula tonics for reinforcement of yin or yang deficiency on the inhibition of whole blood aggregation

Ko BS, Jeon WK, Lee JH
Quality Control of Herbal Medicine Department, Korea Institute of Oriental Medicine, 461 – 24 Jeonmin-dong, Yuseong-gu, 305 – 811, Daejeon, Korea

Herbal formula tonics for reinforcement of yin or yang deficiency are commonly used in Korea traditional medicine [1, 2]. This study examined the possible inhibitory effects of 30 kinds of these herbal formula tonics on platelet aggregation induced by collagen in human whole blood using the impedance method of aggregometry [3]. Among them, 4 kinds of yin-tonic and 3 kinds of yin-yang-tonic water extracts were selected to be the most effective candidates ($p < 0.001$). Also, through *in vivo* study, the anti-thrombotic effects of Igyeongtang-, Gamisipjeondaebotang-, and Gamisoyosan-treated groups, with recovery rate of 60 %, 50 %, 45.45 %, respectively, were observed to be higher than the control group (36.8 %) in a mouse acute thrombosis. The results from this experiment provide pharmacological evidence for the traditional use of tonics with yin-yang theory of traditional medicine, suggesting that yin-tonics could be

used to help problems of blood circulation more than yang-tonics. *Acknowledgements*: This study supported in part by the Inter-Institutional Collaboration Research Program under the Korea Research Council for Industrial Science & Technology (KOCI), Korea. **References:** 1. Liu, Y., Dong, L. (2002), Basic Theories of Traditional Chinese Medicine, 2nd ed., Academy Press, Beijing. 2. Heo, J. (1610), translated by Yoon, S. H. *et al.* (2005), Donguibogam. Donguibogam Press. Hadong. Korea. 3. Armida, P. T. *et al.* (1995) Thromb Res 78: 107 – 115.

P 316

Inhibitory effect of compounds from Rhus chinensis on generation of oxygen species generation in YPEN-1 cells

Kim GS[1], Chung HY[2], Kim JM[2], Lee SE[1], Seong NS[1], Song KS[3], Baek NI[4]
[1]National Institute of Crop Science, Rural Development Administration, 441 – 857, Suwon, South Korea; [2]College of Pharmacy, Pusan National University, 609 – 735, Busan, South Korea; [3] Division of Applied Biology & Chemistry, College of Agriculture & Life Sciences, Kyungpook National University, 702 – 701, Daegu, South Korea; [4]Graduate School of Biotechnology, Kyung Hee University, 449 – 701, Suwon, South Korea

Rhus chinensis Mill. (Anacardiaceae) is a broad leaf tree, which is widely distributed in Korea, Japan and China. Its barks and gall have long been used traditionally for remedies of dysentery and diarrhea. This study has been carried out as a part of our research on antioxidative and antidemential compounds from *R. chinensis*. Ethylacetate extract from the stems of *R. chinensis* was chromatographed on silica gel glass column to yield dammarane triterpene compounds **1**-**4**. Their structures were identified by spectral techniques as 20-hydroxy-24-dammaren-3-one (**1**), 3-oxodammar-20,24*E*-dien-26-oic acid, (**2**), semialatone, (**3**) and semialatic acid (**4**), respectively. Compound **2** was previously isolated and identified as a new dammarane triterpene and compound **1** was first isolated in *R. chinensis* by us. The other compounds were previously reported in *R. chinensis.* Compound **5** was isolated as a major compound from the leaves of *R. chinensis* and identified as methyl gallate (**5**)**.** Compounds **2** and **5** strongly inhibited the oxidation of 2,7-di-chlorodihydrofluorescein diacetate (H_2DCFDA) to 2,7-dihydrofluorescein (DCF) with IC_{50} values of 26.8 and 12.7 μM, respectively. Compounds **2** and **5** also showed the inhibitory effect on intracellular ROS (reactive oxygen species) generation induced by 20 μM *t*-BHP (*tert*-butyl hydroperoxide) in YPEN-1 cells. Compared to untreated control, *t*-BHP treated cell increased ROS generation to 135.7 %. Pretreatment with compounds **2** and **5** decreased the ROS generation to 86.7 and 60.2 % at the concentration of 10 μM, respectively. In conclusion, compounds **2** and **5** maybe potent ROS scavengers. **References**: 1. Kim, A. R. *et al.* (2005), J. Pharm. Pharmacol. 57: 475 – 481. 2. Kim, J. Y. *et al.* (2004), Free Radical Res. 38 (7): 761 – 769. 3. Kim, A. R. *et al.* (2002), J. Pharm. Pharmacol. 54: 1385 – 1392. 4. Hsieh, T. J. *et al.* (2004), Food Chem. Toxicol. 42: 843 – 850.

P 317

Effects of Apple Consumption on Plasma and Erythrocyte Antioxidant Parameters in Elderly Subjects

Öztürk HS[1], Avci A[1], Ergüder IB[1], Atli T[2], Varli M[2], Devrim E[1], Aras S[2], Turgay M[2], Durak I[1]
[1]Department of Biochemistry, Ankara University School of Medicine, Ankara, Turkey; [2]Department of Geriatric Medicine, Ankara University School of Medicine, Ankara, Turkey

Aim: Effects of apple consumption on plasma and erythrocyte antioxidant parameters of elderly subjects were investigated in this study. **Methods:** Fifteen elderly subjects (mean age 71.86 ± 4.17) participated in the study. They consumed an apple a day for 1 month. Before and after this period, fasting blood samples were obtained, and oxidant (malondialdehyde, (MDA)) and antioxidant (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) and antioxidant potential (AOP)) parameters were studied. MDA and AOP levels were studied in plasma, and SOD, GSH-Px, CAT activities and MDA levels were measured in the erythrocytes. **Results:** In the erythrocytes, GSH-Px and SOD activities were found to be higher ($p < 0.001$), but MDA levels lower in the second samples relative to the first ones. In the plasma, AOP value was found to be higher in the second samples relative to first ones ($p < 0.001$). **Conclusion:** Our results suggest that consumption of apple leads to a significant increase in the activities of some antioxidant enzymes and in the antioxidant potential values of the blood, does decreasing oxidation reactions in the body in significant amount. It is quite possible that reduced peroxidation processes owing to consumption of this fruit may play a part in some of their beneficial effects in the elderly subjects.

Table: Oxidant and antioxidant parameters in erythrocytes (RBC) and plasma from elderly subjects who consumed apple (Mean ± SD; n = 15).

Groups	GSH-Px (RBC)IU/mL	CAT (RBC)IU/mL	SOD (RBC)U/mL	MDA (RBC) nmol/mL	AOP (Plasma) (nmol/mL.h)$^{-1}$	MDA (Plasma) nmol/mL
Before apple consumption	6.24 ± 1.23	58877 ± 8588	7337 ± 1623	394.3 ± 72.3	3.43 ± 2.13	2,6 ± 1.0
After apple consumption	7.65 ± 1.29*	62220 ± 11224	9299 ± 1015*	380.9 ± 60.6	8.11 ± 2.29*	3.3 ± 1.0

* $p < 0.05$; paired t-test

P 318

Analysis of essential oils in Chrysanthemum zawadskii in Korea

Shin S, Byun Y
College of Pharmacy, Duksung Women's University, Seoul, 132 – 714, Korea

The essential oils of *Chrysanthemum zawadskii* Herbich, *C. zawadskii* var. *latilobum* (Maxim.) Kitam, *C. zawadskii* Herb. ssp. Naktongense (Nakai) T. Lee, *C. zawadskii* var. *leiophyllum* (Nakai) T. Lee, and *C. zawadskii* var. *tenuisectum* Kitaqawa were obtained by steam distillation, using a simultaneous steam distillation-extraction apparatus, from the above ground parts of plants cultivated in the herbal garden of Duksung Women's University. The compositions of the oils were analyzed by gas chromatography-mass spectrometry and compared. Additionally, their inhibiting activities were investigated by broth dilution method against antibiotic-susceptible and resistant strains of *Streptococcus pneumoniae*. Analyses resulted in tremendous diversity in composition of essential oils among the species and the varieties. The predominantly contained compound of essential oils from *Chrysanthemum* species were camphor (13.00 %), myrtenol (11.97 %) and germacrene D (17.02 %). Additionally, all of the tested *Chrysanthemum zawadskii* essential oils significantly inhibited growth of *S. pneumoniae* with MICs ranging from 0.5 mg/mL to 1 mg/mL. **Reference**: 1. Shin, S., Lim, S. (2005), Arch. Pharm. Res. 7: 765 – 769.

P 319

Preparation of official reference standards from herbal medicines

Kim EK, Ze KR, Seong RS, Lee JP, Park JY, Kang IH, Kim JH, Lee HY, Kim JS, Heo MH, Chang SY
Herbal Medicine Standardization Team, Korea Food and Drug Administration, 231 Jinheungno, Eunpyung-gu, 122 – 704, Seoul, Republic of Korea

The reference standards are very important and essential for quality control of herbal medicines. They are required for identification, assay and purity test etc. Unlike general chemical references, which are used for conventional pharmaceutical products, reference standards for herbal medicines are difficult to obtain or have relatively higher price. We have prepared botanical samples of herbal materials and marker substances for reference standards and intended to promote the quality control of herbal medicines. By botanical scientists, herbal materials are collected, identified its origin and then evaluated its quality and specification. Marker substances

are prepared from medicinal plant materials through extraction, separation and purification. All materials are tested and characterized [1, 2]. We review and reevaluate the herbal materials and marker substances and then establish them as official reference standards. Particularly, several herbal materials and marker substances such as gardenia fruit and saikosaponin A etc., were reviewed and reevaluated, and we will describe the establishment process of these reference standards. *Acknowledgements*: Chung-Ang University[1], WonKwang University, Wann Kyunn Whang[1], Youn Chul Kim. **References**: 1. (2002), The Korean Pharmacopoeia 8th edition, KFDA 2. (2005), The Korean Herbal Pharmacopoeia, KFDA.

P 320

Toxicity assessment of the aqueous root extract of Sanseviera Liberica (Agavaceae)

Adeyemi OO[1], Amida MB[1], Yemitan OK[1,2]
[1]Department of Pharmacology, College of Medicine of the University of Lagos, Idi-Araba, P.M.B. 12003 Lagos, Lagos, Nigeria; [2]Department of Pharmacology, Lagos State University College of Medicine, P.M.B. 21266 Ikeja, Lagos, Nigeria

The aqueous root extract of *Sanseviera liberica* Ger, & Labr. (SL) is used in African folklore medicine for ailments including chronic pain and inflammatory conditions, and convulsive disorders [1]. Because of the potential for long term uses, the study investigated the acute and subchronic toxicity patterns of the plant. Acute oral & *i.p.* toxicity tests were carried out in mice; and the median lethal dose estimated [2]. Subchronic (52 days) studies were conducted in rats with oral daily doses of 80, 400 & 2000 mg/kg, which represented 1/ 5 [3], active dose and 5x the pharmacologically active dose [4], respectively. Parameters observed at the end of chronic tests included changes in body & vital organ weights, mortality, haematological, biochemical, hepatic and male reproductive effects [3, 4]. SL did not produce any visible toxicities or mortality with oral doses up to 20 g/kg within 14 days of single treatment, but i.p. administration caused mortalities with LD_{50} of 668.3 mg/kg. In the chronic tests, neither mortality nor visible signs of lethality was seen in rats. No significant change in the weight of the kidney, liver, heart and spleen, but at 400 mg/kg, a significant reduction in weight of the lungs. Significant increases in the weight of testes, sperm count and motility was produced. There were no changes in the sperm head and tail abnormalities, but significant increases in the % normal sperm cells. Biochemical parameters like the AST, ALT and uric acid were not affected, but significant increase in ALP level at 2 g/kg was produced. Significant increase in RBC was recorded, but no changes in levels of PCV and Hb. Results indicate that oral doses of SL are safe, but assessment of hepato-biliary function be done during chronic uses. **References**: 1. Watt, J.M., Breyer-Brandwiijk, M.J. (1962), Medicinal and Poisonous Plants of South and Eastern Africa 2nd ed. pp 197 – 198. 2. Miller, L.C., Tainter, M.L. (1944), Proc. Soc. 24: 839 – 840. 3. Tanira, M.O.M. *et al.* (1988), Int. J. Crude Drug Res. 26: 56 – 60. 4. Yemitan, O.K., Adeyemi, O.O. (2004), Nigerian J. Hlth. Biomed Sci. 3 (1): 20 – 23.

P 321

Seasonal variation in the essential oil composition of Salvia fruticosa Mill. cultivated in Portugal

Braga PSC[1], Vicente AM[2], Fernandes-Ferreira M[1]
[1]Department of Biology, University of Minho, Campus de Gualtar, 4710 – 057 Braga, Portugal; [2]Estação Regional de Hortofloricultura, Quinta S. José – S. Pedro de Merelim, 4700 – 856 BRAGA, Portugal

Salvia fruticosa Miller (Greek sage) is a well-known medicinal plant endemic of the Eastern Mediterranean region. Also named three-lobed sage (*Salvia triloba* L.) due to its leaf morphology, Greek sage has been commercialized since ancient times for use in therapy but also as a spice to flavour meats such as pork, sausage and poultry [1]. Its essential oil has shown antimicrobial, cytotoxic, antiviral and anti-tumor properties [2]. However, the composition of the essential oil varies geographically and seasonally and these variations seem to affect its properties [3, 4]. Our group has already studied the seasonal and geographical variation of the essential oil composition of *S. officinalis* plants grown in Portugal [5]. We now report on the seasonal variation of *S. fruticosa* essential oil composition. Aerial parts of *S. fruticosa* plants (~30 cm) were collected every two months, during a year period, from an experimental field from DRAEDM located in Merelim, Braga (Portugal). The aerial parts were then divided in an upper (UAP) and lower segment (LAP) at ~10 cm from the top and approximately 25 g of each sample were hydrodistilled in a Clevenger type apparatus. The resulting essential oils were analyzed by GC and GC/MS. They were rich in oxygenated monoterpenes and monoterpene hydrocarbons and, despite some variation, 1,8-cineole was the major compound in all samples, representing 29 % to 46 % of the oil. Myrcene was present in high percentages throughout the year. However, its levels fell during flowering, especially in the UAP's. The levels of *cis*- and *trans*-thujone were always low (< 3 %). Camphor percentages were also low but rose in August and October, being higher in the LAP's. *Acknowledgements:* This work was sponsored by EU (FSE/FEDER) and Portuguese Republic Government (FCT) through the Grant SFRH/BD/18908/2004 and the Project SageBiotech (POCTI/AGR/62040/2004). **References**: 1. Gali-Muhtasib, H. *et al.* (2000), J. Ethnopharmacol. 71: 513 – 520. 2. Gali-Muhtasib, H.U., Affara, N. I. (2000), Phytomedicine 7: 129 – 136. 3. Skoula M. *et al.* (2000), Biochem. Syst. Ecol. 28: 551 – 561. 4. Farhat, G.N. *et al.* (2001), Toxicon 39: 1601 – 1605. 5. Santos-Gomes, P.C., Fernandes-Ferreira, M. (2001), J. Agric. Food Chem. 49: 2908 – 2916.

P 322

Effects of Chelidonium majus extracts in human hepatocytes in vitro

Adler M[1], Appel K[2], Canal T[3], Corvi Mora P[3], Delfino R[4], Gennaro R[4], Gritzko K[5], Pascolo L[4], Ruzzier F[3], Tiribelli C[4], Wallner B[5]
[1]Sohlbacher Str. 20, 57078 Siegen, Germany; [2]VivaCell Biotechnology GmbH, Ferdinand-Porsche-Str. 5, 79211 Denzlingen, Germany; [3]Actimex SrL, Ed. Q – Area Science Park, S.S. 14, Km 163.5, 34012 Basovizza, Trieste, Italy; [4]Center for Liver Studies (CSF) and Dept. BBCM, University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy; [5]GenPharmTox BioTech AG, Fraunhoferstr. 9, 82152 Planegg, Germany

In recent years there has been a discussion whether reversible hepatitis may be a rare potential side effect in *Chelidonium majus* L. extracts used in the therapy of biliary and hepatic dysfunctions [1]. Therefore, earlier *in vitro* studies of cytotoxic effects of these extracts in rat hepatocytes have found increased attention of regulatory agencies. With the aim to establish a better database, two studies in human hepatocytes have been conducted. A study in primary human hepatocytes was conducted under GMP/GLP conditions with *C. majus* extract (extraction medium 30 % ethanol (v/v), 1:2.5 – 3.5, total alkaloids 5.9 mg/g). For indication of cytotoxicity, MTT and neutral red assay were used. EC_{50} over 24 h was calculated as 0.83 ± 1.69 mg/mL and 0.82 ± 2.49 mg/mL, respectively, which was equivalent to 4.9 µg/mL total alkaloids. In a concentration of 0.74 mg/mL 86 % of the cells survived (MTT). The EC_{50} of ascorbic acid in this assay is in a similar range (0.8 – 3.5 mg/mL). A second study was conducted in the human-derived Chang liver cell line, testing *C. majus* extract with 6.2 mg/g total alkaloids. The vitality of the cells was determined from MTT assay and morphological appearance. EC_{50} (24 h) was 0.96 ± 0.49 mg/mL, equivalent to 5.9 µg/mL total alkaloids. The EC_{50} of *Ginkgo biloba* extract and paracetamol, assayed for comparison, was 0.31 and 2.49 mg/mL. These data show a clear dose dependency of all observed effects and do not point to special hepatotoxicity of *Chelidonium majus* extracts, compared to other drugs with established therapeutic safety. They are in accordance with available toxicity data for oral application, which show no hepatotoxic effects and give no indication for ex-

tended pharmacovigilance risk limitation. **Reference**: 1. Nahrstedt, A., Weber, C. (2005), Deutsche Apotheker-Zeitung 145:3890 – 3892.

P 323

Topical anti-inflammatory activity of Plantago major L. leaves

Della Loggia R[1], Sosa S[1], Cateni F[2], Zacchigna M[2], Tossi A[3], Tubaro A[1]
[1]Dipartimento dei Materiali e delle Risorse Naturali, Università di Trieste, Via A. Valerio 6, 34127, Trieste, Italia; [2]Dipartimento di Scienze Farmaceutiche, Università di Trieste, P.le Europa 1, 34127, Trieste, Italia; [3]BBCM, Università di Trieste, Via L. Giorgieri 1, 34127, Trieste, Italia

The leaves of *Plantago major* L. (Plantaginaceae) are traditionally used for the topical treatment of skin inflammations, infections and wounds [1]. Their constituents, such as terpenoids, phenols and iridoids, possess immunostimulant activity and/or inhibit enzymes involved in inflammation [2, 3]. Although the main therapeutic use of *P. major* is in cutaneous inflammatory diseases, their topical antiphlogistic properties were not yet investigated. Therefore, *P. major* leaves were studied for their topical anti-inflammatory activity. *P. major* leaves were sequentially extracted with *n*-hexane, chloroform and methanol and the relevant extracts were evaluated for their ability to inhibit the Croton oil-induced ear dermatitis in mice [4]. Each extract (300 $\mu g/cm^2$) provoked a significant edema reduction, the chloroform one being the most active. Its potency was only two fold lower than that of the reference drug indomethacin: their ID_{50} (dose giving 50 % edema inhibition) values were 177 and 93 $\mu g/cm^2$, respectively. By column chromatography, the extract was separated in four fractions (I-IV), concentrating its activity into fraction III, a mixture of ursolic acid and oleanolic acid, in the ratio 1:2. Each compound induced a dose-dependent edema inhibition, being ursolic acid (ID_{50}= 56 $\mu g/cm^2$) more active than oleanolic acid (ID_{50}= 132 $\mu g/cm^2$) and indomethacin. The antimicrobial activity of the plant is under investigation, in order to identify possible active compounds, different from the well known aucubin. **References**: 1. Samuelsen, A.B. (2000), J. Ethnopharmacol. 71: 1 – 21. 2. Chiang, L.C. *et al.* (2003), Planta Med. 69: 600 – 604. 3. Ringbom T. *et al.* (1998), J. Nat. Prod. 61: 1212 – 1215. 4. Tubaro, A. *et al.* (1985) Agents Actions 17: 347 – 349.

P 324

Release of soy isoflavones from commercial capsule preparation

Cvejić J[1], Stepanov I[1], Pocuca M[2]
[1]Laboratory for Analysis of Natural and Pharmaceutical Products, Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia and Montenegro; [2]Laboratory for Biopharmacy, Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Serbia and Montenegro

Herbal medicinal products can be biopharmaceuticaly characterized by dissolution tests, provided that there was identification of the active pharmaceutical ingredient. This study describes the determination of the concentration of released soy isoflavones (dissolution test) for commercial capsules containing soybean extract using an HPLC method. In analysed preparation four different isoflavones were detected and total of their concentrations was evaluated. Two major compounds were daidzein and genistein glucosides, most probably malonyl-glucosides. Aglycones daidzein and genistein were also present, but in much smaller amounts. Release of isoflavones from commercial capsules (Menosoya, Belgrade) was measured with in 90 minutes (six time-points). The dissolution tests were performed in a Erweka DT800 multi-bath (n = 6) dissolution test system, in accordance with the United States Pharmacopeia (USP) general methods. Conditions applied to carry out the dissolution tests were 900 mL of 0.05M potassium dihydrogen phosphate buffer pH4.5 (KH_2PO_4) as dissolution medium, basket at 100 rotations per minute (rpm) stirring speed and bath temperature of 37 °C. Quantification of isoflavones was performed using gradient reversed phase (150 x 4 mm, 5 µm LiChrospher RP-18) high-performance liquid chromatography and detection at 270 nm. All dissolution profiles obtained for six capsules were similar. Values of standard deviation for all measurements (10, 20, 30, 40, 60 and 90 min) were between 3.0 10^{-4} and 5.3 10^{-4}. Analysis of samples showed that more than 50 % of isoflavones were dissolved from capsules after 40 minutes. Approximately 30 % of the total is released in the first 10 min and 65 % after 90 minutes. Individual isoflavones showed slight differences between their dissolution curves. In the first 20 min release of glucosides from formulation was more important than of aglycones.

P 325

Evaluation of the hepatoprotective effect of Ocimum lamiifolium methanolic extract on acetaminophen-induced hepatotoxicity in rats – precision cut liver slices

Mukazayire MJ[1], Allaeys V[2], Buc Calderone P[2], Duez P[1]
1. Université Libre de Bruxelles (ULB), Laboratory of Pharmacognosy, Bromatology and Human Nutrition, Institute of Pharmacy, CP 205/9, Bd du Triomphe, 1050 Brussels, Belgium; 2. Université Catholique de Louvain (UCL), Unité de Pharmacocinétique, Métabolisme, Nutrition et Toxicologie, Ecole de Pharmacie, Woluwe, Brussels, Belgium

Numerous plant species are used to treat hepatitis in the indigenous health care system of Rwanda. Notable among these is *Ocimum lamiifolium* Hoechst. ex Benth. in DC. The present study aims to evaluate the hepatoprotective effects of the methanolic extract of leaves of *O. lamiifolium* against hepatotoxicity induced by acetaminophen in rats – precision cut liver slices (PCLS). The fresh rats-PCLS were incubated in Williams medium E for 24 h with acetaminophen (hepatotoxicant) concomitantly with the plant extract or N-acetylcysteine (NAC) (reference antihepatotoxicant). The measurement of ATP level and CYP2E1 activity were used as endpoints to assess liver toxicity and activity. The *O. lamiifolium* methanolic extract was found to be free of hepatotoxic effects in concentrations up to 10 mg/mL. The severe depletion of intracellular ATP by acetaminophen (10mM) was prevented by treatment with the extract at a dosage of 1 mg/mL, to the same extent as with NAC treatment (20mM). **Reference**: 1. Evdokimova, E. *et al.* (2001), Toxicology in Vitro 15: 683 – 690.

P 326

Modulation of the peristaltic reflex of rat ileum segments by STW 5 (Iberogast®)

Kelber O[2], Yuece B[1], Wallbach J[1], Sibaev A[1], Weiser D[2], Goeke B[1], Storr M[1]
[1]Medizinische Klinik II, Klinikum Grosshadern, Ludwig-Maximilian University, Marchioninistr. 15, 81377 Munich, Germany; [2]Scientific Department, Steigerwald Arzneimittelwerk GmbH, Havelstr. 5, 64295 Darmstadt, Germany

Dyspepsia and motility related disorders of the gastrointestinal tract are increasingly treated with herbal drugs. This especially applies to STW 5 (Iberogast®), a fixed combination of standardized plant extracts, for which clinical efficacy has been proven in several modern randomized controlled double blind studies [1, 2]. But there still remain unanswered questions about the possible mechanisms of action by which the plant extracts induce the beneficial effects. Aim of the present study was to investigate whether plant extracts influence the reflex pathways underlying the peristaltic reflex of rat small intestine. The myenteric pathways of the peristaltic reflex were studied in 10 cm ileum segments and peristaltic activity was stimulated by electrical stimulation in the middle of the segments. Ascending and descending reflex responses were recorded by using force transducers. Areas under the contraction curves were evaluated. Drugs were added in a cumulative manner. Concentrations are given as dilution of the standardized fluid plant extract in organ bath medium. The extracts had individual, reproducible effects on the elicited reflex responses, showing a clear concentration depen-

dency (e.g. peppermint leaf extract on ascending contraction: 1:1000: -10.1 ± 4.0 %; 1:500: -8.8 ± 6.6 %; 1:250: – 17.3 ± 4.7 %; 1:167: – 39.5 ± 13.5 %; 1:83: -55.4 ± 15.5; 1:50: -58.9 ± 15.6). All observed effects were fully reversible after washout and were blocked by tetrodotoxin proving the underlying neuronal mechanisms. Contractile responses were abolished by atropine proving that they are mediated by cholinergic mechanisms. The observed effects of STW 5 and its components on gastrointestinal motility are a clue for understanding the pharmacological mechanisms underlying the relief in patient symptoms. **References**: 1. Holtmann, G. *et al.* (2004), Wien Med Wochenschr 154:21 – 22. 2. Madisch, A. *et al.* (2004), Aliment. Pharmacol. Ther. 19:271 – 279.

P 327

Concentration of Grapefruit Essential oil by Fractional Distillation

Monsef-Esfahani HR[1], Amanzade Y[1], Hajiaghaee R[1], Mahdavi F[1]
[1]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, P. O. Box 14155 – 6451, Tehran, Iran

Grapefruit, *Citrus paradisi* M. (rutaceae) the tree grows in north and south part of Iran with a height about 3 – 5 m, it's fruit is globular, with nipple at apex, mostly big and bright yellow or lemon colored with a mild acid or slightly bitter taste. Fruit of grapefruit of north of Iran were purchased during winter 2003. Grapefruit oil was obtained from peel of the fruits by hydro distillation method. GC and GC/Mass analysis performed on the sample and computer library and kovates index used to identify the compounds. Limonene (96.11 %), beta-Myrcene (1.89 %), alfa-Pinene (0.58 %) detected as the major components in the essential oil. 20 fold concentrate was prepares by a fractional distillation process from hydro distilled oil and analyzed quantitivly by GC and GC/Mass. major and minor constituents were identified by computer library and kovats index. The influence of the concentration process on oxygenated flavor compounds, primarily aldehydes and alcohols and monoterpene hydrocarbons was evaluated by comparing the results. Concentration of Limonen in 20 fold concentrate decreased 21.86 %, whereas alfa-Pinen, Sabinene and beta-Myrcene were completely removed. Concentration of Decanal and Linalool in the 20 fold oil increased 41.96 and 11.94 times respectively.

P 328

Improved isolation of α-mangostin from the fruit hull of Garcinia mangostana and its antioxidant and antifungal activity

Puripattanavong J, Khajorndetkun W, Chansathirapanich W
Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai Campus, Songkhla 90112, THAILAND

The fruit hull of mangosteen, *Garcinia mangostana* L. (Family Clusiaceae), has been used for many years as a folk medicine for treatment of skin infection, wounds and diarrhea [1]. In this study, the HPLC analysis for lphα;-mangostin was developed and validated in terms of resolution (Rs), capacity factor (k'), selectivity factor (α), and tailing factor (T_f). In addition, the effect of solvents on quantity of α-mangostin, total phenolic content [2], antioxidative [3] and anti-tinea activities were examined. Seven types of solvent (hexane, dichloromethane, chloroform, acetone, ethyl acetate, methanol and ethanol) were used for extraction giving % yields of 0.03, 1.87, 1.39, 3.00, 0.90, 7.74 and 6.07, respectively. The EtOAc extract was found to possess the highest content of α-mangostin (91.92 %). The acetone extract showed the highest phenolic content of 0.5713 mg/g as gallic acid equivalent (GAE). The best antioxidant activity (DPPH-assay) was found in the chloroform extract with the EC_{50} of 3.44 µg/mL. The study of antifungal activity showed that extracts (ethanol, acetone and methanol) provided antifungal activity against three species of tinea: *Trichophyton rubrum, T. mentagrophyte* and *Microsporum gypseum*. *Acknowledgements*: Thailand Research Fund (TRF), Thailand., Sirirat Pinsuwan **References**: 1. Chomnawang, M.T. *et al.* (2005), J. Ethnopharmacol. 101: 330 – 333. 2. Folin, O., Ciocalteu, V. (1927), J. Biol. Chem. 27: 627 – 650. 3. Yamasaki, K. *et al.* (1994), Chem. Pharm. Bull. 42: 1663 – 1665.

P 329

Secondary Metabolites from Hypoglycaemic fraction of Treculia africana Decne (Moraceae)

Moody JO[1], Oyelola OO[1], Fukushi Y[2], Tahara S[2], Hashidoko Y[2], Sakihama Y[2]
[1]Department of Pharmacognosy, University of Ibadan, Ibadan, NIGERIA;
[2]Laboratory of Ecological Chemistry, Hokkaido University, Sapporo, JAPAN

Treculia africana Decne (Moraceae) commonly known as African breadfruit is a plant food native to tropical West and parts of East Africa. Ethnomedicinally, it is used as a vermifuge, febrifuge, galactogogue and laxative (Irvine, 1961). The plant is also an important component of some ancient anti-diabetic recipe used in Western and Middle Belt areas of Nigeria as shown by our survey among herbalists and a number of patients attending diabetic clinics in the University College Hospital, Ibadan. Bioactivity –monitored fractionation of the hydroacetone extract of the bark of *T. africana* revealed that 'the non-aqueous soluble fraction (10 mg/kg) exhibited the highest activity by giving a significant reduction in blood sugar level (69.4 % at 240 hours, $p < 0.05$) which was in comparable range with reference standard glibenclamide (65.8 % reduction at 0.5 mg/kg dose level). Column Chromatographic separation (Silica gel, hexane:ethyl acetate mixtures) and reverse-phase preparative thin-layer chromatography of the ethyl-acetate fraction resulted in the isolation, for the first time from *T. africana*, of secondary metabolites characterized as 3-prenyl – 2'-4,4'- trihydroxy- chalcone and bergapten (IR, NMR, MS). **Reference**: 1. Irvine, F.R. (1961), Woody Plants of Ghana, Oxford Univ. Press.

P 330

Volatile Composition and Cyclooxygenase (COX) inhibitory effect of Stachys setifera C. A. Mey

Khanavi M[1], Hadjiakhoondi A[1], Sharifzadeh M[2], Shafiee A[3], Rustaiyan A[4]
[1]Department of Pharmacognosy, Medicinal Plant Research Center and Pharmaceutical Sciences Research Center (a), Faculty of Pharmacy, Tehran University of Medical Sciences, P.O.Box: 14155/6451, Tehran, Iran;
[2]Department of Toxicology and Pharmacology, Medicinal Plants and Pharmaceutical Sciences Research Centers (b), Faculty of Pharmacy, Tehran University of Medical Sciences, P.O.Box: 14155/6451, Tehran, Iran;
[3]Department of Chemistry, Medicinal Plant and Pharmaceutical Sciences Research Centers (c), Faculty of Pharmacy, Tehran University of Medical Sciences, P.O.Box: 14155/6451, Tehran, Iran; [4]Department of Chemistry (d), Sciences and Research campus, Islamic Azad University, P.O.Box: 14515 – 775, Tehran, Iran

The genus Stachys L. from lamiaceae family comprises about 300 species in the world [1]. Iran is an area particularly rich in taxa with more than 34 species including *Stachys setifera* C.A.Mey [2]. The chemical composition of the essential oil and COX inhibitory effect of aerial parts of *S. setifera* has not yet been described. The oil obtained by steam distillation of the aerial parts of the plant was analyzed by GC and GC/MS. It was rich in eugenol (21.1 %), hexadecanoic acid (12.5 %) and linoleic acid (11.0 %). Because of some previous reports about modulatory effect of eugenol on cyclooxygenase pathway [3], anti-inflammatory activity of methanolic extract of aerial parts of *S. setifera* was investigated in this study. For assessment of anti-inflammatory properties, two well-characterized inflammatory models, formalin test and carrageenan-induced paw edema were used. Interaperitoneal injections of methanolic extract (50,100, 200 mg/kg), 30 min before formalin injection, had no effects against the first phase of the formalin- induced pain, but all three doses caused a significant blockade on the second phase ($P < 0.01$, < 0.001). In the carrageenan-induced paw edema, each extract revealed dose-related inhibitory effects over the dose range 50 –

200 mg/kg. The anti-inflammatory activity of *S. setifera* was comparable with high dose of indomethacin (5 mg/kg). In conclusion, the present findings provide further evidences for inhibitory effects of these extracts in inflammatory processes via possible interactions with cyclooxygenase (COX). **References**: 1. Rechinger, K.H. (1982), Flora Iranica. Akademiche Druck-u. Verlagsanstalt, Graz-Austria. 2. Mozaffarian, V. (1996), A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran-Iran. 3. Kelm, M.A. *et al.* (2000), Phytomedicine 7:7 – 13.

P 331

Discrimination of Piper longum and Piper retrofractum fruits by chromatographic fingerprint analysis

Bauer R[1], Stöhr J[2]
[1]Institute of Pharmaceutical Sciences, Department of Pharmacognosy, Karl-Franzens-University Graz, Universitätsplatz 4/I, A-8010 Graz, Austria;
[2]Institute of Pharmaceutical Biology, Heinrich-Heine-Universität, 40225 Düsseldorf, Germany

In the Pharmacopoeia of the Peoples Republic of China the fruits of *Piper longum* L. are considered as "Bibo". According to our investigations with a series of commercial batches of bibo, most of the samples were derived from *Piper retrofractum* Vahl. although this is not an accepted substitute. Several books dealing with the Chinese Materia Medica clearly describe properties of *Piper retrofractum* under the chapter "*Piper longum L.*" and even illustrate them with pictures of this allied species. The macroscopic description of the drug in the pharmacopoeia does not unambiguously allow the destinction of both species. Therefore chromatographic methods have been developed for a clear identification. Peaks have been identified by LC-MS [1]. The HPLC-fingerprint of *P. retrofractum* only shows one predominant peak (piperine) and a very small one of retrofractamide A. In contrast to the chromatogram of *P. longum* retrofractamides B and D, and N-isobutyl-2*E*,4*E*-octadecadienamide can be detected. The HPLC-chromatogram of *Piper longum* shows a homogeneous distribution of numerous peaks, with piperine and pellitorine as the predominant compounds. *Piper retrofractum* and *Piper longum* can also easily be distinguished by TLC under UV_{254nm} and UV_{365nm} [2]. **References**: 1. Stöhr, J. *et al.* (2001), J. Ethnopharmacol. 75:133 – 139. 2. Wagner, H. et al. (2006), Chinese Drug Monographs and Analysis, in press.

P 332

In vitro effect of BA and NAA on growth and development of Pueraria candollei Grah. ex. Benth. var. mirifica

Chawapun A, Dheeranupattana S, Jatisatienr A, Jatisatienr C
Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Shoot of *Pueraria candollei* were cultured on MS medium with 0, 0.25, 0.5, 1.0 and 2.0 mg/L BA found that medium supplemented with 0.5 and 1.0 mg/L BA induced the greatest number of multiple shoots formation at 5.3 and 5.2 shoots/explant respectively for 12 weeks. Shoot of *Pueraria candollei* from natural were cultured on MS medium supplemented with 1 and 2 mg/L BA plus 1 mg/L NAA. It was found that MS medium with 2 mg/L BA plus 1 mg/L NAA induced the greatest diameter of callus (2.0 cm) for 4 weeks and shoots formation were induced the greatest number of 4.4 shoots/explant and root formation were induced the highest at 60 % on MS medium with 1 mg/L BA plus 1 mg/L NAA for 10 weeks. *Acknowledgements*: Chiang Mai University, Plant Tissue Culture Unit **References**: 1. Dougall, O.K. (1981), Tissue culture and the study of secondary (nature) product, In Conn, E.E. (ed.), The biochemistry of Plant. Vol. 4 New York: Academic Press. 2. Kashemsanta, L., Subatabandhu, K. and Bartlett, S. (1963), Estrogenis substance Zmiroestrol from the tuberous roots of *Pueraria mirifica*. Proc. Pacific Sci Assoc 9th Bangkok Thailand. 5: 37 – 40. 3. Matkowski, A. (2004), J. Plant Phys. 161: 3. 343 – 346. 4. Rao, A.N., Lee, S.K. (1986), An overview of the *in vitro* propagation of woody plants and plantation crops. In Wither, L.A. and Aldersan, P.C. (eds.), Plant cell tissue and its agricultural application. Singapore: Butterworths. 5. Thiem, B. (2003), Plant Science. 165 (5): 1123 – 1128. 6. Yu, S., Li, L. (1999), Plant Resoures and Environment 8: 63 – 64.

P 333

In vitro secondary compound production from roots of Stemona curtisii

Palee J[1], Dheeranupattana S[1], Jatisatienr A[1], Jatisatienr C[1]
[1]Department of Biology, Faculty of Science, Chiang Mai University, 50200, Chiang Mai, Thailand

Stem explants of *Stemona curtisii* Hook. f. plantlets from *in vitro* plantlets were cultured on MS agar media supplemented with 11 concentrations of NAA (0 – 6.0 mg/L) and in liquid media supplemented with 6 concentrations of NAA (0 – 6.0 mg/L) for 12 weeks. It was found that the MS agar medium containing 1.0 mg/L NAA and the MS liquid medium containing 3.0 mg/L NAA induced 100 % roots formation with the highest average number of 21.53 and 17.44 roots per explant, respectively. Apical roots were then cultivated in the MS liquid media supplemented with 10 concentrations of NAA (0 – 3.0 mg/L) for 8 weeks. It was found that the MS medium containing 0.3 mg/l NAA induced 60 % lateral roots with the highest average number of 8 lateral roots per explant. Secondary compounds were extracted with 95 % ethanol from *S. curtisii* roots cultured on the agar and in liquid MS media supplemented with 7 concentrations of NAA (0 – 1.0 mg/L). The crude extracts was then separated by TLC using dichloromethane: methanol: ammonia (95: 5: 1) as the mobile phase. In the initial experiment, stemocurtisine was not found in the tissue cultured roots. However, the unknown nitrogenous secondary compound was detected. *Acknowledgements*: We are grateful to the Ministry of National Research and Environment for supporting this project. **Reference**: 1.Mungkornasawakul, P. *et al.* (2003), J. Natural Pros. 66(7): 980 – 982. 2.Babu, K.N. *et al.* (1993), Hort. Abstr. 63(7): 5386.

P 334

Ascorbic acid in Iranian Rose species (2)

Asgari T, Amin G, Hamidian Jahromi A
Faculty of Pharmacy, Tehran University of Medical Sciences, Pursina Ave, 14155 – 3451, and Faculty of Pharmacy, Azad university, Tehran, Iran

Fruits of Roses specially *Rosa canina* L. are one of the richest source of Ascorbic acid (AA) and have the best effect on human body. Iran is the main habitat of Roses in the world, and there are many native and endemic species. Previously three species (*R. foetid* J. Herrm., *R. boissierii* Crep., *R. hemisphaerica* J. Herrm.) were investigated and AA was (1000 – 4000) mg/100 g. In the recent study two other species (*R. canina, R. beggeriana Schrenk.*) were collected from Taleghan in N.W of Tehran, identified and their voucher specimen were deposited in the Herbarium of faculty of pharmacy, Tehran university of medical sciences. Their pulps were dried and extracted via decoction method. AA were assayed via two methods of titrimetric (by2, 6-dichlorophenolindophenol) and spectrometric (by 2, 6-dinitrophenylhydrazine) with providing of calibration curve. Range of AA in these two species was 2200 and 4658 mg/100 g, respectively. While there is reported that the range of AA or Rosa species is 1095 – 6694 mg/100 g. In conclusion the range of AA and diversity of *R. canina* and *R. beggeriana* in Iran is very noticeable and will a good source of natural products. *Acknowledgments*: Safar Ali asgari, Lina mehrabadi-e-yari. **Refrence**: Joublan, J.P., M. Berti, H. *et al.* (1996), Wild rose germplasm evaluation in Chile. p. 584 – 588. In: J. Janick (ed.), Progress in new crops. ASHS Press, Arlington, VA.

P 335

Flavonoids, volatiles and biological activities of the aerial parts of Calliandra haematocephala Hassk

Zeid AA[1], Hifnawy M[2], Saleh M[1], Sleem A[3], Mohamed R[1]
[1]Pharmacognosy Dept., National Research Centre, El-Tahrir St, Dokki (12622), Cairo, Egypt; [2]Pharmacognosy Dept, Faculty of Pharmacy, Cairo Univ, Kasr El-Einy, Cairo, Egypt; [3]Pharmacology Dept, National Research Centre, El-Tahrir St, Dokki (12622), Cairo, Egypt

The genus Calliandra (Fabaceae) contains 132 species. Most of them are native of America, but few of Asia and Africa. The current study deals with isolation and identification of flavonoids, as well as, investigation of volatile constituents and biological activity of extracts of the aerial parts of *Calliandra haematocephala*. The total ethanol extract, as well as, successive extracts (petroleum ether, chloroform, ethyl acetate and methanol) were prepared from the dried powdered plant. The flavonoids were isolated from the ethyl acetate extract by using Silica gel column chromatography. The isolated compounds were finally purified by Sephadex LH – 20 column [1]. Three flavonoid aglycones, quercetin, kaempferol, myricetin, as well as, three flavonoid glycosides, quercetin-3-O- rhmnopyranoside, keampferol-3-O-(2"-O-galloyl)- rhamnopyranoside and myricetin-3-O-(2",3"-di-O-galloyl)-rhamnopyranoside were identified by determination of UV, ^{1}HNMR, ^{13}CNMR spectra and hydrolytic products [1, 2]. The volatile constituents of fresh aerial parts were prepared by hydro-distillation using Nikerson's apparatus and analyzed by GC/MS analysis. Sixty four compounds were identified representing 93.32 % of the total volatiles of the plant. The oxygenated and non-oxygenated compounds constituted 72.34 % and 20.98 %, respectively. The LD_{50}, analgesic, antipyretic, anticonvulsant, anti-ulcer activity of the successive extracts, as well as, the antioxidant activity of the isolated compounds were investigated. The results of the biological activity tests were statistically analyzed using the student's "t" test [3]. Most of the tested extracts were found to be significantly active. **References**: 1. Mabry, J., Markham, K. (1970), The Systematic Identification of Flavonoids, Springer Verlag, Berlin. 2. Markham, K. (1982), Techniques of Flavonoid Identification, Academic Press, London. 3. Snedecor, W., Cochran, G. (1982), Statistical methods 10th ed, Iowa State, University Press, USA.

P 336

Efficient production of Sundew (Drosera rotundifolia L.) in vitro using a temporary immersion system

Kopp B, Wawrosch C, Buol I, Dorfer T
Department of Pharmacognosy, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria

The carnivorous plant *Drosera rotundifolia* has been used since centuries against affections of the respiratory tract. Its spasmolytic, antibacterial and antiinflammatory properties are attributed to naphthoquinones and flavonoids [1]. In the last decades this species became very rare due to degradation of the natural habitats. The crude drug obtained from various other species is of poor quality, and adequate quantities are difficult to obtain [1]. *In vitro*-culture can be an alternative in that uniform plants for further field culture can be produced [2]. In addition, the contents of active compounds in material obtained *in vitro* can be higher than under field conditions [3]. Thus, a biotechnological approach to the production of fresh plant material (*e.g.* for homeopathic use) offers interesting perspectives. Frequently, a major obstacle to a more widespread application of tissue culture for plant production is elevated costs resulting from labour [4] and expensive nutrient media. The use of temporary immersions systems with liquid nutrient medium can be highly efficient in reducing production costs. Furthermore, the overall efficiency of this micropropagation technique concerning multiplication, biomass yields, and plant quality is substantially higher than in conventional systems using semisolid media [4]. In this contribution an *in vitro*-culture system based on temporary immersion is presented. By adjusting frequency and duration of the immersion multiplication and biomass yield could be improved when compared to the control in submerged culture. The results indicate that this system would allow for the efficient production of plants for field culture as well as crude drug material. **References**: 1. Krenn, L., Kartnig, T. (2005), Z. Phytotherapie 26: 197 – 202. 2. Wawrosch, C. *et al.* (1996), Sci. Pharm. 64: 709 – 717. 3. Wawrosch, C. *et al.* (2005), Sci. Pharm. 74: 251 – 262. 4. Etienne, H., Berthouly, M. (2002), Plant Cell Tissue Organ Cult. 69: 215 – 231.

P 337

Is the Alkaloid Pipermethystin Connected with Liver Toxicity of Kava Products?

Lechtenberg M[1], Quandt B[1], Schmidt M[2], Nahrstedt A[1]
[1]Institute of Pharmaceutical Biology and Phytochemistry, Univ. of Münster, Hittorfstr. 56, D-48149 Münster, Germany; [2]Herbresearch Germany, Wartbergweg 15, D-86874 Tussenhausen-Mattsies, Germany

Recently, a worldwide discussion on the potential liver toxicity of extracts obtained from Kava (Piperis methystici rhizoma) was initiated by a series of reports resulting in a ban by the German Federal Institute for Drugs and Medicinal Products (BfArM) that was followed by other countries [1]. However, most cases were evaluated as "doubtful" on causality assessment [2]. Several theories evolved as to why liver failure may have occurred [1]. Dragull et al. [3] suggested the alkaloid pipermethystin being responsible for hepatotoxicity. We therefore investigated various kava preparations including a series of retain samples of kava extract containing products from the German market, self-produced extracts from root and stem material obtained from two identified kava cultivars ("noble kava" Ava La'au from Samoa, "Tudei kava" Palisi from Vanuatu; extracted with ethanol 96 % respectively acetone 75 % or 100 %), and an extract from the leaves of *Piper methysticum* G. Forst. (as a positive control). Samples were analyzed for their content of pipermethystin by GC-ESI-MS using total ion currency (TIC) and selective ion monitoring (SIM) detection. Limit of detection (LOD) was about 0.009 %. As a result, pipermethystin was detected in the leaves (0.2 %), but no pipermethystin above LOD was detected in all other samples except one where a peak below 0.02 % was found at the position corresponding to pipermethystin. Thus, if there is hepatotoxicity, it should not be connected to the alkaloid pipermethystin. *Acknowledgemenst*: We thank Dr. K. Dragull, Univ. of Hawaii-Manoa in Honolulu, for a sample of pure pipermethystin. **References**: 1. Anke, J., Ramzan, I. (2004), Planta Med. 70: 193 – 196. 2. Schmidt, M. *et al.* (2002), Wien. Med. Wochenschr (WMW) 152: 382 – 388. 3. Dragull, K. *et al.* (2003), Phytochemistry 63: 193 – 198.

P 338

Effect of drying methods on essential oil content and composition of wormwood (Artemisia absinthium L.)

Khangholi S[1], Rezaeinodehi A[1], Sefidkon F[2]
Department of Horticulture, Faculty of Agricultural Sciences, Shahed University, Tehran, Iran

The aerial parts of wormwood (*Artemisia absinthium* L.) were harvested in full blooming stage in September 2005 from an area between Deylaman and Asiabar villages, around the Siahkal city in Gilan province in north of Iran. In order to complete drying, a sample of aerial parts was placed at shade (room temperature) for several days and a sample placed in ventilated oven at 35°C temperature for 24 h. The aerial parts essential oil was extracted by hydrodistillation in a Clevenger apparatus and analyzed by GC/MS. Results showed that essential oil yields in shade condition and oven condition were 1.3 and 1.1 percent respectively. Also number of chemical components of the essential oil in shade drying and oven drying methods were 28 and 33 components respectively, which were mostly monoterpenes. β- pinene and β- thujone were main components of the both drying methods, which their contents in shade drying and oven drying methods were (23.8 and 18.6 %) and

(18.7 and 27.9 %) respectively. Hydrocarbon monoterpenes content in shade drying method were higher than the other method but in case of oxygenated monoterpenes vice versa. There was not significant difference with respect to sesquiterpenes content between the drying methods. The results proved that chemo type of the studied wormwood essential oil was specific and different from other wormwood essential oil chemotypes, which have been reported.

P 339

Prediction of microbial metabolism of phytochemicals using an in vitro colon model

Aura AM[1], Seppänen-Laakso T[1], Bounsaythip C[1], Oresic M[1], Oksman-Caldentey KM[1]
[1]VTT Technical Research Centre of Finland, Tietotie 2, P.O.Box 1000, FIN-02044 VTT, Finland

Human colon contains 1.5 kg of microbiota, which actively takes part in the degradation and decomposition of the non-absorbable intake. Colonic microbiota changes by age, diet, intestinal diseases and medication causing intra-individual variation in the metabolite pool in addition to the inter-individual variation between subjects. However, a good correlation has been found for dietary phenolic microbial metabolites between the *in vitro* colon model[1] and corresponding metabolite profiles from human body fluids. Traditional prediction of drug metabolism includes several *in vitro* test systems and animal trials. However, microbial metabolism in the colon is not generally used in the pre-clinical stage. To address these challenges, the developed batch *in vitro* colon mode can be coupled with an advanced metabolomics and bioinformatics platform that provide complementary pre-clinical data on the metabolites circulating in the human body. The model can be used for: Identification of toxic metabolites of new phytochemicals Comparison of metabolite profiles of phytochemicals Comparison of the microbial metabolite profile with those from body fluids of man: *in vitro-in vivo* correlation **Reference**: 1. Aura A.M. *et al.* (2002), J. Agric.Food Chem. 50: 1725 – 1730.

P 340

GC-MS Analysis of Eryngium maritumum L. Volatile Oil

Aslan S, Kartal M
Ankara University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Tandogan-Ankara, Turkey

The genus *Eryngium* (Umbelliferae family; Saniculoideas subfamily) is known to contain acetylenes, flavonoids, coumarins and terpenic compounds [1]. *Eryngium* species are represented by 317 species, subspecies and varieties [2]. *Eryngium* species, generally *E. campestre* L. and *E. maritimum* L. are known in Turkish folk-medicine as "Bogadikeni" and widely distributed in all parts of Turkey. Infusions of aerial and underground parts of this species are used in folk remedies as antitussive, diuretic, appetizer, stimulant and aphrodisiac [3]**.** The genus *Eryngium* is represented by 23 species (24 taxa) in the Flora of Turkey and East Aegean Islands which ten of them are endemic [4 – 6]. In the present study, the volatile oil composition of the aerial part of *E. maritumum* was investigated by capillary gas chromatography-mass spectrometry (GC-MS). The oil of was found to be remarkably rich in spathulenol, 1,5-epoxysalvial-4(14)-ene, α-amorphene and caryophellene oxide. **References**: 1. Erdelmeier, C.A.J., Sticher, O. (1986), Phytochemistry 25(3): 741 – 743. 2. Wörz, A. (1999), Stuttgarter Beitr. Naturk. Ser. A, Nr. 596: 1 – 48.3. Baytop, T. (1999), Turkiye'de Bitkilerle Tedavi-Gecmisten Bugune (Therapy with Medicinal Plants in Turkey-Past and Present), 2nd edn. Pp. 169, Nobel Tıp Basimevi, Istanbul, Turkey. 4. Davis, P.H. (1972), Flora of Turkey and The East Aegean Islands. University Press, Edinburgh, Vol. 4: 292 – 304. 5. Davis P.H. et. Al (1988), Flora of Turkey and The East Aegean Islands (Supplement), University Press, Edinburgh, Vol.10: 145. 6. Güner, A. *et. al.* (2000), Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh, vol.11: 136 – 138.

P 341

Onions of the Allium Subgenus Melanocrommyum – the Better Garlic?

Keusgen M[1], Fritsch RM[2]
[1]Philipps-Universität Marburg, Institute of Pharmaceutical Chemistry, Marbacher Weg 6, D-35032 Marburg, Germany; [2]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany

About 200 *Allium* species occur in the mountainous regions of Central and South-West Asia [1]. The use of especially tasteful and curative members of this family has a long tradition with apparently deep historical roots in several Asian countries. Besides cultivated onion and garlic, also a number of wild species are collected and eaten by local populations of the above mentioned regions. In this area, especially members of the subgenus *Melanocrommyum* are widely distributed and highly estimated as vegetable and medicinal plant. In Tajikistan and in some parts of neighbouring countries where related tribes settled, leaves of *A. rosenbachianum* auct. – this name is used in some scientific literature for *A. rosenbachianum* Regel in a strict sense as well as for *A. rosenorum* R.M. Fritsch – are extensively used. Leaves are often collected and eaten in form of traditional dishes because consumption "refreshes the body after the winter period" [2]. *Allium komarowii* Lipsky owns obviously a rather strong medical activity, because it is used as anabolic drug for horses [2]. Also this kind of activity could not be correlated to high cysteine sulphoxide contents, but these species contain a conspicuous red dye, which is chemically a sulphurpyrrol. *Allium motor* Kamelin et Levichev is often used in a similar manner as *A. rosenbachianum* in parts of Uzbekistan. The term 'motor' means 'health', but application may cause problems for people suffering from high blood pressure [29. The above mentioned examples demonstrate that members of the subgenus *Melanocrommyum* are used as medicinal plants with huge variety of applications and are even higher estimated as garlic. However, the active principle of these plants is still unknown and needs further investigation. *Acknowledgements*: Research was supported by the German VolkswagenStiftung as part of the "PharmAll"-project. **References**: 1. Khassanov, F.O. (1996), Plant life in Southwest and Central Asia, EGE University Press. Izmir.141 – 159. 2. Keusgen, M., Fritsch, R.M. *et al.* (2006), J. Ethnobiol. Ethnomed. 2: 18.

P 342

Matrix free MALDI mass spectrometry for phytochemical investigations

Hashir MA[1], Stecher G[1], Abel G[2], Popp M[2], Bonn GK[1]
[1]Institute of Analytical Chemistry and Radiochemistry, University of Innsbruck, Innrain 52a, 6020, Innsbruck, Austria; [2]Bionorica AG; Kerschensteinerstr. 11 – 15, 92318 Neumarkt, Germany

Matrix assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF-MS) is a very sensitive mass spectrometric technique which utilizes acidic materials as matrices for laser energy absorption, desorption and ionisation of bio-molecules such as peptides and proteins. These matrix materials produce background peaks particularly in the low mass range and make the detection and identification of small molecules difficult. Therefore, some efforts have been made to develop matrix free MALDI for the analysis of small molecules. For this purpose a neutral substance was immobilized on a carrier system in order to enable absorption of laser energy sufficient for successful desorption and ionization of analytes of low mass without interference and fragmentation [1]. For comparison some already existing other systems [2 – 5] were reproduced and compared with the new system, resulting in spectra of highest quality by means of pure spectra with high signal intensity

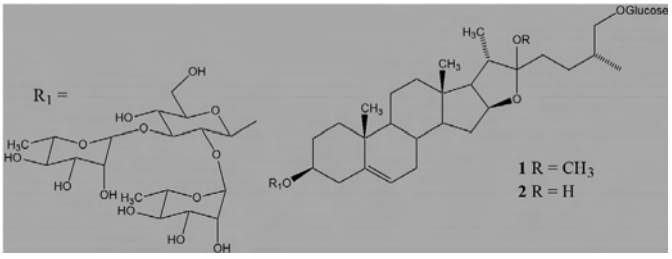
and low noise for the newly synthesized material. **References:** 1. Bonn, G.; Hashir, M.A.; Stecher, G., Bakry, R. (2006), Patent pending. 2. Mohr, M.D., Börnsen, O.K., Widmer, H. M. (1995), Rapid Commun. Mass Spectrom. 9: 809 – 814. 3. Papac, D.I., Wong, A., Jones, A.J.S. (1996), Anal. Chem. 68: 3215 – 3223. 4. Shen, Z., Thomas, J.J. *et al.* (2001), Anal. Chem. 73: 612 – 619. 5. Zhang, Q., Zou, H. (2001), Rapid Commun. Mass Spectrom. 15: 217 – 223.

P 343

New Furostanol Glycosides from the Rhizomes of Tacca integrifolia

Heilmann J[3], Htay Shwe H[1], Aye M[2], Sein MM[2], Kreitmeier P[1], Reiser O[1]
[1]Institute of Organic Chemistry, University of Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany; [2]Department of Chemistry, University of Mandalay, Mandalay; Myanmar; [3]Institute of Pharmacy, University of Regensburg, Universitätsstr. 31, 93053 Regensburg, Germany

The rhizomes of *Tacca integrifolia* Ker. Gawler (Taccaceae), a perennial plant growing in central region of Myanmar, are used in traditional medicine for the treatment of carbuncles, skin abrasion, skin diseases and various kinds of cancer. Previous studies on other *Tacca* species revealed the presence of highly oxygenated steroids named taccalonolides [1, 2] as well as sterol saponins [3]. Phytochemical investigation of the methanol extract of the rhizomes of *T. integrifolia* let to the isolation of two new furostanol type saponins, namely (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-methoxyfurost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (**1**) and (25*R*)-26-[(β-D-glucopyranosyl)oxy]-22α-hydroxyfurost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside (**2**), along with the known spirostanol type saponin, diosgenin-3β-O-α-L-rhamnopyranosyl (1→2)-O-α-L-rhamnopyranosyl-(1→3)-O-β-D-glucopyranoside.



References: 1. Tinley T.L. *et al.* (2003), Cancer Res. 63, 3211 – 3220. 2. Mühlbauer A. *et al.* (2003), Helv. Chim. Acta 86: 2065 – 2072. 3. Yokosuka A. *et al.* (2004), Chem. Pharm. Bull. 52: 1396 – 1398.

P 344

Cosmetic applications of selected Various Citrus fruits

Lee CW[1], Kim HS[1], Kim DH[1], Kim GO[2], Choi SY[3], Kim SJ[4], Chang IS[1]
[1]R&D Center, Amore-Pacific Corporation, 314 – 1, Borari, Kiheung-eup, Yonin-si, Kyounggi-do, 449 – 729, South Korea; [2]Hi-Tech Industry Development Institute, Jeju, 690 – 121, South Korea; [3]Technology Innovation Center for Life Science, Cheju National University, Jeju, 690 – 756, South Korea

Because tyrosinase catalyzes melanin synthesis, tyrosinase inhibitors are important in cosmetic skin-whitening. Oxidative stress contributes to skin aging and can adversely affect skin health, which means antioxidants active in skin cells may support skin health. Total 50 citrus species involved jeju citrus fruits 17 species were cultivated in Jeju-island (in South Korea). And Citrus Unshiu is the most cultivated with compared other species of citrus in Jeju-island. We examined traditional citrus 17 species that might be useful for skin-whitening and skin health. Extracts (50 g/mL) were tested for cytotoxicity on B16 melanoma cells; 17 exhibited low cytotoxicity. Their effects on tyrosinase and melanin inhibitory activities and free radical scavenging activities were further assessed. Three Citrus fruits, Immature *Citrus unshiu* Marc., *Citrus hassaku* Hort. ex Tanaka and *Citrus sinensis* x *reticulata* exhibited potent inhibitory effects on melanin formation. Immature *Citrus unshiu, Citrus hassaku, and Citrus sinensis* x *reticulata* showed good antioxidative activities. Among active making them the strongest candidates for cosmetic application found in the current study. **References**: 1. Aburjai, T., Natsheh, F.M. (2003), Phytotherapy Research 17: 987 – 1000. 2. Baurin, N., Arnoult, E., Scior, T. *et al.* (2002), J. Ethnopharmacol. 82: 155 – 158. 3. Briganti, S., Camera, E., Picardo, M. (2003), Pigment Cell Research 16: 101 – 110. 4. Lee, S.H., Choi, S.Y. *et al.* (2002), Biol. Pharmaceutic. Bull. 25: 1045 – 1048. 5. Lee, K.T., Lee, K.S. *et al.* (2003), J. Cosmetic Science 54: 133 – 142. 6. Roh, J.S., Han, J.Y. *et al.* (2004) Biol. Pharmaceutic. Bull. 27: 1976 – 1978. 7. Sawai, Y., Moon, J.H. (2000), J. Agric. Food Chem. 48: 6247 – 6253. 8. Yoon, J.S., Sung, S.H. *et al.* (2004), Arch. Pharm. Res. 27: 589 – 592.

P 345

Quality caracterisation of propolis tinctures by pharmacopoeial parameters and wax content

Cvek J[1], Zubčić S[1], Vitali D[2], Vedrina-Dragojević I[2], Tomić S[1], Medić-Šarić M[2]
[1]Agency for medicinal products and medical devices, Ksaverska cesta 4, 10000 Zagreb, Croatia; [2]Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia

The increasing use of propolis preparations nowdays requires setting of clear criteria for their quality control. For that purpose, ten tinctures of propolis samples from different Croatian regions were subjected to analysis of general pharmacopoeial parameters wich are fundamental for the creation of quality specification. These are relative density (determined using instrument Mettler Toledo DE40 Density Meter), dry residue of extract (determined according to Ph. Eur. 5.0 method), and content of ethanol and its possible impurities – methanol and isopropanol (developed and validated gas chromatography method for their simultaneous analysis was applied). Additionally, by the method of Woisky and Salatino we determined the content of waxes as the main inactive constituents in order to determine the level of their migration from crude propolis samples to the prepared tinctures (extraction solvent: 80 % V/V ethanol; drug – preparation ratio = 1:10) [1]. Relative density values increased along with the increase of dry residue of extract (*e.g.* the lowest values were determined in propolis tincture from South Dalmatian Islands: d_{20} = 0.8688, $RSD_{(n=2)}$ = 0.42 % and dry residue = 4.40 % w/w, $RSD_{(n=2)}$ = 0.80 % while the highest values were obtained for propolis tincture from central Croatia: d_{20} = 0.8841, $RSD_{(n=2)}$ = 0.01 % and dry residue = 7.62 % w/w, $RSD_{(n=2)}$ = 0.28 %). Investigated validation parameters for GC method satisfied the acceptance criteria (correlation coefficient more than 0.999; precision: $RSD_{(n=21)}$ = 0.60 %; average $recovery_{(n=21)}$: 99.08 %; DL = 0.001 % for methanol and 0.002 % for isopropanol; QL = 0.003 % for methanol and 0.006 % for isopropanol). Ethanol content was in range from 73.98 to 77.74 % V/V ($RSD_{(n=3)}$ = 0.06 – 2.43 %) which is in accordance with USP 29 requirement for alcohol content in herbal extracts (90 – 110 % of declared amount). Contents of methanol and isopropanol were below detection limits. Presence of waxes was not observed in propolis tinctures indicating the suitability of applied extraction method. **Reference**: 1. Woiski, R.G., Salatino, A. (1998), J. Apic. Res. 37:99 – 105.

P 346

Impact of kava cultivar, plant part and extraction medium on in-vitro cytotoxicity of kava (Piper methysticum) in HepG2 and Hep3B cells

Schmidt M[2], Gebhardt R[1]
[1]Institut für Biochemie, Universität Leipzig, Liebigstr. 16, D-04103 Leipzig, Germany; [2]Herbresearch Germany, Wartbergweg 15, D-86874 Tussenhausen-Mattsies, Germany

Preparations from kava (*Piper methysticum* G. Forst.) have been banned based on the suspicion of adverse liver effects. To date, no convincing proof has been given to substantiate the danger of a relevant toxicity. We systematically tested kava extracts prepared with acetone or ethanol from two different cultivars, both used for kava extract production: Ava Laau from Samoa, a "noble kava", and Palisi from Vanuatu, a "Tudei kava" ("two-day" lasting effect). We also tested the influence of aerial parts (stem peelings) on toxicity. *Methods:* Extracts were prepared and characterized by the working group of Prof. Nahrstedt at the University of Münster (Germany). Kava plant material was obtained from cultivations. Extracts were tested in HepG2 and Hep3B liver cells, using the MTT test, the Rezasurin blue assay, quantification of LDH leakage and measurements of intracellular ATP and GSH contents. *Results:* Only gradual differences in cytotoxicity were found. The sequence of toxicity for ethanolic extracts was roots (noble) < peelings (noble) ≤ roots (Tudei) < peelings (Tudei). In the case of extracts prepared with acetone the toxicity of the Tudei-material was partly reversed: peelings (Tudei) < roots (Tudei). In no case were the EC_{50} values in a relevant dosage range (1250 to > 5000 µg/mL for roots, 800 to > 5000 µg/mL for peelings in the MTT test and rezasurin blue assay, with the highest toxicity found with Tudei peelings in the rezasurin blue test in Hep 3B cells). **Conclusions:** No hint on relevant liver cell toxicity was found in this battery of *in vitro* models.

P 347

Effects of chronic administration of Ginkgo biloba extract (EGb 761®) on levels of dopamine, noradrenaline and serotonin in the prefrontal cortex of the awake rat

Kehr J[1,2], Nöldner M[3], Yoshitake T[1,4]
[1]Dept. of Physiology and Pharmacology, Karolinska Institutet, Nanna Svartz väg 2, 171 77 Stockholm, Sweden; [2]Pronexus Analytical AB, Karolinska Science Park, Fogdevreten 2a, 171 77 Stockholm, Sweden; [3]Preclinical Reseach, Dr. Willmar Schwabe GmbH & Co. KG, Willmar-Schwabe-Str. 4, 76227 Karlsruhe, Germany; [4]Dept. of Pharmaceutical Sciences, International University of Health and Welfare, 2600 – 1, Kitakanemaru, Ohtawara-shi, Tochigi 324 – 8501, Japan

The special Ginkgo biloba extract (EGb 761®) has been shown to exert beneficial effects in the therapy of age-related neurological disorders such as Parkinson's and Alzheimer's disease [1]. Besides neuroprotective effects, EGb 761® has been demonstrated to improve cognitive functions in animal models [2] and in clinical studies [3]. Thus, it was the aim of the present study to investigate whether EGb 761® influences monoamine levels in brain areas implicated in cognitive function, motivation and mood behaviour. For this purpose, the effect of sub-chronic (14 days) daily administration of EGb 761® on basal extracellular levels of dopamine (DA), noradrenaline (NA) and serotonin (5-HT) were monitored by microdialysis in the prefrontal cortex of awake rats. Administration of EGb 761® at a dose of 100 mg/kg p. o., caused a significant increase in DA levels during 40 – 180 min, reaching a maximum level of 163 % of the control group. The extracellular levels of NA increased only by about 120 % and the concentrations of 5-HT were not changed from the pre-drug levels. These findings supports previous observations suggesting that Ginkgo biloba extracts could increase monoaminergic function via inhibition of MAO activity. However, the minimal effects on NA and 5-HT concentrations indicate that EGb 761® may affect brain monoaminergic system also through other mechanisms than direct inhibition of MAO activity. These results suggest that treatment with EGb 761® can lead to increased dopaminergic function in the prefrontal cortex which may be an underlying factor to clinically observed effects on improved cognitive function. **References**: 1. Andrieu, S. *et al.* (2003), J. Gerontol. A Biol. Sci. Med. Sci. 58: 372 – 377. 2. Müller, W.E., Chatterjee, S.S. (2003), Pharmacopsychiatry 36 (Suppl.1): S24. 3. Mix, J.A., Crews, W.D. (2002), Hum. Psychopharmacol. Clin. Exp. 17: 267 – 277.

Author's Index

The numbers refer to the pagination

Author's Index Planta Med 2006; 72: 961–1089 Georg Thieme Verlag KG Stuttgart · New York · ISSN 0032-0943