53rd ANNUAL CONGRESS

FLORENCE, ITALY

AUGUST 21ST-25TH, 2005

PLVS VLTRA

A JOINT CONGRESS WITH

SOCIETÀ ITALIANA
DI FITOCHIMICA

INDEX

ORGANIZATION

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Welcome to the 53rd Annual Meeting of the Society of Medicinal Plant Research (GA) and Joint Congress with the Italian Society of Phytochemistry (SIF) in Florence, with patronage from the Italian Health Ministry, the University of Florence, the Region of Tuscany, the Cassa di Risparmio di Firenze, and ARSIA.

Participation in this Congress by those in the academic world and industry who carry out research and work in the field of drugs of plant origin has been nothing less than massive; thus, affirming that also this year, the GA Congress is the most important event for presenting scientific works in this field, for discussing them and for comparing and exchanging knowledge with other scientists from all over the world.

In this volume of proceedings for the Congress you can find 650 abstracts, which include 9 main lectures, 6 workshops, 53 short lectures, and 582 poster presentations submitted by scientists from about 70 countries, representing the state of the art and most significant scientific progresses in the field of herbal medicinal products and health botanical products. This wealth of material gives us, the Organizing Committee, great pleasure and highlights the topicality of the main areas selected by the Scientific Committee: *metabolomics, biopharmaceutical aspects of herbal medicinal products, prevention of cancer and cardiovascular diseases with botanical health products, traditional herbal medicinal products from non EU countries,* as well as *classical pharmacological research on natural products.*

A new area is being introduced at the Congress with regard to young researchers. A special workshop has been dedicated to them, alongside those of the Permanent Committees on Herbal Medicinal Products of the GA. This initiative was born of the needs expressed by many young researchers to have their own space where it would be possible to discuss not only their research but also the difficulties they face. This workshop will take place in the afternoon on Sunday the 21st, before the Get Together Party. If, as we hope, this initiative meets with success, it could be repeated at future GA Congresses.

The motto for this Congress is *Plus ultra* (Latin expression meaning 'go beyond').

We chose this not as an invitation but to represent our awareness that we must always go forward; in other words, to go past the columns of Hercules, which in antiquity symbolized the impassable limits of the known world. In order to succeed in the endeavour we must combine not only skill and perseverance, but also a constant up-dating of our knowledge.

Yet, going forward does not mean forgetting the past, represented by both the knowledge and people who have handed it down to us. In this sense, I'd like to mention the late Prof. Ivano Morelli, President of the SIF and co-chairman of this Congress. Prof. Morelli was a fundamental point of reference for all of us, both as a scientist and as a man. I had the privilege of being his close friend and, as such, I would like to invite all of you to contribute to the success of this Congress in his memory.

Again, on behalf of the Organizing Committee I would like to wish you a warm welcome to the Congress and to Florence, a city of science and art which I am sure will send leave you with pleasant memories.

Best wishes,
Franco F. Vincieri

ACKNOWLEDGEMENTS

The Organizing Committee wants to express its gratitude to the following companies and institutions for financial support of the 53rd Annual Congress of the Society for Medicinal Plant Research

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SPECCHIASOL

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SCIENTIFIC PROGRAM

SUNDAY, AUGUST 21ST, 2005

14:00-18:30 Congress Center, Palazzo dei Congressi, Secretariat
Registration

14:30-18:00 Congress Center, Palazzo dei Congressi, Sala Verde
Workshop for Young Researchers
Chair : A. Hensel
Co-chairs : A.R. Bilia, A. Deters, M. Kuesgen , J.-L. Wolfender

18:00-18:30 Congress Center, Palazzo dei Congressi, Sala Verde
Donation of SIF Travel Grants

19:00-21:00 Congress Center, Anfiteatro
Get Together Party
(Snacks and drinks will be served)

MONDAY, AUGUST 22ND, 2005

8:00-18:30 Congress Center, Palazzo dei Congressi, Secretariat
Registration

9:00-10:30 Congress Center, Palazzo dei Congressi, Auditorium
Opening Ceremony of the 53rd Annual Meeting of the Society for Medicinal Plant Research

Donation of Awards and Grants

Egon Stahl Award Lecture

10:30-11:00 Congress Center, Palazzo dei Congressi, Passi Perduti
Coffee Break

11:00-12:30 Congress Center, Palazzo dei Congressi, Auditorium
Plenary Lectures: PL001 and PL002
Chairs: R Bauer, F. F. Vincieri

11:00 **R. Verpoorte, Y.H. Choi, H.K. Kim**
Division of Pharmacognosy, Section Metabolomics, Institute of Biology, Leiden, The Netherlands
Metabolomics: New Opportunities for Pharmacognosists

11:45 **E. Holmes**
Biological Chemistry, Biomedical Sciences, Imperial College, London, United Kingdom
Metabolite Profiling of Natural Products and their Metabolic Consequences

12:30-14:00
Break

12:30-14:00 Congress, Center, Palazzo dei Congressi, Auditorium
Workshop 1
Chair: B. Meier

Workshop of the Permanent GA-committee of Manufacturing and Quality Control of Herbal Remedies. The Practice of Dissolution Testing in Herbal Medicinal Products
A. R. Bilia, W. Knoess, H. Sievers

12:30-14:00 Congress Center, Palazzo degli Affari, Ground Floor
Workshop 2
Chair: I. Szelenyi

Herbal Drug Preparations and Rhinosinusitis – Complex Mixtures to Manage a Complex Disease?
St. Maune, A. Pahl, P. Stierna

14:00-14:45 Congress Center, Palazzo dei Congressi, Auditorium
Plenary Lecture: PL003
Chair: G. Abel

14:00 **H. Derendorf**
College of Pharmacy, University of Florida, Gainesville, Florida, U.S.A.
Pharmacokinetics and Drug Interactions of Herbal Medicinal Products

15:00-16:00 Congress Center, Palazzo degli Affari, Ground Floor
Short Lectures: SL001 – SL004
Chairs: E. Holmes, R. Verpoorte

15:00 **Direct Infusion Ion Trap Mass Spectrometry: Method Development and Applications in Metabolomics**
A. Koulman, K. Fraser, L. Johnson, G.A. Lane, S. Rasmussen

15:15 **Potential of Microflow LC/NMR for the Identification of Natural Products at the µg Level**
J.-L. Wolfender, K. Hostettmann

15:30 **Application of GC-MS and HPLC-DAD for Birch Metabolome Analysis**
S. Ossipova, V. Ossipov, K. Pihlaja

15:45 **Metabolomic Analysis of *Strychnos* Species Extracts by ^{1}H Nuclear Magnetic Resonance Spectrometry and Multivariate Analysis Techniques**
M. Frédérich, Y. H. Choi, L. Angenot, G. Harnischfeger, A. W. M. Lefeber, R. Verpoorte

16:00-16:30 Congress Center, Palazzo degli Affari, First Floor
Coffee Break

15:00-16:00 Congress Center, Palazzo dei Congressi, Auditorium

Short Lectures: SL005 – SL008

Chairs: G. Abel, H. Derendorf

15:00 **Bioavailability and Pharmacokinetic Studies on Echinaforce™ Preparations and their Interaction with the Immune System**
K. Woelkart, A. Suter, C. Koidl, R. Raggam, B. Kleinhappl, E. Marth, R. Bauer

15:15 **Solid State Activation and Self-emulsifying Pellets to Improve the In-vitro Bioavailability of Silymarin in Rats**
F. Meriani, G. Zingone, D. Voinovich, L. Garboni, L. Scalise, A. R. Bilia, F. F. Vincieri

15:30 **Liposomes and β-Cyclodextrin Complexes to Improve Stability of Verbascoside**
A.R. Bilia, A.M. Fadda, M. Innocenti, C .Sinico, D. Valenti, F.F. Vincieri

15:45 **Re-evaluation of Bioactivities of Various Plants (*Origanum dictamus, Thymus, Myrtus* Species) of Greek Origin, Before and After Encapsulation in Liposomes**
I. Chinou, Olga Gortzi, C. Liolios, S. Lalas, I. Tsaknis

16:00-16:30 Congress Center, Palazzo dei Congressi, Passi Perduti

Coffee Break

16:30-18:00 Congress Center, Palazzo dei Congressi, Auditorium

Short Lectures: SL009 – SL014

Chairs: R. Della Loggia, A. Hensel

16:30 **Inhibitory Effects of 5-O-DemethylNobiletin on Lymphocyte Proliferation and the Production of Inflammatory Mediators**
E. Bas, M.C. Recio, J.L. Ríos

16:45 **New Water Soluble Indirubin Derivatives as Selective GSK-3 Inhibitors**
P. Magiatis, P. Polychronopoulos, K. Vougogiannopoulou, M. Kritsanida, A.-L. Skaltsounis, L. Meijer, P. Greengard

17:00 ***Panax ginseng* C.A. Mayer (G115) Modulates TLRs Expression in Mice Under Physical Stress**
M.Pannacci, S. Grosso, V. Lucini , F. Scaglione

17:15 **Neural Networks: Tools for the NF-.B Inhibiting Sesquiterpene Lactones**
S. Wagner, A. Hofmann, B. Siedle, L. Terfloth, J. Gasteiger, I. Merfort

17:30 **Shikonin Selectively Inhibits Splicing of Tumor Necrosis Factor Alpha Transcripts**
S.-C. Chiu, N.-S. Yang

17:45 **Anti-depressant, Anxiolytic and Nootropic Activity of *Murraya koeinigii* Leaves**
M. P. Kulkarni, R. C. Hole, R. S. Nachankar, A. R. Juvekar

16:30-18:00 Congress Center, Palazzo degli Affari, Ground Floor

Short Lectures: SL015 – SL020

Chairs: W. Blaschek, H. Stuppner

16:30 **Determination of the Absolute Configuration of Secondary Alcohols by Combining Mosher's Derivatization Technique with LC-SPE-NMR: Polyacetylene Derivatives from Apiaceae as Case Study**
C. Seger, M. Godejohann, F. Hadacek

16:45 **HPLC-SPE-NMR in Natural Products Research**
J. W. Jaroszewski

17:00 **A New HPLC-UV Method for the Analysis of Antocyanins and Anthocyanidins in *Vaccinium myrtillus* Fruit Extracts**
E. de Combarieu, D. Diliddo, M. Falzoni, L. Fumagalli, N. Fuzzati, R. Pace, P. Scandelli

17:15 **Analysis of Cucurbitacins in *Citrullus colocynthis* by MEKC and HPLC/MS**
S. Sturm, A. Danese, C. Seger, and H. Stuppner

17:30 **NMR for the Screening of Plants Water Extracts: a Rapid Approach to Better Link Ethobotanical Data with Further Phyto-pharmacological Investigations**
M. Politi, M.I. Chávez , J. Alvaro-Blanco, F.J. Cañada, J. Jiménez-Barbero

17:45 **NMR-Spectroscopy of Natural Products Using Micro Probes and Cryogenically Cooled Probes**
D. Moskau, T. Kühn

19:00-21:30 Palazzo Vecchio, Salone dei Cinquecento

Welcome Ceremony

Lectures: **Health Benefits of Wine,** H. Glossmann (Innsbruck Medical University)
Two of a kind: Herbal Medicines and Wine, M. A. Popp (Bionorica AG)

(Wine and Oil Tasting will follow)

Participation for booked people only

TUESDAY, AUGUST 23RD, 2005

8:00-18:30 Congress Center, Palazzo dei Congressi, Secretariat

Registration and information

9:00-10:30 Congress Center, Palazzo dei Congressi, Auditorium

Plenary Lectures: PL004 and PL005

Chairs: M. Delbò, M. Hamburger

9:00 **R. Bauer**
Institute of Pharmaceutical Sciences, Karl-Franzens-Universität Graz, Austria
Scientific Evaluation of Traditional Herbal Medicinal Products from Non EU Countries

9:45 **De-an Guo**
School of Pharmaceutical Sciences, Peking University, Beijing, P.R. China
TCM-based Drug Discovery and Main Issues in TCM Modernization

10:30-11:00 Congress Center, Palazzo dei Congressi, Passi Perduti
Coffee Break

11:00-12:30 Congress Center, Palazzo dei Congressi, Passi Perduti and Galleries
Poster Session I - Discussion

P001 – P015	Botany, Systematics
P016 – P041	Breeding, Cultivation, Cell Cultures
P042 – P053	Biosynthesis, Physiology
P054 – P060	Molecular Biology, Biotechnology
P061 – P233	Biological Activity, Pharmacology and Clinical Studies
P234 – P280	Antioxidants

12:30-14:00
Break

12:30-14:00 Congress, Center, Palazzo dei Congressi, Auditorium
Workshop 3
Chair: A.J. Vlietinck

Workshop of the Permanent GA Committee on Regulatory Affairs of Herbal Medicinal Products. How to Implement the New Legislation on Herbal Medicinal Products (HMPs) in Europe?
K. Keller, V. Silano, L. Kabelitz

14:00-15:30 Congress Center, Palazzo dei Congressi, Auditorium
Plenary Lecture: PL006 – PL007
Chair: F. Kemper, B. Kopp

14:00 **G. Appendino, M. Ballero**
DISCAFF, Novara / Consorzio per lo Studio dei Metaboliti Secondari, Cagliari, Italy
A Tale of Bleeding Ship and Ancient Coins. Biodiversity as the Green Gold of Sardinia

14:45 **H. Wagner**
Department of Pharmacy, Center of Pharmaresearch, University of Munich, Germany
Pharmacological Synergy Effects of Phytopreparations and their Relevance for Therapy

15:30-16:00 Congress Center, Palazzo dei Congressi, Auditorium
Short Lectures: SL021 – SL022
Chair: De-an Guo, A. Nahrstedt

15:30 **Psychotropic Activity of Plants Used in South African Traditional Medicine to Treat CNS-related Diseases**
A.K. Jäger, G.I. Stafford, N.D. Nielsen, M. Sandager, A.B. Svenningsen, K.D. Madsen, A. Adsersen, E.E. Elgorashi, J. van Staden

15:45 **In-vitro Antimalarial and Leukotriene Metabolism Inhibitory Activities of Compounds Isolated from Kniphofia Foliosa Roots**
A. Abebe Wube, Franz Bucar, Simon Gibbons, Kaleab Asres, Michael Adams, Rudolf Bauer, Lauren Rattray, S. L. Croft

15:30-16:15 Congress Center, Palazzo degli Affari, Ground Floor
Short Lectures: SL027 – SL029
Chairs: C. Franz, M. Nicoletti

15:30 **Lignans of *Linum* Species - A Chemosystematic Study and Implications on the Evolution of Chemodiversity in the Genus *Linum***
T.J. Schmidt, S. Hemmati, B. Konuklugil, A. Mohagheghzadeh, E. Fuss, A.-W. Alfermann

15:45 **Cysteine Sulphoxide Pattern of *Allium* L. - Relations to Taxonomy**
R. M. Fritsch, M. Keusgen

16:00 **Medicinal Plants from Cerrado Vegetation, Brazil**
R.F. Vieira, M. V. Martins, L. A. Skorupa, O. A. Silva

16:15-16:30 Congress Center, Palazzo dei Congressi & Palazzo degli Affari
Coffee Break

16.30-17.30 Congress Center, Palazzo dei Congressi, Auditorium
Short Lectures: SL023 – SL026
Chairs: De-an Guo, A. Nahrstedt

16:30 **The Reaction of Artemsinin and Semisynthetic Derivatives with Hemoglobin**
L. Messori, C. Gabbiani, M. Siragusa, F.F. Vincieri, A.R.Bilia

16:45 **Bioactive Components of the Uteroactive Medicinal Plant: *Rhoicissus tridentata***
K.B. Brookes, L.C. Katsoulis

17:00 **Novel Biologically Active Triterpenoids from two African *Combretum* Species**
J. Angeh, J.N Eloff, G. Swan, S. Huangi, I. Sattler

17:15 **Exploring Plant Species from São Paulo State Biodiversity: Structure and Neuropharmacological Properties of Alkaloids from *Erythrina mulungu***
O. A. Flausino Jr, I. C. Gamboa, D. H. S. Silva, V. da S. Bolzani

16:30-17:30 Congress Center, Palazzo degli Affari, Ground Floor
Short Lectures: SL030 – SL033

Chairs: C. Franz, M. Nicoletti

16:30 **Identification and Quantification of Melatonin in *Rosmarinus officinalis* in Relation to Chlorophyl a and b, RNA and Proteins**
M. Pedroli, A. Giorgi, M. Cocucci, A. Conti

16:45 **Hydroxyphenylpyruvate Reductase: Structure and Characterisation of an Enzyme Involved in Rosmarinic Acid Biosynthesis**
V. Janiak, A. Heine, G. Klebe, M. Petersen

17:00 **Elicitation of Lignan Biosynthesis in *Linum nodiflorum* L. by Two Synthetic Indanoyl-aminoacid Conjugates**
A. Berim, O. Spring, W. Boland, M. Petersen

17:15 **Application of Innovative Plant Biotechnology for the Production of Active Compounds from Traditional Herbs, Rare Plants and New Medicinal Plants**
A. Gerth, D. Wilken

17:30-19:00 Congress Center, Palazzo dei Congressi, Auditorium
GA Members' Meeting / Elections

WEDNESDAY, AUGUST 24TH, 2005

8:00-18:30 Congress Center, Palazzo dei Congressi, Secretariat
Registration and Information

9:00-10:30 Congress Center, Palazzo dei Congressi, Auditorium
Plenary Lectures: PL008 and PL009
Chairs: N. Mulinacci, C. Pizza

9:00 **C. Gerhäuser, E. Bertl, H. Bartsch**
German Cancer Research Center (DKFZ), Heidelberg, Germany
Anti-angiogenesis as a Mechanism in Cancer Chemoprevention

9:45 **A. D. Kinghorn**
Div. of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State Univ., Columbus, U.S.A.
Potential Cancer Chemopreventive Activity of Botanical Dietary Supplements

10:30-11:00 Congress Center, Palazzo dei Congressi, Passi Perduti
Coffee Break

11:00-12:00 Congress Center, Palazzo dei Congressi, Auditorium
Short Lectures: SL034 – SL037
Chairs: C. Gerhaeuser, A. D. Kinghorn

11:00 **Phenolic Constituents from *Yucca schidigera* Bark Modulate Kaposi's Sarcoma Cell Proliferation and Motility**
M.L. Balestrieri, C. Balestrieri, I. De Maggio, F. Felice, P. Montoro, W. Oleszek, C. Pizza, S. Piacente

11:15 **Multiple Approaches to Identify New Anti-angiogenetic Compounds from Plant Extracts**
F. Dal Piaz, S. Ponticelli, S. De Falco, N. De Tommasi

11:30 **Antioxidative Effect of Active Plant Extract**
A. Grigorov, B. Schaedlich, J. Lichius, H. Kiesewetter

11:45 **Apoptosis Inducing Activity of Willow Bark Extract (Assalix®) and its Bioactivity Guided Fractions Towards Colon and Lung Carcinoma Cells**
K. Hostanska, G. Jürgenliemk, C. Kotalla, A. Nahrstedt, R. Saller

11:00-12:30 Congress Center, Palazzo degli Affari, Ground Floor

Short Lectures: SL038 – SL043
Chairs: S. Canigueral, R. Hiltunen

11:00 **Marine Pharmacognosy in Swedish Cold Waters - Strategy and Examples**
L. Bohlin

11:15 **Isopimaric Acid and Abietic Acid Are Active against Multidrug-resistant and EMRSA Strains of *Staphylococcus aureus* but Show Antagonism with the MDR Inhibitor Reserpine**
E. Smith, E. Williamson, M. Zloh, S. Gibbons

11:30 **New Insights on How Echinacea Alkylamides Interact with Cannabinoid-receptors and Implications for Immunomodulation**
J. Gertsch, S. Raduner, K-H Altmann

11:45 **Supplementation with a Polyherbal Composite Alleviates Clinical Signs of Respiratory Dysfunction in Horses with Chronic Obstructive Pulmonary Disease**
W. Pearson, A.F.Clarke

12:00 **Choleretic Effects of Yarrow (*Achillea millefolium* s.l.) in the Isolated Perfused Rat Liver**
B. Benedek, N. Geisz, W. Jäger, T. Thalhammer, B. Kopp

12:15 **Pharmacogenomics of Artemisinin Derivatives in Anti-Cancer Therapy**
T. Efferth

12:30-14:00
Break

12:30-14:00 Congress, Center, Palazzo dei Congressi, Auditorium

Workshop 4
Chair: C. Franz

Workshop of the Permanent GA Committee on Breeding and Cultivation of Medical Plants. Good Practices, Standards and Certifications of Starting Materials
B. Pätzold, R. Iguera, B. Steinhoff

12:30-14:00 Congress, Center, Palazzo degli Affari, Ground Floor

Workshop 5

Chair: H. Winterhoff

Workshop of the Permanent GA Committee on Biological and Pharmacological Activity of Natural Products. Tests for Improvement of Learning and Memory

C. Vonhoff, M. F. Melzig

14:00-14:45 Congress Center, Palazzo dei Congressi, Auditorium

Short Lectures: SL048 – SL050

Chair: B. Meier, A. J. Vietlinck

14:00 **The Potential of PCR-related Methods to Identify Medicinal Plants in Herbal Medicinal Products**
W. Knoess, T. Kersten, K. Keller

14:15 **To Consider or not to Consider? – Enzymes in Herbal Drugs and Preparations**
W. Kreis, M. Strupf

14:30 **Natural Products and Batch Consistency: a Hurdle for Herbal Drug Development? Example of PX-6518, a Leaf Extract of *Maesa balansae* with Antileishmania Action**
L. Maes, N. Germonprez, K. Kuypers, M. Vermeersch, P. Cos, D. Vanden Berghe, L. Van Puyvelde

14:45-15:30 Congress Center, Palazzo dei Congressi, Auditorium

Short Lectures: SL051 – SL053

Chair: A. Appendino, M. Curini

14:45 **Bioisosteric Modifications of the Potent and Selective k-Opioid Agonist Salvinorin A**
D. Jeremy Stewart, H. Fahmy, B. L. Roth, Fen Yang, J. K. Zjawiony

15:00 **Glucogalloyl Derivatives: Synthesis and Evaluation of their Antimycotic and PARG-inhibition Activity**
P. Arapitsas, S. Menichetti, P. Buzzini, B. Turchetti, A. Chiarugi, L. Formentini, K. Sofou, C. Nativi, A. Romani

15:15 **Reductive Modifications of Hyperforin, the Major Phloroglucinol from St. John's Wort**
L. Verotta, E. Lovaglio, O. Sterner, G. Appendino, S. Gibbons, W. Shiu, E. Bombardelli

15:30-16:00 Congress Center, Palazzo dei Congressi, Passi Perduti

Coffee Break

14:00-15:00 Congress Center, Palazzo degli Affari, Ground Floor

Short Lectures: SL044 – SL047

Chairs: J. L. Rios, H. Wagner

14:00 **Effects of *Cimicifuga racemosa* (L.) NUTT. on Human Breast Cancer Cell Line MCF-7 Determined by Gene Expression Profiling**
F. Gaube, L. Pusch, T. Kroll, S. Wölfl, M. Hamburger

14:15 **Inhibitory Effects of the *Cimicifuga racemosa* Extract BNO 1055 (CR) on Androgen-induced Rat Prostate Growth and on Human Prostate Cancer Cells LNCaP**
W. Wuttke, P. Thelen, L. Pitzel, D. Seidlova-Wuttke

14:30 **Effects of Phytoestrogens and Plant Extracts on Estrogen Responsive Genes in Rat Pituitary Gland In-vitro and In-vivo**
J. Wober, M. Krumbholz, T. Richter, G. Vollmer

14:45 **Effects of St. John's Wort Extract and Single Compounds on Stress Induced Hyperthermia in Mice**
O. Grundmann, O. Kelber, V. Butterweck

15:30-16:00 Congress Center, Palazzo degli Affari, First Floor
Coffee Break

16:00-17:30 Congress Center, Palazzo dei Congressi, Passi Perduti and Galleries
Poster Session II - Discussion

P281 – P367	New Natural Constituents
P368 – P372	Metabolomics
P373 – P394	Herbal Medicinal Products: Pharmaceutical Technology
P395 – P494	Traditional Herbal Medicinal Products from non EU Countries
P495 – P542	Analytical Methods
P543 – P563	Quality, Safety
P564 – P582	Miscellaneous

17:30-18:00 Congress Center, Palazzo dei Congressi, Auditorium
Closing Ceremony and Poster Awards

19.30-24:00 Villa Medicea La Ferdinanda - Artimino
Congress Dinner

Meeting point for bus departure: Piazza Adua (in front of Congress Center)
Participation for ticket holders only

THURSDAY, AUGUST 25TH, 2005

9:00-17:00
Excursions
1. Valtiberina
2. Chianti Area

Meeting point for bus departure: Piazza Adua (in front of Congress Center)
Participation for ticket holders only

S.Gallori - Firenze 2004

LIST OF SCIENTIFIC CONTRIBUTIONS

PLENARY LECTURES

PL001 **Metabolomics: New Opportunities for Pharmacognosists**
R. Verpoorte, Y.H. Choi, and H.K. Kim

PL002 **Metabolite profiling of natural products and their metabolic consequences**
E. Holmes

PL003 **Pharmacokinetics And Drug Interactions Of Herbal Medicinal Products**
H. Derendorf

PL004 **Scientific evaluation of traditional herbal medicinal products from non EU countries**
R. Bauer

PL005 **TCM-based drug discovery and main issues in TCM modernization**
De-an Guo

PL006 **A tale of bleeding ship and ancient coins. Biodiversity as the green gold of sardinia**
G. Appendino, M. Ballero

PL007 **Pharmacological Synergy effects of Phytopreparations and their Relevance for Therapy**
H. Wagner

PL008 **Anti-angiogenesis as a mechanism in cancer chemoprevention**
C. Gerhäuser, E. Bertl, H. Bartsch

PL009 **Potential Cancer Chemopreventive Activity of Botanical Dietary Supplements**
A. D. Kinghorn

WORKSHOPS

W000 **Workshop for Young Researchers**
Chair : A. Hensel
Co-chairs : A.R. Bilia, A. Deters, M. Kuesgen , J.-L. Wolfender

W001 **The Practice of Dissolution Testing in Herbal Medicinal Products**
Chair: B. Meier
Panelists: A.R. Bilia, W. Knoess, H. Sievers

W002 **Herbal drug preparations and rhinosinusitis – complex mixtures to manage a complex disease?**
Chair: I. Szelenyi
Panelists: St. Maune, A. Pahl, P. Stierna

W003 **How to implement the new legislation on Herbal Medicinal Products (HMPs) in Europe?**
Chair : A.J. Vlietinck
Panellists : K. Keller, V. Silano, L. Kabelitz

W004 **Good Practices, Standards and Certifications of Starting materials**
B. Pätzold, R. Iguera, B. Steinhoff, Ch. Franz

W005 **Tests for improvement of learning and memory**
Chair: H. Winterhoff
Panelists: C. Vonhoff, M.F. Melzig

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SL001 **Direct infusion ion trap mass spectrometry: method development and applications in metabolomics**
A. Koulman, K. Fraser, L. Johnson, G.A. Lane and S. Rasmussen

SL002 **Potential of microflow LC/NMR for the identification of natural products at the μg level**
J.-L. Wolfender and K. Hostettmann

SL003 **Application of GC-MS and HPLC-DAD for birch metabolome analysis**
S. Ossipova, V. Ossipov, K. Pihlaja

SL004 **Metabolomic analysis of *Strychnos* species extracts by ^{1}H nuclear magnetic resonance spectrometry and multivariate analysis techniques**
M. Frédérich, Y. H. Choi, L. Angenot, G. Harnischfeger, A.W.M. Lefeber, R. Verpoorte

SL005 **Bioavailability and pharmacokinetic studies on Echinaforce™ preparations and their interaction with the immune system**
K. Woelkart, A. Suter, C. Koidl, R. Raggam, B. Kleinhappl, E. Marth, R. Bauer

SL006 **Solid state activation and self-emulsifying pellets to improve the in-vitro bioavailability of Silymarin in rats**
F. Meriani, G. Zingone, D. Voinovich, L. Garboni, L. Scalise, A. R. Bilia, F. F. Vincieri

SL007 **Liposomes and b-cyclodextrin complexes to improve stability of verbascoside**
A.R. Bilia, A.M. Fadda, Marzia Innocenti, C .Sinico, D. Valenti, F.F. Vincieri

SL008 **Re-evaluation of bioactivities of various plants (*Origanum dictamnus, Thymus*, *Myrtus* species) of Greek origin, before and after encapsulation in liposomes**
I. Chinou, O. Gortzi, C. Liolios, S. Lalas, I. Tsaknis,

SL009 **Inhibitory effects of 5-*O*-demethylnobiletin on lymphocyte proliferation and the production of inflammatory mediators**
E. Bas, M.C. Recio, J.L. Ríos

SL010 **New Water Soluble Indirubin Derivatives as Selective GSK-3 Inhibitors**
P. Magiatis, P. Polychronopoulos, K. Vougogiannopoulou, M. Kritsanida, A.-L. Skaltsounis, L. Meijer, P. Greengard

SL011 **Panax ginseng C.A. Mayer (G115) modulates TLRs expression in mice under physical stress.**
M.Pannacci, S. Grosso, V. Lucini ,F.Scaglione

SL012 **Neural networks: Tools for the NF-κB inhibiting sesquiterpene lactones**
S. Wagner, A. Hofmann, B. Siedle, L.Terfloth, J. Gasteiger I. Merfort

SL013 **Shikonin Selectively Inhibits Splicing of Tumor Necrosis Factor alpha transcripts**
Shao-Chih Chiu, Ning-Sun Yang

SL014 **Anti-depressant, anxiolytic and nootropic activity of *Murraya koeinigii* leaves**
M. P. Kulkarni, R. C. Hole, R. S. Nachankar, A. R. Juvekar

SL015 **Determination of the absolute configuration of secondary alcohols by combining Mosher's derivatization technique with LC-SPE-NMR: Polyacetylene derivatives from Apiaceae as case study**
C. Seger, M. Godejohann, F. Hadacek

SL016 **HPLC-SPE-NMR in natural products research**
J.W. Jaroszewski

SL017 **A new HPLC-UV method for the analysis of antocyanins and anthocyanidins in *Vaccinium myrtillus* fruit extracts**
E. de Combarieu, D. Diliddo, M. Falzoni, L. Fumagalli, N. Fuzzati, R. Pace, P. Scandelli.

SL018 **Analysis of cucurbitacins in *Citrullus colocynthis* by MEKC and HPLC/MS**
S. Sturm, A. Danese, C. Seger, and H. Stuppner

SL019 **NMR for the screening of plants water extracts: a rapid approach to better link ethobotanical data with further phyto-pharmacological investigations.**
M. Politi, M.I Chávez , J. Alvaro-Blanco, F.J. Cañada, J. Jiménez-Barbero

SL020 **NMR-Spectroscopy of Natural Products using Micro Probes and Cryogenically Cooled Probes**
D. Moskau, T. Kühn

SL021 **Psychotropic activity of plants used in South African traditional medicine to treat CNS-related diseases**
A.K. Jäger, G.I. Stafford, N.D. Nielsen, M. Sandager, A.B. Svenningsen, K.D. Madsen, A. Adsersen, E.E. Elgorashi, J. van Staden

SL022 **In *vitro* antimalarial and leukotriene metabolism Inhibitory activities of compounds isolated from *Kniphofia foliosa* roots**
A. Abebe Wube, F. Bucar, S. Gibbons, K. Asres, M. Adams, R. Bauer, L. Rattray, S. L. Croft

SL023 **The reaction of artemsinin and semisynthetic derivatives with Hemoglobin**
L. Messori, C. Gabbiani, M. Siragusa, F.F. Vincieri, A.R.Bilia

SL024 **Bioactive components of the uteroactive medicinal plant: *Rhoicissus tridentata***
K.B. Brookes, L.C. Katsoulis

SL025 **Novel biologically active triterpenoids from two African *Combretum species***
J. Angeh, J.N Eloff, G. Swan, S. Huangi, I. Sattler

SL026 **Exploring Plant Species from São Paulo State Biodiversity: Structure and Neuropharmacological Properties of Alkaloids from *Erythrina mulungu***
O. A. Flausino Jr, I. C. Gamboa, D. H. S. Silva, V. da S. Bolzani

SL027 **Lignans of Linum species - A chemosystematic study and implications on the evolution of chemodiversity in the genus Linum**
T.J. Schmidt, S. Hemmati, B. Konuklugil, A. Mohagheghzadeh, E. Fuss, A.-W. Alfermann

SL028 **Cysteine sulphoxide pattern of Allium L. - Relations to Taxonomy**
R. M. Fritsch, M. Keusgen

SL029 **Medicinal plants from cerrado vegetation, Brazil**
R.F. Vieira, M.V.Martins, L.A.Skorupa, O.A.Silva

SL030 **Identification and quantification of melatonin in *Rosmarinus officinalis* in relation to chlorophyl a and b, RNA and proteins**
M. Pedroli, A. Giorgi, M. Cocucci, A.Conti.

SL031 **Hydroxyphenylpyruvate reductase: structure and characterisation of an enzyme involved in rosmarinic acid biosynthesis**
V. Janiak, A. Heine, G. Klebe, M. Petersen

SL032 **Elicitation of lignan biosynthesis in *Linum nodiflorum* L. by two synthetic indanoyl-amino-acid conjugates**
A. Berim O. Spring, W. Boland, M. Petersen

SL033 **Application of Innovative Plant Biotechnology for the Production of Active Compounds from Traditional Herbs, Rare Plants and New Medicinal Plants**
A. Gerth, D. Wilken

SL034 **Phenolic constituents from *Yucca schidigera* bark modulate Kaposi's sarcoma cell proliferation and motility**
M.L. Balestrieri, C. Balestrieri, I. De Maggio, F. Felice, P. Montoro, W. Oleszek, C. Pizza, S. Piacente

SL035 **Multiple approaches to identify new anti-angiogenetic compounds from plant extracts.**
F. Dal Piaz, S. Ponticelli, S. De Falco, N. De Tommasi

SL036 **Antioxidative effect of active plant extrakt**
A. Grigorov, B. Schaedlich, J, Lichius, H. Kiesewetter

SL037 **Apoptosis inducing activity of willow bark extract (Assalix ®) and its bioactivity guided fractions towards colon and lung carcinoma cells.**
K. Hostanska, G. Jürgenliemk, C. Kotalla, A. Nahrstedt, R. Saller

SL038 **Marine pharmacognosy in Swedish cold waters - strategy and examples**
L. Bohlin

SL039 **Isopimaric acid and abietic acid are active against multidrug-resistant and EMRSA strains of *Staphylococcus aureus* but show antagonism with the MDR inhibitor reserpine**
E. Smith, E. Williamson, M. Zloh, S. Gibbons

SL040 **New insights on how Echinacea alkylamides interact with cannabinoid-receptors and implications for immunomodulation**
J. Gertsch, S. Raduner, K-H Altmann

SL041 **Supplementation with a polyherbal composite alleviates clinical signs of respiratory dysfunction in horses with Chronic Obstructive Pulmonary Disease**
W. Pearson, A.F.Clarke

SL042 **Choleretic effects of yarrow (*Achillea millefolium* s.l.) in the isolated perfused rat liver**
B. Benedek, N. Geisz, W. Jäger, Th. Thalhammer, B. Kopp

SL043 **Pharmacogenomics of Artemisinin Derivatives in Anti-Cancer Therapy**
T. Efferth

SL044 **Effects of *Cimicifuga racemosa* (L.) NUTT. on human breast cancer cell line MCF-7 determined by gene expression profiling**
F. Gaube, L. Pusch, T. Kroll, S. Wölfl, M. Hamburger

SL045 **Inhibitory effects of the Cimicifuga racemosa extract BNO 1055 (CR) on androgen-induced rat prostate growth and on human prostate cancer cells LNCaP.**
W. Wuttke, P. Thelen, L. Pitzel, D. Seidlova-Wuttke

SL046 **Effects of phytoestrogens and plant extracts on estrogen responsive genes in rat pituitary gland in vitro and in vivo**
J. Wober, M. Krumbholz, T. Richter, G. Vollmer

SL047 **Effects of St. John's wort extract and single compounds on stress induced hyperthermia in mice**
O. Grundmann, O. Kelber, V. Butterweck

SL048 **The potential of PCR-related methods to identify medicinal plants in herbal medicinal products**
W. Knoess, T. Kersten, K. Keller

SL049 **To consider or not to consider? – Enzymes in herbal drugs and preparations**
W. Kreis, M. Strupf

SL050 **Natural products and batch consistency: a hurdle for herbal drug development ? Example of PX-6518, a leaf extract of Maesa balansae with antileishmania action**
L. Maes, N. Germonprez, K. Kuypers, M. Vermeersch, P. Cos, D. Vanden Berghe, L. Van Puyvelde

SL051 **Bioisosteric Modifications of the Potent and Selective k-Opioid Agonist Salvinorin A**
D. Jeremy Stewart, H. Fahmy, B. L. Roth, Fen Yang, J. K. Zjawiony

SL052 **Glucogalloyl derivatives: Synthesis and Evaluation of their Antimycotic and PARG-inhibition Activity**
P. Arapitsas, S. Menichetti, P. Buzzini, B. Turchetti, A. Chiarugi, L. Formentini, K. Sofou, C. Nativi, A. Romani

SL053 **Reductive modifications of hyperforin, the major phloroglucinol from St. John's wort**
L. Verotta, E. Lovaglio, O. Sterner, G. Appendino, S. Gibbons, W. Shiu, E. Bombardelli

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P001 **Comparison of botanical characteristic of *Phyllanthus amarus* with other *Phyllanthus species* growing in south Vietnam**
H. N.Thuy, N. K. Q. Cu, Ng.V.Thu et al.

P002 **The black glands of the genus *Hypericum*.**
L. Maleci Bini, A. Paolillo, R. Filippini

P003 **Morphoanatomy and histochemistry of *Guiera senegalensis* leaves, a major west african herbal drug**
O. Silva, R. Serrano, E. T. Gomes

P004 **Standardization of *Pterocarpus soyauxii* Taub (Fam. Leguminosae)**
C. O. Ezugwu, N. J.Nwodo

P005 **A Chemotaxonomic Investigation of two Species of the S*taehelina* Genus**
M. P. Kotsos, N. Aligiannis, S. Mitaku and A. L. Skaltsounis

P006 **Chemical profiling of *Ocimum americanum* using external flavonoids**
R.F. Vieira, R. Grayer, A. Paton

P007 **Secondary metabolites from Anthemis triumfetti**
M. Pavlović, N. Kovačević, M. Couladis, O. Tzakou

P008 **Isoflavonoids in three *Ruta* species**
O. Lapcik, R. Koblovska, M. Vítkova, Z. Mackova, B. Klejdus, L. Kokoska

P009 **Essential oil composition and variations of volatiles composition in eleven varieties of hops *(Humulus lupulus)***
S. Chericoni, G. Flamini, I. Morelli

P010 **Secondary metabolites of *Centaurea deflexa***
M. Tebano, G. Flamini, I. Morelli

P011 **Comparative analysis of essential oils, from leaves and fruit peels of seven cultivars of *Citrus* growing in Greece**
D. Lekkas, G. Panoutsopoulos, I.B. Chinou

P012 **Taxonomy of wild Allium species used in folk medicine in Tajikistan and Uzbekistan**
R. M. Fritsch

P013 **Aspects to be considered in Pharmacognostic studies using South African medicinal plants as a demonstration model**
A.M. Viljoen, S.F. van Vuuren

P014 **Losing knowledge about medicinal plants around the Brazilian Royal Road, Minas Gerais, Brazil**
M.G.L. Brandão, A.C. Alkmin, A.M. Barbosa, C.F.F. Grael, H.F. Gomes, A.C.P. Santos, M. Quinelato, D.S. Pimenta, J.A. Paula, R.L. Monte-Mor, J.R. Stehmann

P015 **The Uses of Wild Growing Allium Species of Central Asia as Spice or medicinal Plant**
M. Keusgen, R. M. Fritsch, H. Hisoriev, P. A. Kurbonova, F. O. Khassanov

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P016 **Agrobiodiversity of medicinal and aromatic species of Iran**
A. Koocheki, M. Nassiri Mahalati, F. Nadjafi

P017 **Propagation and some agronomic practices of Amukkara (*Withania somnifera* L.)**
R.M. Dharmadasa, L.S.R. Arambewela

P018 **The effects of nitrogen supply and repeated harvests on the concentration of flavonoid glycosides and caffeic acid esters in aerial parts of stinging nettle (*Urtica dioica* L.)**
K. Grevsen, X.C. Fretté, L.P. Christensen

P019 **Tha status of exudate species in Iran and existing challenges in their sustainable utilization**
A. Koocheki, F. Nadjafi, A. Ghasem Arian

P020 **Characterization of some bioactivities of verbascoside**
S. Boninsegna, O. Bertaiola, C. Di Felice, R. Dal Toso, R. Dal Monte, N. Crespi Perellino, A. Minghetti.

P021 **In vitro propagation, preservation and stevioside production of Stevia rebaudiana Bertoni**
S. Dheeranupattana, M. Wangprapa, A. Jatisatienr

P022 **Callus cultures and cell suspensions of Pelargonium sodoides and P. reniforme**
E. Neef, R. Bos, S. Batterman, W. Quax, O. Kayser

P023 **Medicarpin production by fenugreek tissue cultures. Expression of isoflavone synthase and vestitone reductase**
P. Sotiriou, K. Haralampidis, I. Chinou, C. G. Spyropoulos

P024 **Isolation of the lignan 6-methoxypodophyllotoxin from *Linum boissieri* and first experiments concerning the biosynthesis of lignans in this species**
B. Konuklugil, E. Fuss, W. Alfermann

P025 **Carotenoid composition of yeasts of the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces* and *Sporidiobolus***
P. Buzzini, M. Innocenti, B. Turchetti, D. Libkind, M. van Broock, A. Martini, N. Mulinacci

P026 **Differences of phenolic responses in two *Hypericum perforatum* L cell lines elicited with *Colletotrichum gloeosporioides*.**
L.F. Conceição, R.M. Tavares, A.C.P. Dias

P027 **Metabolites produced by shoots and calli of Lavandula agustifolia L.**
P. S. C. Braga, C. Araujo, A. Vicente, P. C. R. Valentão, P. B. Andrade, A. C. Gonçalves, R. M. Seabra, M. Fernandes-Ferreira

P028 **Industrial production of verbascoside in suspension cultures of *Syringa vulgaris***
R. Dal Monte, R. Dal Toso, M. Noro, N. Crespi Perellino, A. Minghetti

P029 **Antibacterial, antioxidative and cytotoxic activities of extract from in vitro grown Ruta graveolens**
E. Lojkowska, M. Sidwa-Gorycka, J. Bieda, E. Biskup, E. Loikowska, A. Krolicka

P030 **Antimicrobial activity of secondary metabolites from the in vitro grown Drosera capensis**
A. Krolicka, A. Szpitter, L. Marczak, E. Biskup, M. Stobiecki, E. Lojkowska

P031 **Levisticum officinale hairy root cultures: influence of light and light type on growth and essential oil production**
A. S. B. Lima, M. J. Sousa, L.G. Pedro, A. C. Figueiredo, J. G. Barroso, S. G. Deans and J. J. C. Scheffer

P032 **Nitrogen stress induction on *Levisticum officinale* hairy roots: effect on growth and essential oil production**
M. M. Costa, A. C. Figueiredo, J. G. Barroso, L. G. Pedro, S. G. Deans, J. J. C. Scheffer

P033 **Can Flavour Components of Garlic be produced by Mushrooms?**
C. Kreuzberg, M. Keusgen

P034 **Cytotoxic activity of extracts from Linum cell cultures**
N. P. Vasilev, I. Ionkova

P035 **Optimization of lignan production of in vitro cultures of Linum tauricum ssp. tauricum (Willd.) Petrova**
K. Danova, I. Ionkova, N. Vasilev

P036 **The effect of medium composition on shikonin derivatives accumulation in suspension culture of *Arnebia euchroma***
K. Sykłowska-Baranek, A. Pietrosiuk, S. Wyderska, M. Furmanowa

P037 **Cytochemichal investigation of leaf secretory tissues and cell culture of Inula viscosa – Antifungal activity**
A. Nikolakaki, G. Panoutsopoulos, N.S. Christodoulakis, I.B. Chinou

P038 **Metabolites produced by shoots and cell suspensions of sage (*Salvia officinalis* L.)**
P. S. C. Braga, P. C. Santos-Gomes, P. C. R. Valentão, P. B. Andrade, R. M. Seabra, M. Fernandes-Ferreira

P039 **Production and analysis of bioactives in *in vitro* shoot and hairy root cultures of southern African sages**
N.P. Makunga, J. Van Staden

P040 **Micropropagation of the medicinal plant, *Echinacea purpurea*, in solid and liquid culture systems**
A.M.P. Jones, S.J. Murch, and P.K. Saxena

P041 **Hypericum perforatum subsp. angustifolium: morphological, histological and phytochemical study in in vitro vegetative organogenesis**
N. Mulinacci, C. Giaccherini, R. Caniato, B. Monacelli, A. R. Santamaria, G. Pasqua

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A. Karioti, A. Chatzopoulou, A. R. Bilia, G. Liakopoulos, S. Stavrianakou, H. Skaltsa

P043 **Cultivation of *Myrtus communis* L.: effect of nitrogen on leaves polyphenolic metabolites**
A. Romani, P. Pinelli, F. Ieri, C. Lapucci, F.F. Vincieri, E. Chessa, G. Nieddu

P044 **Effects of Nitrogen Nutrion Seed Yield and Oil Seed Content of Naked Seed Pumpkin (*Cucurbita pepo* subsp. *pepo* convar. *pepo* var. *styriaca*)**
H. Aroiee, M. Azizi

P045 **Preliminary trials on effect of nitrogen nutrition on growth and on secondary metabolism of Achillea millefolium L. spp *collina***
A. Giorgi, G. L. Licheri, M. Cocucci

P046 **Occurrence of amides derived from corresponding cyanogenic glycosides.**
J. Sendker, A. Nahrstedt

P047 **Substrate specificity and kinetic properties of recombinant D^5-3b-hydroxysteroid dehydrogenase from *Digitalis lanata* Ehrh.**
J. Frankenstein, V.Herl , F. Mueller-Uri, W. Kreis

P048 **Biosynthesis of acetate-derived naphthylisoquinolines and naphthoquinones**
K. Springob, A. Jindaprasert, T. Gronauer, T. Noll, M. Greb, G. Bringmann, J. Schmidt, T.M. Kutchan

P049 **Influence of Altitude on the Amount of Antioxidative Flavonoids and Hydroxycinnamic Acids in Flowers of *Sambucus nigra* L.**
G. Rieger, M. Müller, H. Guttenberger, F. Bucar

P050 **Aroma Precursors of Georgian Wild Allium Species**
N. Pekgoz, K. Pistrick, J. Jedelska, M. Akhalkatsi, G. Nakhutsrishvili, M. Keusgen

P051 **Analysis of the polyketide synthase involved in naphthoquinone formation in Plumbago indica L.**
A. Jindaprasert, K. Springob, J. Schmidt, W. De-Eknamkul, T.M. Kutchan

P052 **Medicarpin production by *Trigonella foenum-graecum* L. in response to biotic elicitors. Expression of isoflavone synthase and vestitone reductase.**
D. Tsiri , M. Halabalaki, I. Chinou, C. Spyropoulos

P053 **Investigations on the cardinal temperature for germination of *Plantago ovata* and *Plantago psyllium***
L. Tabrizi , M.Nassiri Mahallati, A. Koocheki

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S. Kaulard, C Pullen, T. Boettcher, H. Müller, A Krechel, G. Berg, E. Leistner

P055 **Gene expression profile in TNF-α stimulated 293 cells after treatment with parthenolide**
M.T. Lindenmeyer, C. Kern, J. Schwager, J. Donauer, C. Kreutz, D. Porath I. Merfort

P056 **Molecular cloning and expression of a recombinant 3β-hydroxysteriod dehydrogenase from Digitalis lanata Ehrh.**
V. Herl, G. Fischer, F. Mueller-Uri, W. Kreis

P057 **Plant propagation through somatic embryogenesis of *Stemona tuberosa* Lour., an Asian antitussive traditional herb**
N.Montri, C.Wawrosch, B.Kopp

P058 **The anti-malarial artesunate and the epidermal growth factor receptor tyrosine kinase inhibitor OSI-774 for the combination treatment of glioblastoma cells**
T. Efferth, T. Ramirez, E. Gebhart, and M.-E. Halatsch

P059 **Seed Transmittance of an Epibiotic Clavicipitalean Fungus Occuring on a Dicotyledoneous Plant (*Ipomoea asarifolia)***
M. Ahimsa-Mueller, A. Markert, J. Gross, E. Jonas, V. Knoop, U. Steiner , E. Leistner

P060 **The possible role of fungi in the accumulation of ergoline alkaloids in *Ipomoea asarifolia* (Convolvulaceae)**
A. Markert , S. Kucht, M. Ahimsa-Mueller, J. Groß, U. Steiner, E. Leistner

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R. Gebhardt, M. Schmidt

P062 **Cytotoxic activity and Comparative Molecular Field Analysis of cycloartanes of Parthenium argentatum**
H. Parra-Delgado, T. Ramírez Apan, C. M. Compadre, and M. Martínez-Vázquez

P063 ***In vitro* cytotoxicity against human cancer cells of plant called Hua-Khao-Yen**
A. Itharat , C.Dechsukum, J.Saecoo , V.Chantharataptawan, J.Taengfug, V.Tongteerapab

P064 **Sesquiterpenes from *Zinowiewia costaricensis* as Chemosensitizing Agents of the Multidrug Resistance Phenotype in Cancer**
C. R. Mendoza, F. Muñoz-Martínez, I. L. Bazzocchi, I. A. Jiménez, F. Gamarro

P065 **Sesquiterpenes from *Maytenus canariensis* and their Inhibitory Effects on Epstein-Barr Virus Early Antigen Activation in Raji Cells**
S. Koteich-Khatib, I. A. Jiménez, I. L. Bazzocchi, H. Tokuda, H. Kushida

P066 **Chemical Composition and Cytotoxic Activity of the Essential Oils of five *ERYNGIUM* species from Crete**
E. Kalpoutzakis, N. Aligiannis, H. Pratsinis, D. Kletsas, E. Tsitsa, S. Mitakou

P067 **In vitro cytotoxicity of extracts from Hungarian Asteraceae**
Zs. Hajdú, B. Csupor-Löffler, J. Hohmann, B. Réthy, I. Zupkó, Gy. Falkay, I. Máthé

P068 **Constituents and antiproliferative activity of the Malaysian plant *Hydnophytum formicarium***
D. Rédei, H. Abdullah, A. Hawariah, P. Forgo, J. Molnár, J. Hohmann

P069 **Effect of terpenic and phenolic compounds on apoptosis induction on mouse lymphoma cells**
N. Duarte, A. Varga, M. Lopes, J. Molnár, M . J. U. Ferreira

P070 **Evaluation of three new macrocyclic lathyrane diterpenes from *Euphorbia lagascae* on the reversal of multidrug resistance on mouse lymphoma cells**
N. Duarte, A. Gonçalves, N. Gyémánt, J. Molnár P. Abreu, M. J. U. Ferreira

P071 **Differential inducibility of CYP1A mRNA expression by green tea extract in two intestinal cell lines**
M. I. Netsch, H. Gutmann, J. Drewe, C. Aydogan

P072 **The effect of *Cousinia shulabadensis* ethanol extract on the matrix metalloproteinase activity**
A.R. Shahverdi, H. R. Monsef-Esfahani, M. R. Khorramizadeh, F. Saadat, S. Vahid, F. Attar, A. Ghahraman

P073 **Isolation of Tumour Modulatory Compounds in Chinese Herbal Remedies Through Activity-Guided Fractionation**
S.Willimott, J. Barker, L. Jones, E. I. Opara

P074 **Apoptotic activity of flavonoids isolated from *Astragalus verrucosus* moris**
L. Pistelli, A. Bertoli, L. Luciardi, J. Buhagiar, I. Morelli

P075 **Chemopreventive activity of natural compounds on nitric oxide donors induced mouse skin carcino genesis**
H. Tokuda, F. Enjo, T. Konoshima, M. Takasaki, J. Takayasu, A. Iida, H. Nishino

P076 **Chemopreventive activity of naturally occuring compounds against adavanced glycation endproducts induced carcinogenesis**
H. Tokuda, M. Ogata, F. Enjo, J. Takaysu, A. Iida, H. Nishino

P077 **Anacardic acids and cardanols from the bark of *Amphipterygium adstringens* Schiede ex Schlecth**
H.Rosas Acevedo, M. Martínez-Vázquez, M. Soto Hernández

P078 **Cytotoxic activity of , 3β,6α-diol-cholest-8-ene, 14α-methyl-3β,6α-diol-cholest-8-ene and 3β, 16β, 22α, 28-tetrol-olean-12-ene.**
A. L. Salgado Orozco, M. Silva Magaña, T. Ramírez-Apan, Juan R. Salazar and M. Martínez-Vázquez.

P079 **Bioactive Lanostanes and Friedolanostanes from the bark of Garcinia speciosa**
I. Mondranonda, L. Vieira, M. Nascimento, A. Kijjoa

P080 **The Cytotoxicity of Naphthazarine Derivatives from *Onosma arenaria***
T. Kundaković,T. Stanojković, Z. Juranić, N. Kovačević

P081 **The Use of Shiitake Mushroom polysaccharides in Cancer Treatment**
A. S. Daba

P082 **A novel polyacetylene inhibits angiogenesis and induces apoptosis in human endothelial cells**
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S.Gallori - Firenze 2004

PLENARY LECTURES

PL 001 Metabolomics: New Opportunities for Pharmacognosists

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Metabolomics is the latest of the –omics technologies in functional genomics. It aims at the chemical characterization of a phenotype by the qualitative and quantitative analysis of all metabolites present. Usually chromatographic methods, mass spectrometry or NMR are used for this purpose. Each of these well known phytochemical methods has advantages and disadvantages. Particularly NMR is a very suitable method for having a macroscopic view on the constituents in a plant under different conditions. NMR is highly reproducible and quantitatition does not require calibration curves for all single components. Mass spectrometry and chromatographic methods are more sensitive, but reproducibility and quantitation are their weak points.
The first step in metabolomic studies is to establish the biological variability of a system, this requires large numbers of analyses. To deal with such large data sets chemometric methods such as multivariate and principle component analysis are needed to define variability and to define signals that can be used as markers to separate different materials. In functional genomics these methods can be combined with proteomics and transcriptomics data e.g. to make links between genes and the biosynthesis of a compound. For medicinal plants metabolomics is an excellent tool in quality control. Metabolomics can also be very useful in systems biology approaches in studying the activity of medicinal plants. If compared with the present day paradigm of drug discovery using the reductionist approach of single target, single compound, the holistic approach of systems biology has the advantage that it also may detect prodrugs and synergy of compounds in plant extracts. All this offers the pharmacognosist, with their extensive expertise in phytochemical analysis, some exciting new possibilities in studying medicinal plants.

Robert Verpoorte

Division of Pharmacognosy, Section Metabolomics, Institute Biology Leiden, Leiden University, PO Box 9502, 2300 RA, Leiden, The Netherlands.
MSc 1970, Pharmacists degree 1972, PhD 1976, lecturer at Leiden University 1976-1987, professor and head of the department of Pharmacognosy, Leiden University, since 1987
Honorary Doctorate University of Amiens, France (2004).
Author/co-author of 525+ scientific papers, 3 books and 4 patent applications. Editor of Journal of Ethnopharmacology, Phytochemical Reviews and Associate Editor of Phytochemical Analysis. Editorial board member of 12 scientific journals.
Vice-Chairman and Chairman of the committee of the Phytochemical Society of Eu-rope (1992-1998).
Research interests: Biosynthesis plant secondary metabolites, metabolic engineering, metabolomics, medicinal plants, isolation natural products.

PL 002

Metabolite profiling of natural products and their metabolic consequences

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Metabonomics is a rapidly emerging field of research combining sophisticated analytical tools such as NMR spectroscopy and mass spectrometry with multivariate statistical analysis to generate complex metabolic profiles of plant and animal tissues and extracts. Metabolic profiling strategies have been successfully applied to the characterization of various herbs and plant products for quality control, determining geographical origin and for detecting adulteration of products. Additionally the metabolic consequences of plant extracts have been demonstrated in experimental animals and in man. Here the application of various spectroscopic and chemometric tools for analysing natural products and their metabolic consequences are described with particular emphasis on deconvolving biological complexity and minimising confounding biological and analytical 'noise'. Examples are taken from chamomile, feverfew, artemesia annua and other traditional Chinese medicine components.

Elaine Holmes

Dr. Elaine Holmes obtained a BSc in Biological Sciences followed by a PhD in Chemistry from London University and currently holds a readership in Biological Chemistry at Imperial College. Her work is centred on the development of NMR and pattern recognition based computerised expert systems for predicting drug toxicity and characterisation of physiological dysfunction. Other research interests involve the direct analysis of intact tissues using high resolution MAS NMR spectroscopy and the application of hyphenated analytical techniques such as LC-NMR-MS to the identification of drug metabolites and biomarkers of pathology.

PL 003

Pharmacokinetics and Drug Interactions of Herbal Medicinal Products

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It is necessary for the rational use of any drug to have a good understanding of the concentrations that will be achieved in the body after its administration. Of particular interest is the question of bioavailability to assess to what degree and how fast the therapeutic agent is absorbed. Whereas there is usually detailed information available about the pharmacokinetics and biopharmaceutics of chemical drugs, this is not the case for herbal medicinal products. However, in principle the same concepts apply since only with a good characterization of pharmacokinetics ('what the body does to the drug') and pharmacodynamics ('what the drug does to the body') it is possible to optimize the therapeutic use of the agent. Knowledge of the bioavailability is essential for the correct *in-vivo* interpretation of *in-vitro* activities that are sometimes the basis of therapeutic claims. One common problem in the assessment of pharmacokinetic properties of natural product is that frequently the pharmacologically active agents are not known. This presents a dilemma since without clearly identified target compounds it does not make much sense to measure concentrations of the product ingredients. Only if a correlation exists between the concentration of an active component of a natural product and its effect or side effect pharmacokinetic studies of individual chemical entities are warranted. If this is not possible, an alternative approach for the characterization of herbal medicinal products is the use of pharmacodynamic surrogates, which should be easily quantifiable and correlate with the therapeutic outcome. These surrogates allow evaluating the overall activity of a complex biological mixture and compare different products. Results from these pharmacodynamic studies may then also be helpful to identify the active ingredients and obtain a better scientific understanding of the pharmacological mechanisms. These studies will lead to appropriate criteria, which can be used to evaluate different natural products and their dosage forms and help to advance the field of herbal medicine from empirical experience to a more rational and safer pharmacotherapy. Finally, herbal medicinal products can cause a number of drug interactions with other medications that may be of clinical significance. Some representative examples will be reviewed.

Hartmut Derendorf

Hartmut Derendorf is Distinguished Professor and Chairman of the Department of Pharmaceutics at the University of Florida College of Pharmacy in Gainesville. He received his B.S. (1976) and Ph.D. (1979, summa cum laude) in Pharmacy from the University of Münster, Germany and then joined the University of Florida, first as a Postdoctoral Fellow (1981/82) and later (1983) as a faculty member. He has been teaching Biopharmaceutics, Pharmacokinetics and Clinical Pharmacokinetics. In 1995, he received the Teaching Improvement Award of the University of Florida. In 2002, he was awarded the University of Florida Research Foundation Professorship and in 2004 the International Educator of the Year Award.
Prof. Derendorf has published over 260 scientific publications and given over 500 presentations at national or international meetings. Prof. Derendorf won the Rottendorf-Award for Pharmaceutical Sciences (1983), the McKeen-Cattell Award for the best publication in J. Clin. Pharmacology (1994) and the Faculty Award of the University of Utrecht (2005). In 2003, he was awarded the Nathaniel T. Kwit Distinguished Service Award of the American College of Clinical Pharmacology (ACCP) and the Research Achievement Award in Clinical Science of the American Association of Pharmaceutical Sciences (AAPS). He is a Fellow, former Secretary, Honorary Regent and President-elect (2006/07) of ACCP. He is currently President (2004/05) of the International Society of Antiinfective Pharmacology (ISAP). He is a Fellow of AAPS, and member of APhA, FPA, DPhG, ASCPT and AACP.

Scientific evaluation of traditional herbal medicinal products from non EU countries

PL 004

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Herbal products are used in traditional medicine all over the world. In recent years, safety, efficacy and quality of the products have become important issues for health authorities and the public. WHO has recently compiled data on the global situation (1). In the European Union, the use of traditional herbal medicinal products has been regulated in Directive 2004/24/EC (2). It requires a medicinal use throughout a period of at least 30 years with at least 15 years use related to the European Union.
As long as the efficacy of these medicinal products is plausible on the basis of long-standing use and experience, clinical studies and pre-clinical tests are not obligatory from the regulatory point of view. Nevertheless, clinical and pharmacological studies support the rational use and promote the acceptance of such products. Special emphasis has to be put on the evaluation of the safety of the products.
Since the quality aspect is independent of the traditional use, the necessary physico-chemical, biological and microbiological tests have to be performed with every medicinal product individually.
Several examples of medicinal plants from Asia, Africa and South America will be presented, which have been scientifically evaluated and which have successfully entered the European market, either as medicinal products or as dietary supplements, or which are still in development.

References: 1. Bodeker, C. et al. (2005) WHO Global Atlas of Traditional, Complementary and Alternative Medicine. World Health Organization, Kobe. **2.** Silano, M. et al. (2004) The new European legislation on traditional herbal medicines: main features and perspectives. *Fitoterapia* 75(2): 107-16.

Rudolf Bauer

Univ.-Prof. Dr. Rudolf Bauer studied pharmacy in Munich, in 1984 obtained the PhD and in 1990 the habilitation in Pharmaceutical Biology at the Uni-versity of Munich. From 1993 to 2002 was university professor at the Uni-versity of Düsseldorf, now is full professor and Head of the Institute of Pharmaceutical Sciences, University of Graz, Austria. He is Co-Editor of Planta Medica. Since 1984 he is regular member of the Society for Medicinal Plant Research (GA)and since 1994 member of the Board of Directors. From 1998 to 2001 was Vice President and since 2002 President of GA.. Awards: Egon-Stahl-Award from the Society of Medi-cinal Plant Re-search (1990), Cipla Distinguished Fellowship in Pharma-ceu-tical Sciences of the Department of Chemical Techno-lo-gy, Uni-ver-sity of Bombay (1994), International Award of the Belgian Society of Pharmaceutical Scien-ces (1996). In 2000 was Guest Professor of the School of Chinese Materia Medica at the Beijing University of Chinese Medicine. Main research areas: Quality control and standardization of herbal drugs and herbal medicinal products; isolation and structure elucidation of plant constituents; phar-macolo-gical screening of plant con-stituents with anti-inflammatory and immunomodulatory and anti-cancer activity; search for compounds with inhibitory activity on cyclo-oxygenase 1 / 2 and 5-lipoxygenase; phytochemical and pharmacological investigation of Echinacea species; phytochemical and pharmacological investigation of Chinese herbal drugs; quality control of Chinese herbal drugs. Publications: 200 research papers and reviews; 4 books.

PL 005 TCM-based drug discovery and main issues in TCM modernization

De-an Guo
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Traditional Chinese medicine (TCM) has over 2000 years of history to treat diseases in China and has played a essential role in the Chinese healthy system. Due to its long practicing history in human body and its efficacy to treat diseases, TCM is a rich resource for new dryg discovery. According to the resources' investigation, there are 12807 species of traditional Chinese medicines, among which 11146 are derived from medicinal plants, 1581 are from animals and 80 from minerals. In the past 30 years, over 1200 TCMs have been phytochemically investigated by the Chinese scientists, from which a number of bioactive principles were developed into new drugs and launched into the market. Several typical new drugs derived from TCM resources - such as artemisinin (an anti-malarial drug from *Artemisia annua*), schisandrin (an anti-heptitis C drug from *Schisandra chinensis*) and indirubin (an anticancer drug from *Isatis tinctorius*), will be exemplified in the lecture.
TCM modernization is currently one of the major emphases by the Chinese government. Some the issues including quality control, efficacy verification, mechanism of action etc. have been addressed. In this lecture, the quality control issue will be focused and the analytical methods involved in the quality control will be discussed. Several commonly used traditional Chinese medicines including the roots of *Salvia miltiorrhiza* (Danshen), the roots of *Panax notoginseng* (Sanqi), the skin secreation of *Bufo Bufo gargarzans* (Chansu), the roots of *Paeonia lactiflora* (Shaoyao), seeds of *Cuscuta chinensis* (Tusizi) and the heart wood of *Dalbergia odorifera* (Jiangxiang) have been investigated by means of phytochemical analysis and their quality control standards were established. The HPLC fingerprints of the above mentioned crude drugs and their representative preparations were formulated and the major peaks were designated. From the roots of *Salvia miltiorrhiza*, 15 salvianolic acids and 10 tanshinones ere unambiguously identified by HPLC-MS-MS analysis and comparing with the authentic compounds. The qualitative and quantitative analyses of bufadienolides in the Chinese drug ChanSu were carried out by HPLC/DAD/APCI-MS/MS technique. The APCI-MS fragmentation behaviors of bufadienolides were studied for the first time. A total of 35 bufadienolides were identified in the crude drug including four new constituents. And the eight major bufadienolides were simultaneously quantified by a well-established HPLC-UV method. The phenolic compounds in Tusizi were thoroughly analyzed. From the methanol extracts of two derived species (*Cuscuta chinensis* and *Cuscuta australis*), a total of 50 compounds, including 23 flavonoids, 20 lignans and 7 quinic acid derivatives were identified or tentatively characterized based on UV spectra and MS fragmentation behaviors. Contradictory to the previous reports, the phenolic patterns of these two *Cuscuta* species were found to be very different. The phenolic constituents of *C. chinensis* and *C. australis* were comprehensively compared for the first time, and the great differences strongly encouraged further comparison of the bioactivities of these two species. Similarly, the saponin compounds in the *Panax ginseng*, the flavanoids in *Dalbergia odorifera*, the active principles of *Paeonia lactiflora* were also phytochemically analyzed and their major active principles were quantified. These studies set up a model for the comprehensive quality control of complicated TCM systems.

De-an Guo

Professor Dr. De-an Guo is Deputy Director of the Modern Traditional Chinese Medicine (TMC) of the Schoold of Pharmaceutical Sciences, Peking University.
He is Professor of Pharmacognosy, involved in Pharmacological and Pharmacokinetic Studies of TMC. He is vice editor in chief of the english edition of the Chinese Pharmacopoeia. Menber of the Drug Evaluation Committee of China and Panel Member of National 863 Hi-tech program.

PL 006

A tale of bleeding ship and ancient coins. Biodiversity as the green gold of sardinia

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With over 10% of endemic plants in its flora, Sardinia is one of the major sources of biodiversity in Europe. Furthermore, owing to geographical isolation, the Sardinian populations of some non-endemic, pan-Mediterranean species have developed unique chemical traits. This chemical polymorphisms is especially marked in umbelliferous plants, as will be discussed for *Ferula communis* L., *Opopanax chironium* L. and *Thapsia garganica* L.. These plants have a long tradition of use in the Greek-Roman medicine and a rich ethnopharmacology, and the chemical characterization, synthetic modification, and biological profiling of their constituents will be presented. The establishment of a University Consortium specifically dedicated to the study of Sardinian biodiversity, its role, and its present activities will be discussed.

Giovanni Appendino

Giovanni Appendino graduated in 1979 at the University of Torino. He became University researcher at this University in 1983, and Associated Professor of Organic Chemistry in 1998. In 2000 he became Full Professor or Organic Chemistry at the Università del Piemonte Orientale in Novara. His research activity is documented by over 200 scientific articles. In 1991 he received the Rhone-Poulenc-Rorer Award of the Phytochemical Society of Europe for his studies on Isoprenoids. He has acted as local coordinator for four EU research programs. His research activity centres on the chemistry of biologically active organic natural products, and has developed according to three lines, methodologically distinct, but correlated as regards their themes: isolation-structural elucidation, chemical modification, and total synthesis. Compounds from different classes and typical of terrestrial organisms, mainly higher plants, were investigated (isoprenoids, alkaloids, coumarins, acetogenins, flavonoids, phenylpropanes). The main research lines have regarded medium-size cyclic compounds, taxoids, phorboids,vanilloids, cannabinoids, toxic prenylated coumarins, and secondary metabolites from edible plants and spices.

PL 007 Pharmacological Synergy effects of Phytopreparations and their Relevance for Therapy

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Many years of experience with phytotherapy have shown that plant extracts very often have better efficacy and reduced side-effects than equivalent doses of isolated, individual plant compounds. This observation has prompted many pharmacological and therapeutic investigations to explain obvious synergy effects among the constituents of plant extracts. The latest results in this particular field are described here, using several examples. According to Williamson (1), the general understanding of synergy is that it is an effect seen by a combination of substances being greater than would have been expected from a consideration of individual contributions. In order to investigate such synergy effects in vitro, the isobol method of Berenbaum can be used, as demonstrated with mixtures of isolated ginkgolides of *Ginkgo biloba*. Analogous pharmacological investigations have been carried out with many plant extracts, including *Hypericum*, *Kava-Kava*, *Cannabis* and *Valeriana*, as well as with many other monoextracts and with fixed extract combinations (including *Passiflora/ Kava-Kava, Humulus lupulus/ Passiflora* and *Ginseng/ Ginkgo*) and outstanding multiextract combinations of the drug market. These investigations were followed by controlled, clinical, double-blind trials with standardized plant extracts for comparison to synthetic drugs at the same indication. These studies have, surprisingly, shown that the standardized plant extracts were therapeutically equivalent to the synthetic drugs and superior in terms of safety. The advantage of these drug combinations is that the individual substances or extracts of the drug mixture can be administered in doses of a relatively low concentration. All results of these investigations suggest that this new concept of multidrug therapy can be described as a multitarget therapy, in which each component of the drug mixture affects another pharmacological target, resulting in a multicausal treatment. This evident therapeutic superiority and advantage give phytotherapy a new legitimation for the application of standardized mono-or multiextract combinations.

References: 1. Williamson E.M. (2001) Phytomedicine 8:401-9

Hildebert Wagner

Professor Dr.Dr.h.c.mult. Hildebert Wagner, is em. Professor at University the Ludwig-Maximilians of Munich, Institute of Pharmacy - Pharmaceutical Biology. After the degree in Pharmacy in 1965 he became Full Professor of Pharmacognosy at the University of Munich. Dr. Wagner was made a Full Professor of Pharmacognosy in 1965, and later served as Director of the Institute of Pharmaceutical Biology in Munich until 1999. He was Dean of the Faculty of Chemistry / Pharmacy from 1981 to 1983 and director of the Institute of Pharmaceutical Biology till 1999. He obtained Ph.D. honoris causae of the University of Budapest and Debrecen (Hungary) in 1989, of the University of Dijon (France) and the University of Helsinki (Finland) in 1997. Professor honoris causae of the Medicinal Faculty of Beijing (China) in 1990 and of the University of Arequipa (Peru) 1992. Research areas are isolation, structure determination, synthesis and analysis of biologically and pahrmacologically active compounds of medicinal plantes, particularly in the fields of alkaloids, hearth glycosides, flavonoids and lignans: drugs with antiviral, antiasmathic , antiphlogistic, immunostimulating and adaptogenic activity, standardisation of Chinese drugs.
He is the author of over 800 other scientific publications, 30 review aricles and 7 books . He is membership of Editorial/Advisory Boards of Phytochemistry, journal of Ethnopharmacology, Journal o9f Natural Products, International Journal of Phytomedicine.

PL 008

Anti-angiogenesis as a mechanism in cancer chemoprevention

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Inhibition of angiogenesis, the formation of new blood vessels from pre-existing microvasculature, represents an innovative approach to cancer chemoprevention. For the identification of novel inhibitors of angiogenesis, we have established a human *in vitro* anti-angiogenic assay premised on the principle of wound healing (1). Subsequently, a series of twelve chemopreventive lead compounds, belonging to the chemical classes of phloroglucinol derivatives, anthraquinones, isothiocyanates, flavanones, diterpenes, chalcones and bibenzyl derivatives of lunularic acid was tested. At concentrations up to 10 µM, all of these agents potently reduced microvessel growth (2). We selected two chemopreventive compounds, xanthohumol (XN) from *Humulus lupulus* L. (hop) (3) and sulforaphane (SFN), an isothiocyanate derived from cruciferous vegetables (Brassicaceae) (4), for more detailed investigation of their angiopreventive potential. Both compounds potently inhibited endothelial steps crucial for angiogenesis, including hypoxia-induced release of pro-angiogenic factors, endothelial cell proliferation, migration and differentiation. *In vivo* efficacy of XN was demonstrated by intravital microscopy of human MX-1 breast tumour xenografts implanted in dorsal skinfold chamber preparations in female severe combined immunodeficient mice. Our data indicate that these novel lead compounds interfere in the angiogenic cascade at multiple relevant steps and should be further evaluated.

References: 1. Bertl, E. *et al*. (2004) Intern. J. Cancer Prev. 1, 47-61. **2.** Bertl, E. *et al.* (2004) Biochem. Biophys. Res. Comm. 325, 287-295. **3.** Gerhauser, C. *et al*. (2002) Mol. Cancer Therap. 1, 959-969. **4**. Heiss, E. *et al*. (2001) J. Biol. Chem. 276, 32008-32016.

Clarissa Gerhäuser

Dr. Clarissa Gerhäuser obtained the degree in Pharmacy from Julius-Maximilians-University of Würzburg, followed by a PhD in Pharmacognosy from Ludwig-Maximilians-University of München. She was postdoctoral research associate with Prof. J.M. Pezzuto at the Department of Medicinal chemistry and Pharmacognosy of University of Illinois at Chicago. In 1990 she was awarded by the Köhnlechener-Fouundation of Munich and in 2003 she obtained from the European Association for Cancer Research the "Young Cancer Researcher Award Higly Commended" and the "Phoenix Pharmacy Scientific Research Price". Since 1996 she is t group leader of the section Cancer Chemoprevention of the German Cancer Research Center in Heidelberg. Areas of her research are identification of novel chemopreventive agents from various sources, investigation of their mode of action by molecular-biological tools, demonstration of efficacy in animal models, focussing on mammary and colon cancer prevention, evaluation of bioavailability and metabolism, participation in human intervention studies to identify biomarkers for cancer prevention. She is author of more than 40 original papers, 5 book chapters and two patents.

PL 009

Potential Cancer Chemopreventive Activity of Botanical Dietary Supplements

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Cancer chemoprevention is an important strategy for managing and controlling cancer, and involves the use of synthetic or natural agents to inhibit, retard, or reverse the process of carcinogenesis. In previous work, using a variety of *in vitro* and *in vivo* assays (1), a large number of potential cancer chemopreventive agents have been isolated and characterized from plants (2). Recently, there has been an increasing interest in the use of certain botanical dietary supplements for "detoxification" by the U.S. public, and many of these products are employed in oriental traditional medicine for treating liver disease or as hepatoprotectants. We are investigating a number of these products in our laboratory for their antioxidant and potential cancer chemopreventive activity, such as Noni (*Morinda citrifolia*), Mangosteen (*Garcinia mangostana*), and Sea Buckthorn (*Hippophae rhamnoides*). In collaborative studies, some of the compounds obtained have been subjected to testing *in vivo* models relevant to cancer chemoprevention.

Acknowledgements: Nature's Sunshine Products, Inc., Spanish Fork, UT, U.S.A. (Drs. William J. Keller and Jerry L. McLaughlin, for provision of botanical dietary supplement materials)

References. 1. Pezzuto, J.M. et al. (2005) In Cancer Chemoprevention, Vol. 2, Strategies for Cancer Chemoprevention, Kelloff, G.J. et al. (Eds.). Humana Press Inc. Totawa, NJ , pp. 3-37. **2.** Kinghorn, A.D. et al. (2004) Planta Med. 70: 691-705.

A. Douglas Kinghorn

Since 2004, Prof. A. Douglas Kinghorn has been the inaugural Jack L. Beal Professor and Chair in Natural Products Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH. Formerly, he was Professor of Pharmacognosy and Associate Director of the Program for Collaborative Research in the Pharmaceutical Sciences at the University of Illinois at Chicago (UIC).

He was designated as the 1993 B. Kenneth West University Scholar (Senior University Scholar) by the University of Illinois Foundation and was awarded the 2002-2003 UIC Award for Excellence in Teaching, the premier peer-reviewed campus teaching award for faculty members. Prof. Kinghorn has served as President of both the American Society of Pharmacognosy (1990-1991) and the Society for Economic Botany (1991-1992). Currently, he is Editor-in-Chief of the Journal of Natural Products (1994-2008) and serves also on the Editorial Advisory Boards of fourteen other scientific journals. He was recently elected as Chair of the Dietary Supplements – Botanicals Expert Committee (2005-2010) of the United States Pharmacopeia.

His research interests are on the isolation, characterization, and biological evaluation of natural products of higher plant origin, and he has worked in particular on compounds with potential oral antimicrobial, cancer chemotherapeutic, cancer chemopreventive, and sweet-tasting effects. He has authored or co-authored about 385 research articles, book chapters, and reviews, and has edited or co-edited four books. In 2001, he was named as a "Highly Cited Researcher" in Agricultural Sciences by the Institute of Scientific Information (ISI), Philadelphia, PA. Prof. Kinghorn has been Major and/or Thesis Advisor for about 40 graduate students and has also directly supervised over 55 postdoctorals and visiting scholars.

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WORKSHOPS

W 000 Workshop for Young Researchers

Chair : A. Hensel[a]

Co-chairs : A.R. Bilia[b]*, A. Deters*[a]*, M. Kuesgen*[c]*, J.-L. Wolfender*[d]

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This special workshop will open the 53rd Annual Congress of the Society for Medicinal Plant Research, a joint meeting with the Italian Society of Phytochemistry. It will be an opportunity for young researchers to present and discuss difficulties, problems and unclear results related to their works. A decade of young scientists working in the field of Pharmacognosy and Analytical Phytochemistry have been selected to present their work. The presentations will be commented on by experienced panellists and in addition there will be time for discussion with the entire international audience.
The workshop will serve as a forum for the participants to get in contact with other researchers, to become familiar with other approaches and future research topics, to share methodologies and knowledge of instruments, and to expand their own studies by acquiring ideas in a constructive and international atmosphere.
A presentation will be selected and awarded.

Acknowledgements: The Workshop will be supported in part by Bionorica AG, Newmark (Germany).

The Practice of Dissolution Testing in Herbal Medicinal Products

W 001

Chair: B. Meier[a]

Panelists: A.R. Bilia[b]*, W. Knoess*[c]*, H. Sievers*[d]

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Dissolution Tests become more and more an essential part in the documentation of Herbal Medicinal Products (HMP's) – independently of the classification of the herbal extract. The link between biopharmaceutical properties and the efficacy of herbal medicinal product is very complex. Therefore, dissolution characteristics of HMP's will be considered more an aspect of the product's quality rather than criteria for measuring bioavailability. The justification of the phytoequivalence of new products and new galenic forms compared with products of traditional and well established use is another tool for the application of dissolution profiles.

However, the development of strategies for testing dissolution in HMP's, especially herbal medicinal preparations classified as "other extracts" in Ph Eur and combination products containing different preparations, is often very complex. Lead compounds are in a low concentration range and it is difficult to analyse them in 900 mL dissolution medium per dosage. Some other compounds are very lipophilic and do not dissolve without a surfactant. Extracts are multi component systems and it is questionable, whether a single compound is characteristic for the preparation.

The goal of the workshop is to discuss different strategies developed by the speakers to test dissolution of HMP's in practice. The results of products of well established (e.g. chestnut, a sedative combination, passion flower, senna, gingko) as well as traditional use (herbal powders in solid dosage form) will be presented. The addition of a surfactant to the medium as well as the dissolution characteristic by unspecific UV/Vis-Spectra of a combination product will be discussed. Considering high extract and low excipient dosage forms in most HMP's, the question, if tests on disintegration are more conclusive than a dissolution profile, is presented to the participants. Furthermore, the actual regulatory status will be presented.

Acknowledgements: The Workshop will be sponsored by **Zeller AG**, Herbal Medicinal Products, CH-8590 Romanshorn und by **PhytoLab GmbH**, D-914867 Vestenbergsgreuth.

W 002 Herbal drug preparations and rhinosinusitis – complex mixtures to manage a complex disease?

Chair: I. Szelenyi[a]

Panelists: St. Maune[b]*, A. Pahl*[a] *and P. Stierna*[c]

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Rhinosinusitis is a continuum of nasal disease including rhinitis, which may be allergic or non-allergic, sinusitis and possibly polyposis. Since rhinosinusitis affects about 10 – 15% of the population it is regarded as being among the most prevalent respiratory diseases in the western world. It adds considerably to patients morbidity and socio-economic burden. The treatment strategies for rhinosinusitis can range from the use of intranasal steroids or antibiotics to even surgery dependent on the stage or severity of rhinosinusitis. An additional and increasingly popular option to treat rhinosinusitis is represented by herbal drug preparations.

The first part of the workshop will concentrate on the classification of rhinosinusitis, defining and describing the various stages of sinusitis ranging from acute to chronic. The up-to-date standard therapies for each stage will be presented. The presentation will particulary focus on those stages of rhinosinusitis during which herbal therapies are indicated as well as on the advantages offered by the herbal drug preparations. Finally, results derived from preclinical studies are presented to offer a pharmacodynamic rationale for the use of herbal drug preparations in rhinosinusitis.

The second part of the workshop will focus on the difficulties encountered when trying to preclinically model complex diseases such as rhinosinusitis and to investigate molecular-biological and pharmacodynamic mechanisms of herbal drug preparations. Selected examples of experimental approaches will be presented and the significance of preclinical standard models for selecting herbal drug preparations will be discussed.

How to implement the new legislation on Herbal Medicinal Products (HMPs) in Europe?

W 003

Chair : A.J. Vlietinck[a]

Panellists : K. Keller[b]*, V. Silano*[c]*, L. Kabelitz*[d]

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[b] European Medicines Agency (EMEA), Herbal Medicinal Products Committee (HMPC), London, GB.

[c] Ministero della Salute, Roma, Italia

[d] PhytoLab, Vestenbergsgreuth, Germany.

The widespread and increasing worldwide use of herbal medicinal products (HMPs) demands that appropriate regulatory actions are undertaken to regulate and harmonize the legal status of plant preparations throughout Europe.

At the level of the European Medicines Agency (EMEA) the mutual recognition and bibliographic applications for HMPs have been adapted in the light of the most recent experiences gathered by the competent national authorities of the European Union. (Annex 1 to CD 2001/83 amended by CD 2003/63 (25.06.2003)).

Recently a new legislation for the simplified registration of traditional herbal medicinal products (THMPs) (CD 2004/24/EC) was approved (31.03.2004).

The newly constituted committee on HMPs (HMPC) met for the first time in September 2004 to discuss the working methodology and installed three drafting groups (ORGAM, Quality and Safety Efficacy). The HMPC is fully operational since November 2004. Its most urgent tasks are the establishment of a list of traditional herbal substances and the drafting of community herbal monographs with well-established as well as traditional uses.

It is the aim of the workshop to discuss the first experiences and the perspectives taking into account the new legislation. Viewpoints from both the European (K. Keller) and the national authorities (V. Silano) as well as from the pharmaceutical industry (L. Kabelitz) will be presented.

W 004 Good Practices, Standards and Certifications of Starting materials

Chair: Ch. Franz[a]

Panelists: B. Pätzold[b], *R. Iguera*[c], *B. Steinhoff*[d]

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[b] IWWF Germany, Rebstöcker Str. 55, D-60326 Frankfurt/Main

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An estimated 40.000 to 50.000 plant species are used in traditional and modern medicine systems throughout the world, of which a considerable portion is collected from the wild – partly over-harvesting and threatening the respective species – and a smaller number is large scale or small plot cultivated. From the viewpoint of the herbal industry, a guaranteed supply of herbal raw materials in requested quantity and price as well as of high and consistent quality is of great importance. The producer – either collector or farmer – is challenged to respect all above aspects and fulfil all requirements from nature protection and conservation via standard operating procedures to quality control, certification and audit systems.

The workshop will deal with these topics from three points of view:

WWF / TRAFFIC elaborating actually an International Standard for Sustainable Wild Collection,

EUROPAM /EHGA representing the farmers and known as editor of GA' / GWP Guidelines (Guidelines for Good Agricultural resp. Good Wild Crafting Practice for Medicinal and Aromatic Plants, adopted by EMEA as Ponts to Consider...), and

AESGP WSMI since manufacturers of medicinal (herbal) products do have to provide a complete documentation "from the seeds to the final product" for getting the marketing authorisation also for HMP's.

References: www.floraweb.de/proxy/floraweb/map-pro, www.europam.net, www.emea.int. Máthé, Ch. Franz: GAP and Quality of Phytomedicines. J. Herbs, Spices & Med.Plants 1999, 6(3):101-113. Steinhoff: SOP for Medicinal Plants. J. Herbs, Spices & Medicinal Plants 2003, 10(3): 109-125

Tests for improvement of learning and memory

W
005

Chair: H. Winterhoff[a]
Panelists: C. Vonhoff[a]*, M.F. Melzig*[b]
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After a general introduction lectures will be hold by Prof.Dr. Melzig, Berlin on "Test systems of Alzheimer´s Disease" and by Dr. Chr. Vonhoff, Münster on "Animals models in testing learning behaviour and memory-its application in testing phytotherapeuticals and examples of plants and phytochemicals".
With the increasing age of the population in Western countries the part of people with loss of cognitive function, esp. with Alzheimers disease, increases exponentially. Therefore there is an enormeous demand for medications reducing the velocity of memory loss effectively.
The lecture of Prof Melzig deals with the neurodegenerative process in Alzheimers disease which is triggered either by the processing of β-amyloid plaques or by cytoskeletal changes. Treatment strategies based on the knowledge of the molecular events in the development of Alzheimer´s Disease will be presented as well as a spectrum of biochemical and cell-based assays. In addition different relevant in vivo models screening for diverse points of attack will be presented.
The talk of Dr. Vonhoff deals with diverse animal models testing for an improvement of learning and memory. Experiments using positive or negative stimuli are presented and their relevance discussed. An important part of the talks deals with the benefit and the disadvantage of the diverse test systems.

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ABSTRACTS OF SHORT LECTURES

SL 001 Direct infusion ion trap mass spectrometry: method development and applications in metabolomics

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The rise of metabolomics has demanded the development of fast and reliable unbiased analytical methods that are able to yield qualitative and quantitative data on as many metabolites as possible. Direct infusion electrospray ionisation mass spectrometry is one of the applied strategies, often employing time of flight technology to resolve as many compounds as possible with accurate mass resolution. This is a fast method and the accurate mass helps in the identification of the metabolites.
We have applied a different strategy, using ion trap mass spectrometry. The principle of the method is based on the idea that the fragmentation of each ion in the mass spectrum delivers highly discriminative information about the chemistry of the metabolites present. To obtain this information crude solvent extracts were infused into the mass spectrometer (1). Each experiment was run for 6 minutes during which time the mass spectrometer collected a full mass spectrum of the extract together with spectra from a series of collision-induced dissociation reactions. Within a 6 minute run this process yielded around 250 MS^2 spectra, of which around 60 % yielded a MS^3 spectrum, depending on the concentration of the metabolites.
The method has proved to be extremely sensitive in determining metabolomic differences between samples in a number of experiments. The collected fragmentation patterns facilitate rapid identification of the ions of interest to at least the chemical class level. With this method we mapped metabolic differences between different strains of fungi with a disrupted unknown non ribosomal peptide synthetase. On the basis of the multivariate statistics the differences could be pinpointed to the levels of specific ions, which could be identified by their MS2 and MS3 spectra. Although the first candidate ions were characterised as phosphatidylcholine lipids, research is in progress to discover the metabolites that are the direct result of the unknown NRPS. The high density of chemical information obtained with this method makes it extremely useful in metabolomics studies.

References: 1. Smedsgaard, J. et al. (1996) J. Microbiol. Meth. 23: 2-17.

SL 002 Potential of microflow LC/NMR for the identification of natural products at the μg level

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The combined use of LC/UV-DAD, LC/MS and LC/NMR represents a strategic element for the rapid identification of natural products in crude plant extracts and for dereplication prior to the isolation process (1). These techniques have also shown to be very efficient for the study of unstable constituents or compounds not isolable at the preparative scale. In this approach however, compromises have to be made for LC/NMR measurements since this technique is by far the much less sensitive than LC/MS and solvent suppression is needed when HPLC grade solvents are used. The recent development of flow probes with reduced coil volumes (CapNMR) has enhanced the sensitivity of NMR and change the concept of hyphenation (2). With such probes the idea is not anymore to work *on-line* but *off -line* directly on the peaks of interest collected from the HPLC. While more sample handling is needed for this approach the quality of NMR spectra is considerably enhanced. Samples can be directly measured in small amount of deuterated solvent (5µl) and the sensitivity is increased thank to the important concentration obtained. The use of CapNMR give the possibility of recording ^1H spectra in the low microgram range while more demanding experiments such as HSQC or HMBC can be recorded overnight ($>$ 20 µg of sample is needed). The possibility of acquiring NMR data on sample scales equivalent to biological screening amounts can considerably accelerated lead finding process. Different examples of applications at the small scale will be illustrated in the case of metabolomic studies.

References: 1. Wolfender, J.-L. et al. (2003) *J. Chromatogr. A* 1000, 437. **2.** Olson, D. L., et al. (2004) Anal. Chem. 76, 2966.

Application of GC-MS and HPLC-DAD for birch metabolome analysis

SL 003

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Plant metabolome is the quantitative complement of all the low molecular weight compounds present in the plant object in a particular physiological or developmental state. The metabolome, with all of its individual concentrations and quantitative inter-relationships, forms the biochemical phenotype of plant. Metabolites are not chemically homogeneous and, because of huge chemical diversity, measuring many or even an entire set of metabolites from a single sample is a critical aspect of metabolomics. For analysis of birch leaf metabolome, we developed experimental technique that includes a sample extraction, a single fractionation step of leaf extract into lipophilic and polar phases, external silylation of metabolites, and their separation and quantification with GC-MS which combines high chromatographic separation power with a universal detector to produce excellent sensitivity and selectivity. About 500 distinct polar and lipophilic compounds could be detected in a single extract. After rigorous comparison of mass spectra with commercially available libraries and with spectra of reference compounds, many birch leaf metabolites were identified. However, GC-MS technique was not useful for analysis of polar phenolics. Therefore, for identification and quantification of this segment of birch leaf metabolome (45 - 80 individual compounds), HPLC system couples to mass spectrometry detector or diode-array detector (DAD) was used. The metabolomic approach was applied for determination and identification of ecologically and pharmacologically active metabolites (polar and lipophilic phenolics, triterpenoids and etc.) in the leaves of different birch species.

Metabolomic analysis of *Strychnos* species extracts by ^{1}H nuclear magnetic resonance spectrometry and multivariate analysis techniques

SL 004

M. Frédérich [a,b], *Y. H. Choi* [a], *L. Angenot* [b], *G. Harnischfeger* [c] *A.W.M. Lefeber* [b], *R. Verpoorte*[a].
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[c] Schafer and Brummer GmbH and Co KG, D-38251 Salzgitter, Germany

^{1}H Nuclear magnetic resonance spectrometry and multivariate analysis techniques were applied for the metabolic profiling of three *Strychnos* species: *Strychnos nux-vomica* (seeds, stem bark, root bark), *Strychnos ignatii* (seeds), and *Strychnos icaja* (leaves, stem bark, root bark, collar bark). The principal component analysis (PCA) of the ^{1}H NMR spectra showed a clear discrimination between all samples, using the three first components. The key compounds responsible for the discrimination were brucine, loganin, fatty acids, and *Strychnos icaja* alkaloids such as icajine and sungucine. The method was then applied to the classification of several "false angostura" samples. These samples were, as expected, identified as *S. nux-vomica* by PCA, but could not be clearly discriminated as root bark or stem bark samples after further statistical analysis. The method was then applied to the discrimination of *S. usambarensis* tree form (east Africa) and liana form (west Africa).

Acknowledgments: Belgian National Fund for Scientific Research, Post-doctoral Fellowship Program of the Korea Science Engineering Foundation (KOSEF).

SL 005 Bioavailability and pharmacokinetic studies on Echinaforce™ preparations and their interaction with the immune system

K. Woelkart[a], A. Suter[b], C. Koidl[c], R. Raggam[c], B. Kleinhappl[c], E. Marth[c] and R. Bauer[a]

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[b]A. Vogel Bioforce AG, CH-9325 Roggwil, Switzerland; [c]Institute of Hygiene, University of Medicine, 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, 2) The alkamides have recently been shown to be quite fast absorbed and nanomolar quantities have been detected in the blood after oral application of a tincture from *Echinacea angustifolia* roots. (3) In order to compare the bioavailability of alkamides from liquid and tablet preparations of *E. purpurea* (Echinaforce™) and to study the *ex vivo* effects in humans, we performed a randomised, open, single-dose, crossover study with 8 volunteers. They received either 4 ml of the standardized liquid Echinaforce™ or 12 Echinaforce™ tablets. Both doses contained the same amount (0.07 mg) of the major alkamides, dodeca-2*E*,4*E*,8*Z*,10*E*/Z-tetraenoic acid isobutylamides. Liquid chromatography electrospray ionisation ion-trap mass spectrometry was used to determine the content of alkamides in serum. We found, that the mean C_{max} of dodeca-2*E*,4*E*,8*Z*,10*E*/Z-tetraenoic acid isobutylamides absorbed after oral application of the Echinaforce™ drops appeared after 30 min with ca. 0.40 ng/ml serum. In comparison the T_{max} of tablets was 45 min with a C_{max} of 0.12 ng/ml. An *ex vivo* study was conducted to measure the influence on the innate and adaptive immune system. Both preparations released the same effects on the immune system based on concentration of proinflammatory cytokine TNF-α and the chemokine IL-8 or IL-6, respectively. 23 hours after oral application we found a significant downregulation of LPS-stimulated TNF-α and IL-8. However, we observed no induction of B cell response.

References: 1. Woelkart, K., Xu, W. et al. (2005) Planta Med, submitted. **2.** Gertsch, J., Schoop, R. et al. (2004) FEBS Lett 577: 563-9. **3.** Woelkart, K., Koidl, C., et al. (2005) J Clin Pharmacol 45, in press.

SL 006 Solid state activation and self-emulsifying pellets to improve the in-vivo bioavailability of Silymarin in rats.

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Silymarin is a mixture of flavonolignans extracted from the fruits of *Silybum marianum* (L.) Gaertn. (Asteraceae). They are well known for their excellent activity in protecting liver cells from harmful effects caused by smoking, alcohol, overworking, environmental contaminants, stress and other liver-damaging substances. However, the bioavailability of orally administered flavonolignans is low due to their solubility problems in water and to the spontaneous formation of non-absorbable microcrystals. Hence, the aim of this work was to develop an oral Sylimarin formulation through its solid state activation with different polymeric excipients. Furthermore, self-emulsifying pellets were prepared using the 10-l Roto-J Zanchetta high shear mixer, by incorporating a mixture of mono- and di-glycerides, polysorbate 80 and water (oil to surfactact ratio of 1:4 w/w), in a powder mixture of microcrystalline cellulose, lactose and Sylimarin. After preparation, the formulations were evaluated for their in vitro dissolution and absorption properties. The results demonstrated that all the performed systems showed an improved in vitro dissolution and permeation of flavonolignans with respect to an extract of *S. marianum* (L.) and its commercial products. Comparing the pharmacokinetics of the solid complexes and the self emulsifying pellets to that of commercially available "Silymarin" preparations, after administration of single oral dose of silybin (200 mg/kg) to rats, a seven-fold higher relative bioavailability of the Silymarin from self emulsifying pellets was measured. Therefore, this approach allowed to improve the dissolution and permeation properties of the hepatoprotective flavonolignans from *S. marianum*.

Liposomes and β-cyclodextrin complexes to improve stability of verbascoside

SL 007

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Verbascoside is a phenylpropanoid widely spread in nature having various biological effects: antioxidant (1,2), anti-inflammatory (3), antitumour (4) and *in vitro* immunomodulatory (5) activities. No investigations are instead reported concerning its low chemical stability in aqueous solutions at different pH. Our investigation has been focused on the possibility to use liposome or complexes with β-cyclodextrin in order to improve the stability of the molecule. Thus, liposomes are self-assembled colloidal particles tipically which are extensively studied as carrier systems because can improve activity or protect sensitive drugs. Cyclodextrins are widely used to increase water solubilty, or improve stability and bioavailability of drugs. Liposomes were prepared from soya phosphatidylcholine and cholesterol as large multilamellar vesicles (MLV) and small unilamellar vescicles (SUV). SUV were prepared from the MLV by sonication under a nitrogen steam. Vesicle dispersions were characterised by transmission electron microscopy (TEM) for vesicle formation and morphology, by dynamic light scattering (DLS) for mean size and polydisperivity index, and HPLC evidenced a good incorporation efficiency (60-65%) preventing the degradation of verbascoside. Inclusion complexes with β-cyclodextrin were obtained by freeze-drying method. The complex was structurally characterized by one- and two-dimensional nuclear magnetic resonance experiments by the application of NMR advanced techniques such ROESY and DOSY. The studies evidenced the caffeic moiety was included in the Cdβ-cyclodextrin. Stability testing at room temperature evidenced that verbascoside was very stable (<90%) in water solution after 8 months.

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Re-evaluation of bioactivities of various plants (*Origanum dictamus, Thymus*, *Myrtus* species*)* of Greek origin, before and after encapsulation in liposomes

SL 008

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The biological activities (antimicrobial and antioxidant ones) of 10 natural extracts (*Origanum dictamnus*, several endemic *Thymus* species and *Myrtus communis*) as well as of the essential oil of *O. dictamnus*, have been evaluated before and after their encapsulation in liposomes(1). Among them, *Myrtus* and *T. leucotrichus* (methanolic extracts), *T. longicaulis* (CH_2Cl_2) showed the higher antioxidant action using three different methods (Rancimat, MDA and DSC) and compared with common commercial antioxidants BHT and α-tocopherol. Especially, myrtle showed superior antioxidant activity than BHT. The volatiles of *Origanum. dictamnus,* (wild and cultivated in Crete), have been also studied through GC-MS analyses. All samples were encapsulated in liposomes and their antioxidant action was again estimated. Thermal-oxidative decomposition of the samples (liposomes pure and encapsulating extracts) was studied by DSC method. In all cases their antioxidant activities proved to be superior from the same in pure form, exhibiting superior activity even than BHT and α-tocopherol. Differential Scanning Calorimetry was employed to study the phase transition characteristics of MLVs liposomes (2). Besides, almost all plants have been exhibited antimicrobial activities against all the assayed microbia (6 Gram positive and negative bacteria and three human pathogenic fungi), while the oil of *O. dictamnus* appeared as the most active mostly due to its carvacrol content. After their encapsulation in liposomes all samples, showed also stronger antimicrobial spectrum of action, against all tested micro organisms.

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SL 009 Inhibitory effects of 5-*O*-demethylnobiletin on lymphocyte proliferation and the production of inflammatory mediators

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5-*O*-Demethylnobiletin (DMN) is a polymethoxyflavone isolated from *Sideritis tragoriganum* sp. *mugronensis* (1). We previously reported on its anti-inflammatory and anti-allergic effects using different experimental models, such as acute ear oedema induced by carrageenan and PLA_2, chronic ear inflammation induced by repeated application of TPA, as well as on the delayed-type hypersensitivity (DTH) induced by different agents in mice (2). In order to gain insight into the mechanism of action of DMN, we examined its effect on lymphocyte proliferation and their cell cycle, as well as on its influence on the production of several cytokines and other mediators involved in inflammation, such as NO and LTB_4 (3,4). DMN was found to strongly inhibit lymphoproliferation ($IC50 = 2.8$ μM), arresting the cell cycle in the G_1 phase. The production of mediators such as IL-1β, IL-2, TNF-α, and IFN-γ by human lymphocytes was also significantly inhibited by DMN ($IC_{50} < 2.5$ μM for all the mediators), whereas IL-10 production was actually increased (by 43% at 5 μM). DMN down-regulated the NOS-2 induction in lipopolysaccharide-treated RAW 264.7 murine macrophages, reducing the NO production with an $IC50 = 8.3$ μM, but it did not inhibit the activity of the enzyme. In addition, DMN decreased the LTB_4 production ($IC_{50} = 0.35$ μM) in rat polymorphonuclear leukocytes by directly inhibiting the enzyme 5-lipoxygenase. Our results indicate that the effect of DMN on inflammatory cells, mainly lymphocytes, and their inflammatory mediators explain its anti-inflammatory and anti-allergic activity previously demonstrated *in vivo* (2).

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SL 010 New Water Soluble Indirubin Derivatives as Selective GSK-3 Inhibitors

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Gastropod mollusks of the Muricidae family (Hexaplex trunculus, Murex brandaris and Thais haemastoma) have been used for more than 3,500 years to produce the "Tyrian purple" dye. In an effort to identify new kinase inhibitors we investigated the natural indirubins produced by the three aforementioned Mediterranean mollusks. Bioguided fractionation of the extracts recently led to the isolation of 6-bromo-indirubin (1) that showed very strong inhibitory activity against glycogen synthase kinase (GSK-3) and was used as a lead compound for the synthesis of several derivatives (>60), with various substituents at positions 1,4,5,6,3′,6′. The product 6-bromo-3′-oxime (BIO) have showed the most powerful activity ($IC_{50} = 5$ nM) combined with very good selectivity for GSK-3. Co-crystal structures of GSK-3/BIO and CDK5/p25/indirubin-3′-oxime have been resolved, providing a detailed view of indirubins' interactions within the ATP-binding pocket of these kinases and permitting the design of more soluble derivatives. New derivatives with an amino side chain attached to BIO showed similar activity, ameliorated selectivity and dramatically increased water solubility.
Recently, there is growing evidence that the selective inhibition of GSK-3 would be of great pharmacological interest especially for the treatment of neurodegenerative diseases. It should also be noted that through the GSK-3 inhibition, BIO has already found a very innovative application in stem cells cultures (2).

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Panax ginseng C.A. Meyer root extract (G115) modulates TLRs expression in mice under physical stress.

SL 011

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Significant changes in the level and functional activity of immune parameters have been observed in athletes linked to a higher incidence of infection and illness. These mechanisms seem to be related to a different expression of Toll-like receptors (TLRs). TLRs are transmembrane receptors conserved throughout evolution and they play critical role in host defence. The root of *Panax ginseng* C.A. Meyer is widely used as a general tonic as well as agent improving the resistance against infections. We studied the action of Ginseng on TLRs expression in mice underwent to training exercise.Male 6 week-old BALB/c were treated with G115, a standardised extract *P. ginseng* root, and kept swim 1 hour a day for 4 weeks. Suitable control groups were performed. Total RNA was purified from peritoneal macrophages. cDNA was synthesized and subjected to Real Time quantitative PCR to study the expression levels of TLRs. *Results:* TLR4: a peak of expression of about 4 times higher, was observed at first week of exercise in control group, declining up to basal value at 4th week. In treated animals was observed a progressive increase of expression peak, starting from 2nd week and with a maximum (5 times higher *vs* sedentary) at 3rd week. TLR3: a peak was observed at 3rd week of exercise (9 times higher vs basal value in untreated animals and 23 times higher in treated animals). TLR 2 was non-affected by stress as well as by treatment. G115 significantly increases TLR3 and TLR4 expression compared to controls. Particularly the TLR3 appears massively increased after three weeks of exercise. These data support the hypothesis that TLRs could be a target of Ginseng in modulating the immune response.

Neural networks: Tools for the NF-κB inhibiting sesquiterpene lactones

SL 012

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Sesquiterpene lactones (SLs) are the active components of a variety of traditionally used medicinal plants from the Asteraceae family. We could recently demonstrate that SLs inhibit DNA binding of the central transcription factor NF-κB probably by alkylating cysteine38 of its p65 subunit (1). NF-κB plays a pivotal role in controlling the expression of multiple inflammatory and immune genes involved in toxic shock, asthma, rheumatoid arthritis, or cancer. Thus, NF-κB might be an interesting target in drug research (2).
SLs have been suggested to serve as lead compounds for the design of new anti-inflammatory drugs. To pursue this approach models have to be generated to predict the anti-inflammatory activity of the SLs. We here report the development of a quantitative model using counterpropagation neural networks to predict their NF-κB inhibitory properties. We used a data set of 103 structurally different SLs representing 6 structural groups for which the NF-κB inhibitory activity was previously determined (3). The molecules were described by different atom properties and molecule surfaces either coded by Radial Distribution Function (RDF) or by Auto Correlation Coefficient. To prove the predictive power training and prediction sets were generated. The results correlated well with those recently obtained by a QSAR study using multiple linear regression analyses (3).
This neural network is the basis to predict the NF-κB inhibitory activity of other SLs or synthetic compounds for which SLs have served as lead compounds.

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SL 013 Shikonin Selectively Inhibits Splicing of Tumor Necrosis Factor alpha transcripts

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An *in vivo* phytocompound-screening system was developed in a previous study of our laboratory, and we identified shikonin as a potent suppressor of TNF alpha gene expression. Although this phytocompound was shown to interfere with the binding of basal transcription complex to TATA box, the mechanism on how shikonin can achieve the specific regulation of TNF alpha gene expression remains unknown. Here we report that shikonin can selectively inhibit the expression of TNF alpha at the RNA transcripts splicing level. When shikonin is present in test cell culture or *in vivo* system during induction, we show that the flow of both basal pool and induced precursor transcripts into mature TNF alpha mRNA can be blocked, and unspliced TNF alpha precursors accumulate at the expense of functional mRNAs. This effect is very sensitive to the shikonin concentration and is highly specific because neither housekeeping genes nor other inflammatory cytokine genes tested exhibited such a similar regulatory response. Our results also suggest that the PKR signaling pathway may serve as a primary target for this shikonin's action. Based on these findings, we evaluate the potential of shikonin as a candidate in anti-inflammatory therapeutics and propose a possible action site for shikonin on its multifactorial anti-inflammatory activities.

SL 014 Anti-depressant, anxiolytic and nootropic activity of murraya koeinigii leaves

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Anxiety and depression are debilitating multifacetic disorders for which effective treatment strategies are inadequate due to complexities of the ailment. *Murraya koeinigii* (Family Rutaceae) [MK] leaves contain carbazole alkaloids, which resemble neurotransmitters structurally; hence the present work investigated its methanolic extract for anti-depressant, anxiolytic and nootropic activity. The anti-depressant activity was evaluated in Swiss albino mice using tail suspension test, despair swim test, reserpine antagonism, amphetamine-induced excitation and anorexia, potentiation of nor-epinephrine toxicity and by quantitative estimation of catecholamine levels in mice brain. Anxiolytic activity was assessed using plus maze model, open field test and light dark model. Further, the nootropic potential of extract was evaluated using Morris water maze test and estimating cholinesterase levels in mice brain. In-vitro antioxidant potential was evaluated by estimating TBARS levels in mouse brain homogenate. One-Way ANOVA followed by Dunnett's test was applied for statistical significance. Pretreatment with MK extract resulted in decreased immobility time in tail suspension and despair swim test. Reduction in degree of ptosis and catalepsy revealed reserpine antagonism while enhancement of amphetamine induced excitation and anorexia as well as potentiation of nor-epinephrine toxicity revealed anti-depressant activity, which was confirmed by increased catecholamine levels in mice brain. Administration of MK 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. It also showed in vitro antioxidant activity and reverted the scopolamine-induced alterations in cholinesterase levels in mice brain. In conclusion, the MK extract exhibited anti-depressant, anxiolytic and memory-enhancing (nootropic) activity with utility in oxidative cognitive impairment due to its antioxidant potential.

Determination of the absolute configuration of secondary alcohols by combining Mosher's derivatization technique with LC-SPE-NMR: Polyacetylene derivatives from Apiaceae as case study

SL 015

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The formation of diasteromeric Mosher ester derivatives has a been used for more than three decades to aid the absolute configuration determination of secondary alcohol derivatives (1,2). Usually, this methodology is based on two separate reactions of mg quantities of the chiral analyte with an excess of pure enantiomeric Mosher acid chlorides – namely (2*R*)- and (2*S*)-2-Methoxy-2-trifluoromethylphenylacetyl chloride (MPTA-Cl). After preparative separation of the desired derivative from residual amounts of alcohol and acid, the formed esters can be characterized by ^{1}H and ^{19}F-NMR spectroscopy. Shift differences can be correlated with the absolute configuration of the alcohol by empirical rules (2). This technique found broad application in natural product structure elucidation and has been applied to a variety of substance classes. Recently, LC-NMR was introduced to avoid the laborious analyte isolation (3,4). Within this case study, the analysis of eleven chiral polyacetylene derivatives is described. Besides using 1D and 2D NMR methods for signal assignment of these sometimes hardly described analytes, the novel LC-SPE-NMR hyphenation (5,6) was utilized to process the derivatized samples. This technique allowed to use significantly less Mosher reagent and to keep analysis times below 15 minutes/sample. This is the first report on SPE peaks transfers using $CDCl_3$ as NMR solvent. This technique allowed assigning the absolute configuration of 6 polyacetylene derivatives. LC-NMR derived ^{19}F-NMR spectra were recoded in selected cases to aid the configuration assignment. The advantages and limitations of this approach will be discussed briefly.

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HPLC-SPE-NMR in natural products research

SL 016

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Natural products are the most consistently successful source of leads for drug candidates due to their high chemical diversity and broad biological functionality. However, the bottleneck prohibiting a broader use of natural product libraries in industrial pharmacological screens is the high level of cost and labor associated with isolation and purification of natural products. Traditionally, structure elucidation of natural products has been performed by spectroscopic analysis of purified components and thus the structural information was obtained at the end of an often very lengthy isolation- and purification process. This lecture will describe state-of-the art LC-NMR methods for natural products dereplication. Various HPLC-NMR implementation schemes will be described and discussed, with emphasis on the most recent developments employing solid-phase extraction (HPLC-SPE-NMR/MS), with or without use of cryogenically cooled probe-heads. These techniques provide a rapid access to complete sets of 2D NMR data directly from complex mixtures, following repeated injections of sub-milligram amounts of crude extracts directly into analytical HPLC columns. Applications include drug discovery, quality control of herbal drugs, food science, metabolomics/metabonomics, and may others. Examples of the use of hyphenated methods will be presented showing, that time required for extract dereplication may be reduced from many months to weeks or days. When coupled with an automatic compound identification, the hyphenated NMR methods will constitute a productivity tool, completely changing the way natural products research is being conducted today.

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SL 017 A new HPLC-UV method for the analysis of antocyanins and anthocyanidins in *Vaccinium myrtillus* fruit extracts

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The *Vaccinium myrtillus* fruits (bilberry) are well-known anthocyanins source and the extracts are widely used in dietary botanicals and pharmaceutical market for the treatment of vascular and vision disorder.
Different analytical methods employed for standardization of the bilberry extracts and their preparations are available from Pharmacopoeias and from literature. The most common analytical methods employ UV-visible spectrophotometry technique that allows the quantification of anthocyanins by detection in the visible region. In spite of the fact that these methods are the most popular they lack in specificity and do not allow the identification of each anthocyanin. As a consequence these methods are not suitable for the differentiation among extracts produced with different plant materials (raspberry, blackberry, black currant, elderberry, etc.).
High-performance Liquid Chromatography is the best technique for standardization of anthocyanin extracts because allows the evaluation of the individual anthocyanins. However the methods reported in the literature do not allow the detection the free anthocianidins which are markers of the product degradation (e.g. inappropriate storage conditions of the plant material).
A new HPLC method was developed and validated for the identification and the quantification of both anthocyanins and anthocyanidins present in the bilberry plant material and extracts. The method shows a good reproducibility and due to its high specificity is suitable to identify unequivocally the botanical raw materials used for manufacturing and evaluation of the extract composition hence assuring a high degree of product consistency and quality.

SL 018 Analysis of cucurbitacins in *Citrullus colocynthis* by MEKC and HPLC/MS

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Citrullus colocynthis, Schrad. (Cucurbitaceae), a plant distributed throughout North Africa, Syria, Persia, and North-West India, and cultivated in Spain and Cyprus, has been known for its medicinal properties since ancient times, particularly as a powerful hydragogue cathartic (1). Most of the reported bioactivities as well as the toxicity of the plant have been associated with the presence of cucurbitacin glycosides, implicating the demand for a reliable analytical assay for these compounds. Several methods have been published in this connection, but none of them allowed sufficient separation of the constituents present in *C. colocynthis* (2,3). Therefore we decided to establish the first CE-method for this class of compounds and as alternative an optimized HPLC/MS-assay.
In the course of isolation and structural elucidation of reference compounds, cucurbitacin J- and K-glycoside have been identified for the first time in this plant species, beside already reported cucurbitacin I-, L-, E- and dihydrocucurbitacin E-glycosides.
Baseline separation of all compounds was achieved by MEKC with a borat buffer solution (15 mM, pH 9.6) containing 14% of acetonitril and 30 mM of SDS. The voltage was 30 kV, injection was performed in the pressure mode (50 mbar, 3 s) and the operating temperature was 25°C.
The best HPLC/MS-separation and sensitivity was observed using a Phenomenex Synergi Polar-RP column (250 x 4.6 mm, 4 µm) and a linear gradient of water and acetonitril, both containing 0.1% of acetic acid.
As detection limits concentrations of 7-22 µg/mL were determined, linearity was observed in a range from 10 to 1000 µg/mL. Correlation factors for all compounds were > 0.999. The validity of the presented methods was confirmed by standardisation of cucurbitacin glycosides in crude methanolic extracts of *C. colocynthis*. The results obtained by the two alternative methods will be compared.

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SL 019

NMR for the screening of plants water extracts: a rapid approach to better link ethobotanical data with further phyto-pharmacological investigations.

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Several NMR experiments are currently exploited for the study of protein-ligand affinity(1). These analyses can be used for the identification of a bound ligand in mixtures of unbound ligands. Although this method is routinely performed to screen potential drugs especially derived from a synthetic chemistry approach, it can also be applied to many others sources of potential medicaments, like, for instance, crude natural extracts from medicinal plants. In such kind of NMR analysis, the ideal solvent for the proteins is deuterated water. This fact induced us to screen directly water-based plant extracts (infusions and decoctions, for example) as sources of potential bioactive ligands. The water-based extracts represent the most quoted ways to administer natural remedies in many traditional medical systems.
In this work, we present the first example of application of saturation transfer difference (STD) and transferred-NOESY experiments to the crude hot water extract of the fruiting bodies of *Pleurotus ostreatus*(2), a well known edible and medicinal mushroom. We have used the mannose specific lectin from *Lens culinaris* as target protein. The identification of different ^{1}H NMR signals of a bound ligand within the ^{1}H NMR spectra of the whole crude extract was considered an useful data for further phytochemical analyses. For example, the identification of the bioactive compound directly from the crude natural mixtures by a series of 1D selective and 2D NMR experiments, allowed us to recognise an α-trehalose derivative as the major bioactive constituent in terms of affinity with the lectin here chosen as target protein. The already characterised ^{1}H NMR signals of the bound ligand can also be exploited to guide the further bio-assay oriented fractionation of the mixture analysed. The claimed immunomodulatory activity of the hot water extracts of many medicinal mushrooms(3) can be tested by using the approach here described once important lectin-like receptors implicated in the immune-system(3) are available for NMR analysis.
We also present the application of this method to the protein-ligands system represented by the cholera toxin beta-pentamer (CTB) as target receptor and the hot water extract of the bulbs of *Allium sativum* (garlic). Garlic has been mentioned as basic remedy against cholera infection(4). The result of the STDD analysis(5) indicated the interaction between different polysaccharides derivatives from garlic with CTB. A 2 kDa fructan and a 50 kDa galactan were partially identified within the crude extract as the possible interacting ligands. This preliminary proof stimulated the further purification, that is now on going, of these potential bioactive natural products.

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SL 020 NMR-Spectroscopy of Natural Products using Micro Probes and Cryogenically Cooled Probes

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Due to low sample amount, highest possible sensitivity is mandatory for NMR-spectroscopy of natural product. A high sensitivity can be reached by two techniques: a) the use of a micro probe, with an increased filling factor and b) the usage of cryogenically cooled probes, were the thermal noise of the detection coil is reduced. The 1mm MicroProbe, which is currently available at fieldstrengths from 400 to 700MHz, allows NMR measurements of smallest sample volumes in separate NMR tubes. The probe has an extremely high mass sensitivity. This results in up to four times higher signal to noise ratios when measuring the same sample amount compared to a standard 5mm probe at the same field strength. But there are even more advantages going to smaller volumes such as better solvent suppression or less salt dependency. Cryogenically cooled NMR probes are very common to enhance the sensitivity of the NMR experiment and multifarious applications can be found for natural product research. Those probes are available both, for optimum proton and carbon sensitivity. New models of cryogenically probes even allow th detection of nitrogen, phosphorus and fluorine at highest possible sensitivity. In this communication the results obtained from the analysis of some plants such as *Fagopyrum esculentum*, neem tree, *Taxus brevifolia* and *T. peltatum*. The data obtained with the main and characteristic constituent of callus cultures of *T. peltatum*, namely dioncophylline A, are of special interest, as INADEQUATE experiments have been used on a CryoProbe to study biosynthetic pathways to alcaloids plants.

SL 021 Psychotropic activity of plants used in South African traditional medicine to treat CNS-related diseases

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Traditional healers in southern Africa treat many patients for mental and CNS-related afflictions, often with success. We decided to start a project to investigate the medicinal aspect, i.e. the medicinal plants, of the holistic treatment healers utilize. In the first part of the project a database on plant usage was established, currently having over 300 entries. From this database plants were assigned to various bioassays based on their traditional usage: plants used for epilepsy and convulsions, and for sedative purposes were tested for affinity to the GABA-benzodiazepine receptor; plants used for depression and anxiety were tested for affinity to the serotonin transporter (SERT) and for inhibition of MAO-A; plants used for memory-related problems were screened for acetylcholinesterase activity. After plants with promising activity were identified, bioassay guided isolation was carried out, leading to the isolation of two alkaloids, buphanadrine and buphanamine, from *Boophane disticha* with affinity (K_i = 132 and 1799 µM) to the SERT; two biflavones, agathisflavone and amentoflavone, from *Rhus pyroides* with high affinity (K_i = 28 and 37 nM) to the GABA-benzodiazepine receptor; and a new Amaryllidaceae alkaloid from *Crinum moorei*, 1-*O*-acetyllycorine, with an IC_{50} for acetylcholinesterase of 0.96 µM , half of the value of the clinically used galanthamine.

In vitro antimalarial and leukotriene metabolism Inhibitory activities of compounds isolated from *Kniphofia foliosa* roots

SL 022

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The roots of *Kniphofia foliosa* Hochst (Asphodelaceae), which have long been used in Ethiopian ethnomedicine for the treatment of abdominal cramps and wound healing (1), afforded five compounds, namely 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene,10-(chrysophanol-7´-yl)-10-(ξ)-hydroxychrysophanol-9-anthrone, chryslandicin, knipholone and chrysophanol. Although the anthraquinones, anthraquinone-anthrone dimers and their derivaties were reported from *K. foliosa* previously (2, 3), the naphthalene derivative, 2-acetyl-1-hydroxy-8-methoxy-3-methylnaphthalene, was isolated from the genus *Kniphofia* for the first time. These compounds were examined for their antimalarial and cytotoxic activities against *Plasmodium falciparum* and KB cells, respectively, as well as inhibition of leukotriene formation using activated human neutrophile granulocytes. The compounds inhibited the growth of the chloroquine-sensitive 3D7 strain of *P. falciparum* with ED_{50} values ranged from 0.26 µg/ml to 15.4 µg/ml. The anthraquinone-anthrone dimers showed high inhibition of the growth of the malarial parasite *P. falciparum* with very low cytotoxic activity. Among the compounds tested for inhibition of leukotriene formation only knipholone displayed potent inhibitory activity with an IC_{50} value of 4.2 µM, which is twice as active as the commercial 5-LOX inhibitor zileuton.

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The reaction of artemsinin and semisynthetic derivatives with Hemoglobin

SL 023

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Artemisinin (Qinghaosu), largely used in China and East Asia to treat multidrug-resistant Plasmodium falciparum malaria, is obtained from an indigenous plant, *Artemisia annua* L. (sweet wormwood) (1). Due to its low solubility both in oil and water, several semisynthetic derivatives were prepared and characterized, and are now available for clinical use. The reactions of artemisinin and its derivatives, sodium artesunate and dihydroartemsinin, with hemoglobin were investigated by various spectroscopic methods under standard solution conditions (phosphate buffer 50 mM, pH 7, 37°C). Remarkably, all these antimalarial drugs were found to react eagerly with hemoglobin, but not with methemoglobin. The reaction consists of the progressive, slow decay of the Soret band, as a consequence of heme alkylation and subsequent loss of π electron delocalization. For the various drugs the process is complete within about 30-70 hours, at 37°C. Additional experiments were carried out under the solution conditions reported by Selmeczi et al. (2) and by Kannan et al. (3) in their recent studies. Results very similar to those reported above were obtained when adopting the experimental conditions described by Meunier and coworkers(2); in contrast, we observed that under the experimental conditions employed by Kannan, (50% v/v acetonitrile), a dramatic perturbation of the protein structure occurs that leads to destabilization and extensive detachment of the heme group. Analogous reactions were performed with myoglobin, and metmyoglobin. The obtained results confirmed the view that artemisinins do react with myoglobin but not with metmyoglobin. A unified description for the reaction of artemisinin and its derivatives with hemoproteins is provided. Thus, hemoproteins may be correctly considered both potential triggers and targets for artemisinin and derivatives, as recently suggested (4).

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SL 024 Bioactive components of the uteroactive medicinal plant: *Rhoicissus tridentata*

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Rhoicissus tridentata subsp. *cuneifolia* or "wild grape" (Vitaceae) is one of the most commonly selected species for South African traditional medicines used during pregnancy and childbirth. Twenty compounds novel to the species were isolated from extracts of *Rhoicissus tridentata*, which has had virtually no prior chemical investigation. The majority of these compounds promote good health. Water extracts of roots show notable *in vitro* activity on isolated rat uterine smooth muscle tissue. Extracts exhibiting the highest activity were found to contain proanthocyanidin monomers: (-)-epigallocatechin, (+)-gallocatechin, (+)-catechin hydrate, (+)-mollisacacidin, (+)-epicatechin, (-)-fisetinidol and epicatechin-3-O-gallate and dimers: procyanidin B3, procyanidin B4, fisetinidol-(4α-8) catechin and fisetinidol-(4β-8)catechin, as well as gallic acid and 74% polymeric proanthocyanidins. The relative amounts of proanthocyanidins, determined colorimetrically, were higher in summer than in the winter season, and corresponded to a greater uterine activity in summer. Glucose, and a partially identified hydrogel of glucose which greatly stimulated uterine muscle contraction, were isolated. Sitosterol and sitosterolin exhibited only slight oestrogenic activity. Oleanolic acid was isolated from a chloroform extract. Two further triterpenoids, 20(29)-lupen-3-one and 20-epi-ψ-taraxastananol, as well as γ-sitosterol, were identified by GC-MS. The plant growth hormone, triacontanol, was purified from an extract of young branches. The previously reported CNS depressant paralysis attributed to *Rhoicissus tridentata* preparations is possibly linked to sitosterol, sitosterolin and proanthocyanidins present in extracts.

SL 025 Novel biologically active triterpenoids from two African *Combretum species*

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Combretum imberbe (Leadwood, Hardekool) has been used for several medicinal purposes in southern Africa. Preliminary experiments indicated that leaves of this plant contain antibacterial compounds that do not occur in other *Combretum* species (1). In our search for antibaceterial compounds that might have pharmaceutical potential, leaves of *Combretum imberbe* and the closely related *Combretum padoides* were extracted and fractionated by bioassay-guided fractionation. Two new antibacterial triterpenoids (1α, 23β-dihydroxyl-12-oleanen-29-oic acid-3β-O-α-L-2, 4-diacetylramnopyranoside and 1α, 5β-dihydroxyl-12-oleanen-29-oic acid-3β-O-α-L-4-acetylramnopyranoside) along with six known triterpenoids were isolated. The structures of the compounds were elucidated on the basis of NMR and mass spectromety data. All compounds showed moderate (MIC of 62 µg/ml) to strong (16 µg/ml) antibacterial activity against *Staphylococcus aureus, Bacillus subtilis,* and *Mycobacterium vaccae* with compound 2, 5 and 7 being most active. Compound 2 and 3 also had strong anti-inflammatory activity against 3α-hydroxylsteriod dehydrogenase enzyme with an IC_{50} of 10 µg/ml and 7.8 µg/ml as well as moderate cytotoxicity (CC_{50} = 17.6 µg/ml and CC_{50} = 10.5 µg/ml) against Hela cell lines. The results of this study have added new compounds to the global database of phytocompounds , have added new biological activities of compounds isolated earlier and validate the ethnomedicinal use of *Combretum imberbe*.

Acknowledgements: NRF and DAAD provided funding, Hans-Knöll Institute provided facilities.
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Exploring Plant Species from São Paulo State Biodiversity: Structure and Neuropharmacological Properties of Alkaloids from *Erythrina mulungu*

SL 026

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The overall research goals of Biota-FAPESP program have been to discover bioactive natural products and their analogs to be further used as lead molecules in the development of new drugs. These bioprospecting contributions also deal with conservation of natural resources and sustainable economic growth, fundamental for the maintenance of biological diversity from Cerrado and Atlantic Forest, two genuine tropical biomes occurring in Brazil. In our investigations, 1684 extracts obtained from 794 species have been screened for a panel of biological activities including antifungal, antioxidant, potentially antitumoral, and AchE inhibitory activity. To date, some promising plant species have been selected for further phytochemical and bioactivity studies. Among these, *Erythrina mulungu* showed potential activity on central nervous system (CNS) and was selected for bioactivity-directed studies, leading to isolation of two known and one new alkaloid, which showed strong ansiolitic activity, when compared with standard drug diazepam.

Acknowledgements: Research sponsored by Biota-FAPESP Program, CAPES, CNPq
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Lignans of *Linum* species - A chemosystematic study and implications on the evolution of chemodiversity in the genus *Linum*

SL 027

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A chemosystematic study of lignans in *Linum* species was conducted. Analysis of 29 accessions from different geographic origins by combined HPLC/UV- and HPLC-MS analysis (1) led to identification of three major clusters of species. Cluster A contains lignans of the aryltetralin (podophyllotoxin-) type, while cluster B contains instead arylnaphthalenes (justicidin type) and/or biogenetically simpler lignans of the butyrolactone- and furofuran types. Several species (cluster C) did not contain any detectable amounts of lignans. The lignan-containing groups are in good agreement with a very recent study on the molecular phylogeny (2). Based on our chemical data it appears that a major branching point in the evolution of *Linum* lignans occured early in the genus' phylogeny leading to the aryltetralin group (sct. *Syllinum, Cathartolinum, Linastrum/Linopsis*) and the arylnaphthalene group (sct. *Linum*, *Dasylinum*).

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SL 028 Cysteine sulphoxide pattern of *Allium* L. – relations to taxonomy

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The content of the cysteine sulphoxides methiin, alliin, isoalliin and propiin was studied in the genus *Allium*, a few related genera and the Brassicaceae *Alliaria petiolata*. Methiin dominated throughout. In the genus *Allium* high variation and flexibility, which showed remarkable correlation to use as spice or vegetable, was found. Two major chemical types could be recognized: Isoalliin dominates in the widely used "onion-type". Chive (*A. schoenoprasum*), top onion (*A. proliferum*), pearl onion and leek (*A. ampeloprasum*) belong to this group. Alliin dominates in the "garlic-type", which is also widely used, and includes also wild leek (*A. obliquum*) and sand leek (*A. scorodoprasum*). Alliin and isoalliin rarely co-dominate as in the cultivated Chinese leek (*A. tuberosum*). Another unusual feature is a triple mix of almost equal amounts of methiin, alliin and isoalliin present in the traditionally used ramson *(A. ursinum*). Other chemical types are less well separable. Methiin-dominated species are rarely used by man.
Some evolutionary trends were suggested. Among the most ancestral groups, high amounts of methiin were more frequent and highest amounts of propiin also occurred here. Most of the species at moderately advanced evolutionary level showed only traces of cysteine sulphoxides. Among species of the most advanced level the "onion-type" dominates, the "garlic type" is characteristic for subgenus *Allium* and co-dominating alliin and isoalliin also occur. Generally, the total cysteine sulphoxide amount increased and the complexity of cysteine sulphoxide patterns decreased in the transition from the most ancestral to the most advanced groups.

Figure. Chemical structures of typical cysteine sulphoxides.

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SL 029 Medicinal plants from cerrado vegetation, Brazil

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The Brazilian Cerrado (*latu sensu*) biome contains a flora estimated in seven thousand species, which have been under strong anthropogenic action in the last decades. The cerrado vegetation occupies an area of 23% of the Brazilian territory, yet less than 1% of the flora has been chemically and/or pharmacologically examined. This study surveyed the wild species from the Brazilian cerrado used in traditional medicine. Twenty-six sites across six states and the Federal District were surveyed resulting in a total of 42 botanical families and 90 species reported as medicinal plants. The outstanding species are: *Brosimum gaudichaudii, Caryocar brasiliensis, Copaifera langsdorfii, Croton antisiphilliticus, Dimorphandra mollis, Hymenaea courbaril, Lafoensia pacari, Lychnophora ericoides, Macrosiphonia velame, Pterodon emarginatus,* and *Stryphnodendron adstringens.*

Acknowledgements: Environment National Fund - FNMA, National Research Council -CNPq
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Identification and quantification of melatonin in *Rosmarinus officinalis* in relation to chlorophyl a and b, RNA and proteins.

SL 030

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Melatonin, a well-known animal hormone, has been identified in extracts from several plants (1,2,3) species but, up to date, its function in vegetables is not well obvious (1,2). In fresh *Rosmarinus officinalis* plants melatonin was identified and quantified using HPLC-MS in relation to chlorophyl a and b, proteins and RNA contents which levels were measured spectrophotometrically. *Rosmarinus officinalis* plant organs grown at different altitudes (255 and 900 m a.s.l.) were sampled every week from April to December. The difference of melatonin amount on extracts sampled in the light-dark cycle shows the light influence on its level. The study related to melatonin distribution on different organs shows that stems and roots contain substantially more melatonin than leaves.

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Hydroxyphenylpyruvate reductase: structure and characterisation of an enzyme involved in rosmarinic acid biosynthesis

SL 031

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Rosmarinic acid (RA) is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid and is found in different plant families [1]. Many studies with RA could show, besides other effects, a potent antioxidant activity. This makes RA an interesting substance for pharmaceutical and cosmetic use.
One of the enzymes involved in RA biosynthesis is hydroxyphenylpyruvate reductase (HPPR) which catalyses the reduction of hydroxyphenylpyruvates to hydroxyphenyllactates in dependence of NAD(P)H [2]. A HPPR cDNA from cell cultures of *Coleus blumei* (Lamiaceae) was ligated into the expression vector pET15b and transformed into the *E.coli* strain BL21(DE3)pLysS [3]. The expressed protein was purified with help of a Ni-NTA column. First enzyme tests could show a substrate specifity for 4-hydroxyphenylpyruvate and 3,4-dihydroxyphenylpyruvate. Phenylpyruvate is only accepted on a low level.
Further purification by affinity chromatography lead to very pure enzyme that could be crystallised. One suitable crystal was measured at the Synchrotron in Berlin (Germany) to a resolution of 1.47 Å. Initial phases were determined using molecular replacement and the structure was solved (PDB code: 1Z46). The HPPR protein shows high structural similarity to other enzymes of the D-isomer specific 2-hydroxyacid dehydrogenase family.

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SL 032 Elicitation of lignan biosynthesis in *Linum nodiflorum* L. by two synthetic indanoyl-amino-acid conjugates

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The aryltetralin lactone lignan podophyllotoxin (PTOX) is used as a precursor for the semi-synthesis of several anti-cancer drugs. The substance is still being isolated from *Podophyllum spec*. that get increasingly scarce. A biotechnological production procedure would ensure reliable podophyllotoxin supply on one hand and protect the endangered species on the other.
The accumulation of 6-methoxy-PTOX (MPTOX) by cell suspension cultures of *Linum nodiflorum* could be enhanced by the application of two synthetic elicitors, coronalon and indanoyl-isoleucine (1,2). While MPTOX contents rose from 0.21 % to about 2.50 % of the dry weight, the production of a related lignan, 5'-demethoxy-6-methoxypodophyllotoxin (5'-dMPTOX), increased from 0.10 % to 4.50 % of the DW.
Besides the lignan accumulation, the catalytic activities of two enzymes involved in lignan biosynthesis, deoxypodophyllotoxin 6-hydroxylase (3) as well as β-peltatin 6-O-methyltransferase (4) were elevated more than 10-fold, delivering evidence for a changed gene expression in the elicitor-treated cells. This finding may help to develop a differential screening system leading to the identification of the enzyme-encoding genes.

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SL 033 Application of Innovative Plant Biotechnology for the Production of Active Compounds from Traditional Herbs, Rare Plants and New Medicinal Plants

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Plants have always been a suitable source for the production of pharmaceuticals. However today the production of modern pharmaceuticals is problematic when using collected or cultivated plant material. The content and spectrum of the active substances varies depending on the environmental conditions. Pests and diseases additionally lead to a decrease of the quality of the plant material. To solve the problem a base technology for biotechnical production of plant extracts and phytochemicals was developed at BioPlanta.
The aim of our work was to apply this technology for the production of active substances from traditional herbs, rare plants and new medicinal plants. Therefore the cultivation conditions had to be optimized for each plant species and the content and spectrum of metabolites had to be customized.
Several species of traditional herbs, rare plants from Chile and anti cancer plants have been taken into *in vitro* culture. Different culture types (callus, shoot and root cultures) were established and compared regarding biomass productivity and content of active substances. For biomass production BioPlanta's proprietary bioreactor technology basing on the Temporary Immersion Principle was applied and optimized.
In most cases cell cultures of the selected plant species show a low contents of metabolites. In opposite organ cultures cultivated on solid medium show a high and reliable metabolite production but a low biomass productivity. It has been demonstrated, that the production capacity of secondary metabolites in the new developed bioreactor system was very high. Moreover, the control of environmental parameters *in vitro* has been used successfully to modify the content as well as the spectrum of the produced metabolites. This is a promising strategy for a reliable production of high quality extracts and phytochemicals *in vitro*.

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Phenolic constituents from *Yucca schidigera* bark modulate Kaposi's sarcoma cell proliferation and motility

SL 034

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Resveratrol (3,5,4'-trihidroxylstilbene), a natural phytoalexin found in grapes, peanuts, and in some medicinal plants is known for its chemopreventive, antimutagenic, antiplatelet, and antioxidant properties. Recently, five phenolic constituents have been identified in *Yucca schidigera* bark, such as *trans*-3,3',5,5'-tetrahydoxy-4'-methoxy-stilbene, resveratrol, and yuccaols A-C related to resveratrol [1]. These phenolic compounds showed high antioxidant activity in blood platelets [2]. Vascular Endothelial Growth Factor (VEGF) acts on endothelial cells by promoting cell proliferation, shape change and migration. VEGF is also able to induce Platelet-Activating Factor (PAF) synthesis in endothelial cells [3]. PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a potent lipid mediator involved in inflammation, allergic reaction, platelet aggregation, and cell adhesiveness**,** is also known for its ability to induce and sustain in vivo angiogenesis and migration on Kaposi's sarcoma cells [4]. In the present study we examined the effect of *Y. schidigera* phenolics on the VEGF-induced KS cell proliferation and on the PAF-induced cell motility to evaluate their possible chemopreventive and anticancer activity. KS cells were rested for 12 h with serum-free medium and preincubated with *Y. schidigera* phenolics (10-25μM). After preincubation, cells were treated for 24h with PAF (40 ng/ml) or for 48h with VEGF (50 ng/ml). Cell migration was studied under a Nikon Diaphot inverted microscope in a plexiglass Nikon NP-2 incubator at 37°C whereas proliferation was determined by colorimetric assay with XTT. Results indicate that yuccaols A-C are more potent inhibitors of VEGF-induced KS cell proliferation compared to *trans*-3,3',5,5'-tetrahydoxy-4'-methoxy-stilbene and resveratrol. Moreover, treatment of KS cells with yuccaol C completely abrogated PAF-induced KS cell motility in a time-dependent manner.

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Multiple approaches to identify new anti-angiogenetic compounds from plant extracts.

SL 035

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Vessel growth deregulation contributes to the pathogenesis of many disorders such as cancer, psoriasis, arthritis and blindness (1). Two members of the vascular endothelial growth factor (VEGF) family, VEGF-A and PlGF stimulate angiogenesis, interacting with a tyrosine kinase receptors, Flt-1(2).The identification of inhibitors of the interaction between VEGF or PlGF and Flt-1 is one of the main targets in the new antineoplastic strategies.

Plants are a source of an almost uncountable numbers of compounds, many of them characterized by antitumoral activities. We stared a screening of plant secondary metabolites to search for new anti-angiogenetic molecules and to elucidate their mechanism of action. We performed a screening of plant secondary metabolites with a competitive ELISA based assay: the Flt-1 receptor was coated on 96-wells plate, and its binding to VEGF or PlGF was competed using the plant compounds. Direct interaction between VEGF or PlGF and bioactive compounds was investigated by Surface Plasmon Resonance (SPR) (3); PlGF or VEGF was alternatively employed as immobilized ligands, and the testing molecules were injected on them at different concentrations. Mass spectrometry techniques were used to study the nature of the protein/analyte interaction and to investigate the binding region (4). Finally, the efficiency of selected molecules as anti-angiogenetic compounds was tested on endothelial cell systems.

Different classes of plant metabolites were tested. Our results clearly demonstrated that dimeric flavone compounds show a very interesting and specific activity. On this basis a more complete structure-to-function analysis was started.

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SL 036 Antioxidative effect of active plant extract

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The flavonoids and other natural antioxidants exert a powerful in-vitro antioxidant action. In this randomised, double-blind cross-over study we investigate the effect of two formulation of concentrated grape-extract in fruit juice on the oxidative status in vivo. Eighteen women and twelve men (smokers and with non-balanced diet) were recruited from a campus population. The participants ingested 75 ml/daily a high-concentrated resp. low-concentrated extract for four weeks during the study twice: before and after the two-weeks wash-out phase. At the beginning of the study, before and after the first treatment phase and before and after the second treatment phase the following parameter were determined: reactive oxygen metabolites (ROM), total antioxidative status (TAS), C-reactive protein (CRP), Apolipoprotein AI (ApoAI) and Apolipoprotein B (ApoB). The ROM decreased significantly during the treatment with high-concentrated extract in comparison to low-concentrated formulation (from 392 U.Carr. to 360 U.Carr. compared to from 398 U. Carr. to 381 U.Carr.; $p < 0,05$). The rest of the examined parameter show no significant differences between the two concentration formulae. The ingestion of a high-concentrated grape-extract in fruit juice under oxidative stress may have beneficial effects by the preventing oxidative injury.

SL 037 Apoptosis inducing activity of the willow bark extract BNO 1455 and its isolated fractions towards colon and lung carcinoma cells.

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Recently, extensive efforts have been made to evaluate the chemopreventive role of substances present in natural products. We investigated the antiproliferative and apoptosis inducing activity of the standardized willow bark extract BNO 1455 and its three isolated fractions containing mainly derivatives of salicyl alcohol (1),flavonoids (2), and proanthocyanidins (3) on human colon cancer cells HT29 (COX positive),HCT116 (COX negative) and A549 (non-small cell), SW2 (small-cell) lung cancer. Comparative studies indicated quantitative differences concerning the IC_{50} values established by WST-1 assay of the extract and fractions towards the different cell lines. Fraction 3 was most effective (IC_{50}: 33.3-55 μg/ml). Lung cancer SW2 cells responded mostly to BNO 1455 and fractions 1-3 with IC_{50} ($\approx$ 50 μg/ml). HCT116 cells were more sensitive than HT29 ($p<0.05$) to BNO 1455 (122.7 vs 211.7 μg/ml) and 1 (71.7 vs 128.3 μg/ml). Apoptosis induction was confirmed by Annexin V adherence and morphological changes in cell size and granularity using flow cytometry in all cell lines at IC_{50} which exerted low toxicity. The viability of cells was higher than 80% using PI uptake.

Marine pharmacognosy in Swedish cold waters - strategy and examples

SL 038

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The appearance of biologically active metabolites in nature is determined by ecological needs and biosynthetic possibilities.The overall objective of our marine pharmacognosy research is to understand aspects of structure-activity relationships of chemical interactions in the marine environment. Our strategy is based on ecologically-guided search for compounds through studies of physiology and organism interactions coupled to identification of target molecules guided by *in vivo* assays followed by chemical characterization and new synthetic strategies. The marine sponge *Geodia barretti* produces a wide range of secondary metabolites.Two congenerous cyclopeptides, i.e. barettin and 8,9-dihydrobarettin were isolated and structurally elucidated guided by their ability to inhibit settlement of the larvae of the barnacle *Balanus improvisus* (1). Settlement of larvae was reversibly inhibited in a dose-dependent manner without any significant lethal effects in concentrations ranging from 0.5 to 25 µM. In addition to these laboratory tests, a field test showed that both barettin and 8,9-dihydrobarettin also significantly inhibited settlement of the blue mussel *Mytilus edulis* (2). The recent successful total synthesis of the two cyclopeptides are a further reason to making them interesting candidates in new antifouling applications (3). Resolving molecular targets of marine defence compounds is important for a sustainable bioprospecting of nature and can also contribute to an increased understanding of similarities between structure-activity relationships in invertabrates and humans.

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Isopimaric acid and abietic acid are active against multidrug-resistant and EMRSA strains of *Staphylococcus aureus* but show antagonism with the MDR inhibitor reserpine

SL 039

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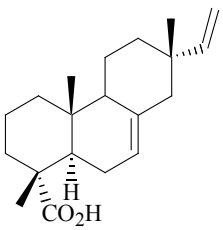

[1]

Isopimaric acid [1] was extracted from the immature cones of *Pinus nigra* (Arnold) using bioassay-guided fractionation of a crude hexane extract. Isopimaric acid and commercially obtained abietic acid showed good activity against multidrug-resistant (MDR) and epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA) with minimum inhibitory concentrations (MICs) of 32 – 64 µg/ml. Pine resin is valued in traditional medicine for its antiseptic properties (1). Both compounds were assayed in combination with the efflux pump inhibitor reserpine. Interestingly, rather than a potentiation of activity by a reduction in MIC, a 2 – 4-fold increase in MIC was seen. We propose that a complex may form between reserpine and the resin acids which is responsible for the reduction in activity (2). This hypothesis is supported by comparison of ^{1}H-NMR spectra of abietic acid and reserpine taken individually and in combination by molecular modelling.

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SL 040 New insights on how Echinacea alkylamides interact with cannabinoid-receptors and implications for immunomodulation

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We have recently shown that alkylamides from *Echinacea* modulate TNF-alpha expression in monocytes/macrophages via CB2 receptors [1;2]. Binding studies on rodent CB receptors have shown to occur with Ki values in the micromolar range for most alkylamides [3]. We have now measured human CB2 and CB1 receptor-ligand interactions and found that certain alkylamides have CB2 affinities in the lower nanomolar range, which indicates that these effects may be physiologically relevant. Interestingly, high affinity alkylamides modulate the receptor status (dimer vs monomer) by a yet unknown mechanism. In vitro experiments show that alkylamides modulate stimulated and constitutive cytokine expression (IL-1, IL-2, IL-3, IL-4; IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, GM-CSF, TNF-alpha) in whole human blood by both receptor-dependent and independent mechanisms as measured by FACS cytometric bead arrays and ELISA. In addition, effects on the expression of CB2 receptors (measured by FACS and real-time PCR) upon stimulation with alkylamides were investigated. Our studies suggest certain alkylamides to be agonistic ligands for CB2 receptors, which at higher concentrations lead to T-cell mediated immunosuppression like other cannabinomimetics. Alkylamide-rich *Echinacea* extracts qualitatively exert the same effects, which may point to possible new areas of therapeutic application of *Echinacea*.

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SL 041 Supplementation with a polyherbal composite alleviates clinical signs of respiratory dysfunction in horses with Chronic Obstructive Pulmonary Disease

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Chronic Obstructive Pulmonary Disease (COPD) is a debilitating respiratory condition accounting for a significant contribution to lost training days in racehorses (1). The objective of this experiment was to evaluate a polyherbal composite in reducing clinical signs of COPD. A polyherbal composite containing garlic, white horehound, boneset, black elder, red clover and hyssop was fed to six horses with symptomatic COPD for 21 days in a cross-over manner. Ventigraphs were used to record respiratory rate and intrapleural pressure; samples of tracheal fluid obtained by tracheal lavage were assessed for changes in microbial populations. Biochemistry and haematology screens were conducted to identify possible adverse effects. Significant differences were identified by paired t-tests; significance was accepted if $p \leq 0.05$. A significant reduction in respiratory rate was observed when horses received herbal composite. No significant changes were observed in horses receiving placebo. All biochemistry and haematology parameters remained within normal reference intervals. It is concluded that signs of respiratory dysfunction may be alleviated in horses with COPD through supplementation with this herbal composite, and no adverse effects were identified. The mechanism by which the herbs provided for a significant decrease in respiratory rate is not related to changes in intrapleural pressure or by alterations in the inflammatory profile of tracheal lavage fluid.

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Choleretic effects of yarrow (*Achillea millefolium* s.l.) in the isolated perfused rat liver

SL 042

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Different species from the *Achillea millefolium* aggregate are used against gastrointestinal disorders in traditional European medicine. While it is known that anti-inflammatory and anti-microbial effects are mediated by sesquiterpenes and essential oil and that flavonoids have spasmolytic effects, the choleretic principles of yarrow are still unknown. Therefore, we investigated a fraction enriched in dicaffeoylquinic acids (DCCAs) on its choleretic effect in the isolated perfused rat liver (IPRL). As a control, cynarin (1,3-DCCA), the main choleretic compound of *Cynara scolymus,* was used.

A fraction containing 3,4-, 3,5- and 4,5-DCCA and luteolin-7-*O*-ß-D-glucuronide was prepared by solid phase extraction on C18-cartridges from a 20% methanolic extract of a commercial sample of yarrow. HPLC analysis with cynarin as internal standard revealed a total amount of 48,8% DCCAs and 3,4% luteolin-7-*O*-ß-D-glucuronide. The IPRL experiment was carried out on Wistar rat livers in a single pass system with Krebs-Henseleit-buffer (KHB) pH 7,4 equilibrated with 95% O_2 and 5% CO_2. Bile flow rate [µl/(g_{liver}.min)] was determined from the time interval between drops and is expressed in % of the basal value. After equilibrium perfusion for 30 min with KHB (basal flow), 10, 20 and 40mg/l of the *Achillea* fraction (n=3) and cynarin (n=3), respectively, were applied for 10 min each with intervals of 10 min with pure KHB.

With the *Achillea* fraction, a dose-dependant increase in bile flow (23-44-47%) was observed. Choleresis was 2 to 3 fold higher than that of cynarin. In conclusion, the combined effect of DCCAs and luteolin-7-*O*-ß-D-glucuronide stimulated bile flow more effective than the single compound cynarin. As, due to their polar structure, these compounds are quantitatively extracted in teas and tinctures, they seem to be the choleretic active principles in the traditional application forms of yarrow.

Pharmacogenomics of Artemisinin Derivatives in Anti-Cancer Therapy

SL 043

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In addition to their well-known anti-malarial activity, artemisinin and its derivatives reveal profound activity against tumor cells in the nano- to micromolar range. By pharmacogenomic and molecular pharmacological approaches, candidate genes were identified, which may contribute to the sensitivity and resistance of tumor cells to artemisinins. Target validation was performed using cell lines transfected with candidate genes or corresponding knockout cells. These genes are from classes with different biological function, e.g., regulation of proliferation (*BUB3*, cyclins, *CDC25A*), of angiogenesis (vascular endothelial growth factor and its receptor, matrix metalloproteinase-9, angiostatin, thrombospondin-1) or of apoptosis (*BCL-2*, *BAX*). Artesunate triggers apoptosis both by p53-dependent and independent pathways. Anti-oxidant stress genes (thioredoxin, catalase, γ-glutamyl-cysteine synthetase, glutathione S-transferases) as well as the epidermal growth factor receptor confer resistance to artesunate. Cell lines over-expressing genes conferring resistance to established anti-tumor drugs (*MDR1*, *MRP1*, *BCRP*, dihydrofolate reductase, ribonucleotide reductase) were not cross-resistant to artesunate, indicating that this drug is not involved in multidrug resistance. The *Plasmodium* translationally controlled tumor protein (*TCTP*) represents a known target protein of artemisinin and its derivatives in the malaria parasite. The microarray-based mRNA expression of human *TCTP* correlated with sensitivity to artesunate in tumor cells, suggesting that human *TCTP* contributes to response of tumor cells to the drug. The multi-factorial nature of cellular response to artemisinin and its derivatives may be beneficial to treat otherwise drug- resistant tumors and may explain, why the development of resistance has not been observed both in cancer and malaria.

SL 044 Effects of *Cimicifuga racemosa* (L.) NUTT. on human breast cancer cell line MCF-7 determined by gene expression profiling

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Extracts from the rhizome of *Cimicifuga racemosa* (L.) NUTT. (black cohosh) are used as an alternative to hormone replacement therapy (HRT) for the relief of symptoms in postmenopausal women. However, the molecular mode of action and the active principles are presently not clear. Published data on mode of action have been largely contradictory. We, therefore, investigated the effects of a lipophilic *Cimicifuga* extract, containing the cycloartane-type triterpenoids, on the estrogen receptor positive human breast cancer cell line MCF-7. Inhibition of cell proliferation could be observed with IC_{50}=15µg/ml. Genome wide expression profiling using Affymetrix HG U133 Plus 2.0 microarrays was carried out to analyze effects at the mRNA level. Some 500 genes appeared to be significantly regulated. Grouping the genes according to function showed that mRNAs coding for gene products involved with cell cycle progression and DNA replication were decreased, while genes coding for inhibitory products were upregulated. This pattern was in accordance with the cell proliferation experiments. We also observed a regulation of gene expression in a pro-apoptotic manner indicating that the extract may sensitize the cells for apoptotic events. Expression of several enzymes with oxidoreductase activity was induced. Some of these genes are known to be regulated via the aryl hydrocarbon receptor (AhR), suggesting that at least a part of the effects of the *Cimicifuga* extract could be via the AhR.

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SL 045 Inhibitory effects of the Cimicifuga racemosa extract BNO 1055 (CR) on androgen-induced rat prostate growth and on human prostate cancer cells LNCaP

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We have recently shown that CR does not bind to estrogen or androgen receptors. Nevertheless, proliferation of the human prostate cancer derived LNCaP cells was significantly inhibited at extraordinary low concentrations. This prompted us to seek for the mechanisms by which CR exerts this putatively important phenomenon. Immature male rats were orally treated with testosterone (T) for 5 days and either co-treated with 0.5 mg of the 5α-reductase inhibitor finasteride (Fin) or with 30 mg of CR. Both, Fin and CR inhibited T-stimulated prostate weight, 5α-reductase gene expression and DHT formation in the prostate ($p<0.05$). These results encouraged us to transplant LNCaP cells into male immune-deficient nu/nu mice. After a subcutaneous inoculation of 1 mio cells 12 of 18 animals developed solid subcutaneous tumours while tumour development was seen in only 5 of 18 CR-treated animals. 5α-DHT as well as PSA in the serum and 5α-DHT in tumour extracts were significantly reduced by the CR treatment. It is concluded that CR is a potent 5α-reductase inhibitor thereby ameliorating the stimulatory effects of T on prostate cancer cell growth. The inhibitory effect by CR on PSA may be an additional antiproliferative effect because PSA is an enzyme which cleaves insulin-like growth factor-1 binding protein-3 (IGF1 BP3) thereby making more IGF1 locally available. IGF1 is a potent mitogen which stimulates LNCaP cell proliferation. Hence, inhibition of PSA secretion prevents the liberation of IGF1 and thereby reduces the stimulation of IGF1-mediated LNCaP cell proliferation.

Effects of phytoestrogens and plant extracts on estrogen responsive genes in rat pituitary gland *in vitro* and *in vivo*

SL 046

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Phytoestrogens and plant extracts containing phytoestrogens are subject of public discussions as alternatives for hormone replacement therapy. To prove effectiveness and to rule out potential risks associated with the intake of these compounds it is crucial to understand the molecular and cellular nature of the effects of these substances. The aim of this study with a pituitary gland derived in vitro model was twofold: a) to identify estrogen responsive genes in the rat pituitary gland and b) to investigate the effects of the rhizomal extracts of *Cimicifuga racemosa* (CR). We established the rat pituitary gland derived somatolactotroph cell line GH3. Using realtime RT-PCR we studied the effects of estrogens, naringenin derivatives and the two isopropanolic (iCR) and ethanolic (eCR) CR extracts on the expression pattern of the candidate marker genes truncated estrogen receptor product 1 (TERP-1), prolactin (PRL) and c-fos in a time and dose dependent manner. E2 and the extracts induced a significant up-regulation of all tested genes which could be inhibited by simultaneous treatment with the pure antagonist ICI 182,780 (Faslodex®). Preliminary results of *in vivo* experiments in ovariectomiced DA/Han rats confirmed TERP-1 and PRL as highly estrogen responsive marker genes. *In vivo*, unlike *in vitro*, iCR did not mimick these gene regulatory propterties. In conclusion, we established a pituitary gland derived *in vitro* and *in vivo* model to study properties of potential estrogenic plant derived substances. This information is important towards risk benefit discussions related to women's health.

Effects of St. John's wort extract and single compounds on stress induced hyperthermia in mice

SL 047

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Emotional or stress induced hyperthermia (SIH) is the rise of body temperature following exposure to psychological stress and has been demonstrated across species (1). In rodents, mild psychological stressors inducing hyperthermia include placing animals in novel environments, restricting an animal's activity, noise, and handling animals in a variety of ways (2). In the present experiments we used exposure to an open field (OF) as inescapable stressor. Exposure of male BL6/C57J mice to OF stress significantly increased body temperature ($\Delta T = 1.3$ °C, $p<0.05$). Home cage animals did not show alterations in body temperature. Distinct classes of drugs probed the mechanism of OF induced hyperthermia (OFIH). Specifically, we tested the ability of an extract of St. John's wort (STW3), hypericin, hyperforin as well as selected flavonoids to inhibit the ΔT rise of OFIH. The effects were compared to diazepam, imipramine, fluoxetine and propranolol as reference compounds. Oral administration of STW3 (500mg/kg) as well as hypericin (0.1 mg/kg) 60 min prior to testing significantly decreased ΔT ($p<0.05$) in the OF group. Hyperforin (8 mg/kg) did not affect ΔT. The effect of different flavonoids on OFIH needs to be further evaluated. Oral administration of diazepam (1 mg/kg) and propranolol (5 mg/kg) significantly suppressed the elevation of body temperature after stress ($p<0.05$) whereas imipramine (20 mg/kg) and fluoxetine (10 mg/kg) had no effect on ΔT during stress. In conclusion, the OFIH is a simple, discrete stress paradigm for detecting stress reactivity. The mediators of psychogenic hyperthermia remain unknown but probably involve a complex integration of GABAergic and β-adrenergic signals.

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SL 048 The potential of PCR-related methods to identify medicinal plants in herbal medicinal products

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A new EU directive on traditional herbal medicinal products was published in March 2004. Basically, this directive introduces a simplified registration procedure. Traditional herbal medicinal products from non EU-countries may have access to the European market under the conditions set out by the directive (article 16a-l of EU-directive 2001/83). Furthermore, in Europe there is an increasing public interest in specific therapeutic systems such as Ayurveda or Traditional Chinese Medicine. During the last years PCR-techniques have been introduced for authentication of medicinal plants and herbal drugs. However, there is a need not only for reliable authentication of raw plant material but also for suitable methods to verify the medicinal plants used in later steps of production, e.g. in herbal preparations and finished products. Our approach is to establish a validated method based on molecular PCR-techniques which should be applicable for a broad range of medicinal plant species. As model for analytical development and validation, we have chosen the genus *Aristolochia* because of its toxic potential and the genus *Matricaria* because of the availability of different preparations in the market. Most methods described in literature focus on either RAPD-techniques or usage of plant specific loci. In addition to RAPD techniques we investigated the potential of amplification of ITS1 and ITS2 (internal transcribed spacer). rDNA is highly conserved, available in numerous copies and thousands of ITS-sequences from plant species are reported in the field of molecular phylogeny. We were able to demonstrate, that DNA could be successfully extracted from finished herbal medicinal products like tablets. However, RAPD-fingerprints were not suitable to identify the raw material: due to the disruption and degradation of DNA during processing of the medicinal products data were not reproducible. Complete fragments of ITS-regions (about 300 bp each) were amplified and sequenced. Thus, comparisons of sequences with data bases may be useful to identify medicinal plants by this method. Future steps include a complete validation of the method to define the limits of detection and to develop strategies do deal with complex mixtures, e.g. by means of real-time-PCR. Until now, the number of ITS-sequences available for medicinal plants is limited. Therefore, a reliable data base should be created, ideally by a network of laboratories.

SL 049 To consider or not to consider? – Enzymes in herbal drugs and preparations

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In the search for so-called active substances and in the efforts made to explain the efficacy of a phytopharmaceutical, products of secondary plant metabolism, for which a biological effect has been proven in pharmacological models, have been the principal objects of study. Phytopharmaceuticals, the efficacy of which can be attributed to an ability to swell and/or the presence of non-degradable polysaccharides, may be regarded as an exception of that rule. The relevance of plant-specific enzymes in the stability and therefore also efficacy of herbal drugs and preparations has not yet been studied extensively. However, it is known that plant enzymes can be active even after harvesting and drying of the plant material. Recent examples (*Echinacea purpurea, Cynara scolymus, Fagopyrum esculentum*) where degrading enzymes have been isolated and characterized from the crude drug (1, 2, 3) will be presented and discussed in a more general context and an adapted commercially available test system (APIZYM, Biomérieux) suitable for checking enzyme activities still present in drugs or extracts will be introduced. Proof will be presented that enzymes are residing in an active form in artificial and real extracts produced from herbs. In conclusion, it is suggested that endogenous enzymes should be considered as important activity-related constituents and therefore be analysed and documented adequately.

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Natural products and batch consistency: a hurdle for herbal drug development? Example of PX-6518, a leaf extract of *Maesa balansae* with antileishmania action

SL 050

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Leaves of the Vietnamese plant *Maesa balansae* were found to possess potent antileishmania activity(1). Since the drug substance material had to be obtained from plant collections in the wild, batch consistency and seasonal variation become important factors that do influence the economical and regulatory feasibility of a development project. A constant composition is particularly relevant as regulators seek firm assurance that the toxicity evaluation is done with the same material as will be ultimately be used in man. In a 1-year study, monthly plant collections aimed to assess reproducibility within- and between batches, influence of different collection sites, seasonal impact and plant condition. Some variability occurred for the total yield and the qualitative composition of active ingredients, endorsing the need for large single-batch collections or planning of agricultural culture of the plant. After scaling-up the manufacturing process, five production batches were evaluated for quality consistency and matching with the Ph. Eur. acceptance criteria. Except for the presence of traces of residual solvents, all batches complied and the relative composition of the active components remained within a 5% range. Important to note is that traces of pesticides that are frequently used in Vietnam could also be detected. Issues relating to batch consistency will be discussed in further detail.

Acknowledgements: WHO-TDR (Geneva, Switserland), DGOS (Brussels, Belgium), Tibotec (Mechelen, Belgium)
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Bioisosteric Modifications of the Potent and Selective κ-Opioid Agonist Salvinorin A

SL 051

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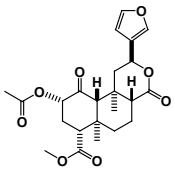
Salvinorin A is a psychoactive secondary metabolite isolated from leaves of *Salvia divinorum* and has been shown to bind with high affinity and selectivity to the κ-opioid receptor (KOR) as an agonist (**1,2**). Bioisosters of the C-2 acetate were developed and biologically evaluated in binding assays and functional assays. Trihaloacetate derivatives showed an inverse relationship between affinity and carbon-halogen bond length and halogen atom size. Replacement of an oxygen atom in the C-2 acetate with sulfur produced comparable activity, but nitrogen substitution had a diminishing effect. Intermediates, which lack a β carbonyl, also had significant affinity. The derivatives were tested against all opioid subtypes and were selective towards KOR.



Salvinorin A

Acknowledgements: National Institutes of Health Grant R01DA017204 and the National Institute of Mental Health, Psychoactive Drug Screening Program.
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SL 052 Glucogalloyl derivatives: Synthesis and Evaluation of their Antimycotic and PARG-inhibition Activity

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The aim of this work was the synthesis of different glucogalloyl derivatives and the evaluation of their antimycotic and PARG-inhibition activities. Several studies on natural extract from green tea and myrtle leaves and synthetic glucogalloyl and galloyl esters, have shown to possess significant biological activities. For example they increase the antimycotic activity of some antibiotics;[1] exhibit a role in protection against cardiovascular diseases; or exert antioxidant protection for human LDL (low density lipoprotein);[2] and are representative poly(ADP-ribose) glycohydrolase (PARG) inhibitors.[3]

Figure 1

On this background, a chemical and biological program was undertaken directed toward the stereo-defined preparation of glucogalloyl compounds where galloyl moieties are selectively linked to the glucose core. In this way the ellagic or depsidic bond was achieved as well (Figure 1). Some of the structurally-defined glucogalloyl derivatives synthesized enhanced the antimycotic activity of Amphoterin B (up to 40 times) and exhibit an effective PARG-inhibition.

Acknowledgments: 'State Scholarships Foundation' (IKY) of Greece for financial support

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SL 053 Reductive modifications of hyperforin, the major phloroglucinol from St. John's wort

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Hyperforin, the major phloroglucinol derivative from St. John's wort (*Hypericum perforatum* L.), shows antidepressant activity, *in-vitro* and *in-vivo* anti-metastatic activity, traced back to the functional and genomic inhibition of various matrix metalloproteases (1). Furthermore, various analogues of the natural products show apoptotic and cytotoxic activity toward cancer cells, with little if any toxicity for primary cells (2). Hyperforin is also the most powerful activator of the Pregnane X Receptor (PXR), a transcription factor that acts as a sensor to maintain chemical homeostasis by regulating the transcription activity of various cytochromes involved in the oxidative metabolism of xenobiotics and drugs, including many anticancer drugs. Given the relevance of the combination between cytotoxic and anti-metastatic drugs, the activation of PXR is a serious drawback for the clinical development of hyperforin as an anticancer agent. This problem can, at least in principle, be solved by the synthesis of analogues that maintain the anti-metastatic activity of the natural product, but are devoid of its activity on enzyme induction. The structure-activity relationships of hyperforin are still unknown for all its biological targets, and the synthesis of analogues is therefore critical to dissect the activity on metalloproteases and enzyme induction. Hyperforin is structurally complex, and its reactivity is difficult to predict. In a series of previous studies, the chemical modification of its acylphloroglucinol core was investigated, obtaining a library of analogues currently under biological evaluation (3,4,5). To complement these studies, reductive modifications of the phloroglucinol core and the polyolefin moiety will be presented, together with preliminary data on the potential of these compounds as MDR antibacterial and anti-metastatic agents.

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S.Gallori - Firenze 2004

ABSTRACTS OF POSTERS

P 001 Comparison of botanical characteristic of *Phyllanthus amarus* with other *Phyllanthus species* growing in south Vietnam

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In Vietnamese traditional medicine, *Phyllanthus amarus (P.a)* Schum.et Thonn. and *Phyllanthus urinaria* L. (*P.u*) have long been used for treatment of kidney and liver diseases. *P.a* is well-known for its anti-hepatitis activity (1) but the bioactivity of other *P.* species remain little known, although they are used interchangeably in many countries(1). Aerial parts of *P.* sp (25 vouchers) were collected in South Viet Nam (March 2004) and morphological characters of these species were compared with *P.a* in order to identify and distinguish them during sample collection (2). *P.a, P.u,* and *P.debilis* were found to grow in South VN, but not *P. niruri* L. They revealed significant differences in morphological and microscopic characters and TLC patterns of main constituents. The plants can be distinguished by their morphological and chromatographic characters.

Organ	*P.amarus*	*P.urinaria*	*P.debilis*
Stem	10-50 cm high, smooth capsule, green	20-50 cm. Reddish-green, not smooth	30-90 cm. Smooth,dark green
Flower, anther	sepals 5,white, apex acute	sepals 6, white, apex obtuse/ round.	Sepals 6, white, apex obtuse.
Fruit	Capsules green, smooth, fruiting pedicels 1-1.5 mm, dilated at apex	Capsules globose, reddish blotches and scurfy- tuberculate, fruiting pedicels 0.5-1.5 mm	Capsules globose, reddish blotches scurfy tuberculate, fruiting pedicels 2 mm
Seeds	Seeds longitudinally rugose.	Seeds transversely rugose	Seeds longitudinally rugose

Acknowledgements: Dept.Botany Univ. Med.& Pharm. of HoChiMinh City, Viet Nam Prof. V.V.Chi
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P 002 The black glands of the genus *Hypericum*.

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Black glands are secreting structures characteristic of many species of *Hypericum*. There is general agreement that hypericin and pseudohypericin, biological active molecules of these plants, are located in the glands. Moreover, the presence of these structures is an important character for the infrageneric classification; indeed they are present only on the taxa of the phylogenetically more advanced sections. In most of the plants, these glands are spread on the whole aerial part of the plant itself, and slight protrude on the epidermal surface (e.g. *H. perforatum* L.). In some species, along the edges of petals and sepals, black glands with a more or less long stalk, are present. In this work the structure and ultrastructure of the black glands without stalk of *H. perforatum* and of those with stalk of *H. richeri* Vill., *H. montanum* L. and *H. elodes* L. are presented. Actually, the glands of *H. elodes* are red in colour; however, also in these structures the presence of hypericin was recently assessed. All the glands examined (with or without stalk) have similar initial developing stages: the cytoplasm bears small, dense, black plastids and smooth reticulum, probably responsible for the secretion production. The secretion, strongly osmiophile, begins to accumulate in the vacuoles of cells in the central part of the gland; successively, also the surrounding cells are filled with the secretion. In the non stalked glands the secretion remains inside the cells, as described by current literature. In the stalked glands, the secretion begins to accumulate into the vacuoles, but soon some cells begin to degenerate, intercellular spaces are formed, and filled with a black osmiophile substance, very similar to that present inside the vacuoles. The secretion is extruded through a hole on the apex of the glands. The significance of this extrusion is presently unknown.

Morphoanatomy and histochemistry of *Guiera senegalensis* leaves, a major west african herbal drug

P 003

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Guiera senegalensis J. F. Gmel (*Combretaceae*) is a medicinal plant, often employed in West African countries, to treat venereal, diarrhoeal, respiratory and fungal diseases. Previous work confirmed *G. senegalensis* antimicrobial activity against *Neisseria gonorrhoeae* (including resistant strains), *Shigella dysenteriae, Vibrio cholerae, Giardia lamblia* and *Cladosporium cucumerinum,* consistent with its traditional uses. Bioguided phytochemical studies permitted the identification of flavonoids, gallic tannins, naphthalene derivatives and terpenoids, among *G. senegalensis*' compounds (1).

Aiming to supply data for the pharmacognostic characterisation of this African herbal drug, we report here results of the leaf morphoanatomy and histochemistry studies.

The leaf is greyish-green with a stipulate base, petiolate and the lamina is pinnatifid, with entire margin, obovate shape, asymmetric base and pinnate venation. Microscopic analysis, by light (LM) and scanning electron microscopy (SEM), showed epidermal cells containing non-glandular trichomes, scales and few anomocytics stomata, mesophyll with clusters crystals of calcium oxalate and marsupiform domatia on the axils of the lower surface primary veins. Histochemical reactions confirm the presence of phenol compounds among secreted substances.

Obtained macroscopic and microscopic characters are significant markers that can be used in the botanical diagnosis of this drug.

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Standardization of *Pterocarpus soyauxii* Taub (Fam. Leguminosae)

P 004

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The morphology and microscopical characters of the leaf, stem and root of *Pterocarpus Soyauxii* Taub are described. Analytical standards of the leaf showed an ash value of 7%, ethanol extractive value of 47%, water extractive value 44% and moisture content of 47.2%. The leaf has a palisade ratio of 6.7, vein-islet number of 12 and stomatal number of 118. Chemical tests revealed the presence of glycosides; Flavonoids, saponins, reducing sugars and tannins.

Microscopical examination of the various parts of *P. soyauxii* revealed striated cuticle, straight walled epidermal cells, collateral vascular bundles and paralytic stomata in the leaf while the stem and root showed reticulately pitted vessels, numerous stone cells (sclerieds) and fibers.

P 005 A Chemotaxonomic Investigation of two Species of the *Staehelina* Genus

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Staehelina (Asteraceae, Tribus Cardueae), is an extremely small genus and consists only of seven species worldwide.(1) *S. petiolata* L. and *S. fruticosa* L. which have been investigated in this work, are both endemic to Greece. The Asteraceae family, and in particular the Cardueae tribe, are known for possessing certain secondary metabolites which serve as chemotaxonomic markers thereof. Such constituents include mono- and diterpenes, sesquiterpene lactones and flavonoids.(2) This investigation has found both species to be rich in pentacyclic triterpenoids, while sesquiterpene lactones were isolated only from *S. fruticosa*. Furthermore, examination of the methanolic extract of *S. petiolata* revealed a prolific pool of flavonoids, especially C-glycosylated flavones. High Pressure Liquid Chromatography (HPLC) was employed to conduct a comparative study and chemotaxonomic analysis of the aforementioned species. Both species were found to share many constituents and due to the modest size of this genus, chemotaxonomic conclusions could be drawn. Numerous C-glycosylated flavones were the key constituents in both species and could be regarded as chemotaxonomic markers of this genus. Other similarities included flavonoid aglycones, chlorogenic acid, eriodictyol-7-O-glucuronide and arbutin, where the latter was found as a major constituent in both species.

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P 006 Chemical profiling of *Ocimum americanum* using external flavonoids

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For the commercial use of culinary and medicinal herbs, quality control is becoming an increasingly important issue. Species belonging to the genus *Ocimum* L., basil (Lamiaceae), are examples of plants that are difficult to distinguish on the basis of just their leaf morphology, because of the wide range of leaf shapes within most species. A HPLC survey was undertaken of the external flavonoids in 111 herbarium specimens of *O. americanum* L. (*O. canum* Sims), which were largely collected from their natural habitats throughout Africa and Asia. The purpose of this study was to establish the flavonoid profiles of this species over the full range of its geographic distribution in order to use these for authentication purposes. Six different external flavonoid chemotypes were found. The major chemotype, present in circa 80% of the specimens of both var. *americanum* and var. *pilosum* collected throughout the distribution area of the species, was characterised by very high levels of nevadensin, slightly lower levels of salvigenin and much lower levels of up to 15 other external flavones. Of the remaining five chemotypes, two were found in var. *americanum* and three in var. *pilosum*. Despite some similarities in profiles, chemical differences were also found among the species, so that it should be possible to authenticate a large proportion of leaf samples of *O. americanum* on the basis of external flavonoid profiles.

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Secondary metabolites from *Anthemis triumfetti* (L.) DC.

P 007

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Anthemis triumfetti (L.) DC (syn.: *Cota triumfetti* (L.) Gay, Compositae) is a perennial herb, 30-90 cm high, growing in woods and rocky places on mountains (1). The flowering aerial parts of *Anthemis triumfetti* (L.) DC. have been studied for the first time. Plant material was collected at the mountain Bjelasica in Montenegro, in July 2003. Dried and finely ground aerial parts of *A. triumfetti* were extracted with CH_2Cl_2-MeOH (1:1) to give crude extract which was suspended in 10% MeOH in water and extracted successively with cyclohexane and CH_2Cl_2. The remaining MeOH/H_2O extract was subjected to column chromatography and preparative TLC.

The isolated compounds were the flavonoids: quercetin, hispidulin, apigenin 7-*O*-glucuronide, luteolin 7-*O*-glucoside, quercetin 3-*O*-glucoside, quercetin 7-*O*-glucoside, quercetin 4'-*O*-glucoside, patuletin 7-*O*-glucoside, rutin; the hydroxycinnamoylquinic acids: chlorogenic acid, 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, and the coumarins: umbelliferone, scopoletin and scopolin.

The structures of isolated compounds were identified on the basis of their chromatographic behavior, spectral data (UV, ^{1}H-NMR, ^{13}C-NMR, COSY, HMBC, HSQC), MS data and confirmed by comparison with literature data (2,3).

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Isoflavonoids in three *Ruta* species

P 008

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Increased attention has been paid to isoflavones during the last decade, due to their importance for human health (1). They are most abundant in the Leguminosae, however they were also recorded in over 20 other families. Recently we have detected isoflavonoids in Rutaceae family (2). In this study, we are reporting presence of isoflavonoids in *Ruta graveolens* L., *R. corsica* DC. and *R. montana* Mill.

The combination of HPLC and radioimmunoassay was used for initial phytochemical screening of plant material as described elsewhere (3). Immunochemically positive samples were subsequently evaluated by HPLC-MS-SIM in order to confirm the identity of individual compounds.

All three species contained numerous isoflavonoids, aglycones as well as glycosides in mg/kg of dry weight (up to 7 mg/kg of sissotrin in leaves of *R. montana*). The 4´-methoxy isoflavones (e.g. sissotrin, formononetin, bichanin A, ononin) and 4´- hydroxy isoflavones (e.g. genistein) were the most abundant in leaves and flowering tips, respectively.

Acknowledgements: Czech Science Foundation (project no. 525/03/0352)

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P 009 Essential oil composition and variations of volatiles composition in eleven varieties of hops *(Humulus lupulus)*

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The importance of hops *(Humulus lupulus)* in beer production is worldwide known. Hops not only provide bitterness to the final product but they also supply aroma and flavour according to the volatile profiles of each variety and the boiling time determining the predominant characteristics of a given beer. Usually, hops are classified in three different categories according to their content in alpha acids: bittering hops (high), aroma hops (low) and general-purpose hops (medium). On the basis of this classification, they are added for different purposes and at different times during the boiling time of the wort. As the importance of alpha-acids is essentially due to their amount in beer, contributing only to its bitterness, the volatiles composition of hops is much more characterizing for the final aroma of the product.
The aim of our work was to evaluate the essential oil and the volatil compositions of hops by GC-MS and SPME-GC-MS and to determine how the relative composition of these substances vary with time and storage.
Eleven varieties of hops were investigated and the results obtained showed that the essential oil composition characterize the different varieties and that the relative composition of volatiles widely changes according to different type of storage.

P 010 Secondary metabolites of C*entaurea deflexa*

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The genus *Centaurea* (Asteraceae) is widely used in the popular medicine. Some species are used for their pharmacological properties: antidiabetic, antidiarrhoeic, antirheumatic, antimalarial, cholagogue, choleretic, digestive, diuretic, astringent, hypotensive, febrifuge, stomachic, etc. (1-2).
Within this genus we have examined an endemic species from Anatolia (Turkey), *Centaurea deflexa,* never previously studied (3).
This species is a perennial plant with woody rhizome and stem up to 30 cm, toothed leaves and yellow flowers. The phytochemical investigation has been performed on a sample collected during July 2000 near Taskent. The flowering aerial parts have been successively extracted in a Soxhlet apparatus with increasing polarity solvents, i.e. *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1) and than, at room temperature, with MeOH. Here we report the results of the study of MeOH, BuOH and AcOEt extracts. By mean of various solvent partitions and chromatographic techniques, some sterols, flavonoids and sesquiterpene lactones were purified and characterized. Among the latter class of compounds, a very rare type of derivatives, a new *nor*-guaianolide has been obtained.

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Comparative analysis of essential oils, from leaves and fruit peels of seven cultivars of *Citrus* growing in Greece

P 011

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Ancient and medieval sources report a much broader spectrum of pharmacological properties and uses of cultivated citrus species than might be expected from modern scientific literature (1). The genus Citrus (Rutaceae) comprises 157 taxa growing in all over the World (Brazil, America, Mediterranean area, India, and Japan). It is also characterized by a large morphological diversity. Its taxonomy is very complicated and undistinguished due to extensive hybridism. *Citrus sinensis* and *Citrus reticulata* have been used as subject to give numerous hybrids (2). Essential oil from mature leaf, and peel of fruit of seven Citrus hybrids growing in Greece (Citrus reticulata x clementine-cleopatra, *Citrus reticulata* x clementine-aurantium, *Citrus reticulata* x clementine-troyer, *Citrus reticulata* x satsuma wase, *Citrus reticulata* x encor-troyer, *Citrus sinensis* x new hall-aurantium, *Citrus sinensis* x aurantium, where cleopatra is Citrus reshini and troyer is *Poncirus trifoliata* x *Citrus sinensis*) were subjected to analyses through gas chromatograph equipped with mass spectrometer. Citrus species contain various kinds of terpenoids, including their derivatives, such as alcohols, esters, and acetates, and their components which vary in species and variety. Eighty one constituents were identified from the above analyses, and the major compounds are, sabinene, myrcene, carene <delta-2->, cymene <para->, limonene, ocimene <beta->, terpinene <gamma->, terpinolene, linalool, terpinen-4-ol, terpineol <alpha->, sinensal <beta->, sinensal <alpha->.

Acknowledgments: Arboricultural Station of Poros, Peloponese, Greece, Director, Mrs Agorastou Th.
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Taxonomy of wild *Allium* species used in folk medicine in Tajikistan and Uzbekistan

P 012

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Common onion, *A. cepa* L., and garlic, *A. sativum* L., are the economically most important *Allium* crop species also in Asia. They play a very important role in the daily diet as spices, vegetables, and medicinal plants. In the mountainous area of Central Asia also more than 200 wild *Allium* species occur which were taxonomically affiliated to three subgenera (1): to the rhizomatous subgenus *Rhizirideum* in the wide sense (more than 80 species), and to the bulbous subgenera *Allium* (about 60 species) and *Melanocrommyum* (more than 60 species). Several wild *Allium* species are traditionally collected and used as food, spices or medicine by the local people. Data on medicinal applications were compiled form sporadically published information and from interviews of the local population during joint research missions in several Asian countries. In Tajikistan and Uzbekistan, altogether 16 wild *Allium* species and subspecies were medicinally used. Most of them are not simply used in place of common onion or garlic, but are very specifically applied. Two of the wild *Allium* species are closely related to common onion (subg. *Rhizirideum*, sect. *Cepa*). They are medicinally applied against stomach-ache and fever. Medicinal application was reported for only one species of subg. *Allium* which is taxonomically rather distantly related to garlic. However, two species from the rhizomatous sect. *Campanulata* (subg. *Rhizirideum*) and four species from the bulbous subgenus *Melanocrommyum* are medicinally applied like garlic. Surprisingly, the leaves of three species of subg. *Melanocrommyum* are collected as food laying special emphasis on a medicinal impact: They are much esteemed for their tonic properties. Important taxonomic characters of the species involved in this study are presented and illustrated by colour photographs. Their taxonomic relationships and positions in the current classification of the genus *Allium* are outlined.

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P 013 Aspects to be considered in Pharmacognostic studies using South African medicinal plants as a demonstration model

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Ethnophamacognosy is a multi-disciplinary science where local communities, botanists, chemists and pharmacologists interface their expertise to research the ethnobotany, chemistry and biological activity of plant extracts. Several problems are often encountered when embarking on such multi-faceted projects. Based on examples emanating from several research projects on indigenous medicinal plants the limitations and pitfalls will be discussed.

1. Sourcing of plant material is often problematic with sampling protocols and plant identification not properly documented. The new biodiversity bill, intellectual property and indigenous knowledge issues complicate and often stifle research on natural resources.
2. The method of extraction and the choice of assay to study biological activity is often not related to the traditional use of the plant. Inhalation therapy is extensively used in African traditional healing and a simple combustion apparatus has been designed to capture the smoke released from the charred plant material. The volatile fraction which more closely mimics that which is inhaled during the healing ritual has been captured, analysed and subjected to biological assays. HPLC-UV-MS data reveals significant differences in the chromatographic profiles and also in the biological activity. The methanol extract for *Pallaea calomelanos,* a plant extensively used for respiratory disorders, has a MIC value of >16 mg/ml against *Klebsiella pneumoniae* while that of the volatile fraction is 2 mg/ml.
3. Plants are rarely used singularly in African traditional healing. Due to complexity plants are most often studied individually in the laboratory. *Artemisia afra* and *Lippia javanic*a are often used in combination therapy and *in vitro* death kinetic data using time kill methods on *Staphyloccosus aurues* cleary demonstrates that the two plants are more effective when used in combination.
4. The choice of bioassay is crucial when evaluating results. The specific physical-chemical properties of essential oils (volatility and lipophylicity) make the study of medicinal aromatic plants in water-based assays problematic. Antimicrobial results for indigenous scented *Pelargonium* species will illustrate the variation encountered when analysing the essential oils, 'deodorised' plant material and the combination.
5. The genus *Eriocephalus* (Cape snowbush) will be used to illustrate the problems which genetic, phytochemical and botanical variation poses in bioprospecting. The within and between population variation is so diverse that product development could only be successful if plant material of a specific geno- and chemotype is cloned and cultivated.

Losing knowledge about medicinal plants around the Brazilian Royal Road, Minas Gerais, Brazil

P 014

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The native vegetation of Brazil has been under a continuous process of destruction, since the time of the discovery of the country more than 500 years ago. The use of several native medicinal plants was noted by European scientists (Burton, Saint-Hillaire, Spix, Martius, Langsdorff, and Pohl) in the XIXth century. In this study, we have evaluated the current use of 26 species mentioned by these scientists around the Royal Road. We have apllied questionaires to 196 people in 150 municipalities. This knowledge is restricted to older people (average 70 years old) and they learned about them by parental influence. Some species as angico (*Anadenathera colubrina*), carqueija (*Baccharis trimera*), fedegoso (*Senna occidentalis*) and imbaúba (*Cecropia hololeuca*) are widely knowed and used. Others as chá-de-pedestre (*Lippia pseudo-thea*) or tingoassuíba (*Zanthoxylum tingoassuiba*) are unknowed. Ipecacuanha (*Psycothria ipecacuanha*) and jaborandi (*Pilocarpus* sp.) were substituted by exotic species. The results showed a strong loss of traditional culture on amerindian plants in this area. Efforts are necessaire to avoid this process in other regions of Brazil.

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The uses of wild growing *Allium* species of Central Asia as spice or medicinal plant

P 015

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In the mountainous regions of Southwest and Central Asia, conspicuously tasting and curative plants are traditionally used by the local population. Also today, wild collected vegetables, spices and medicinal plants play an important role in the daily diet. According to several published reports, also several *Allium* species are among these plants. Own investigations have shown that more wild growing species are collected then formerly reported and hitherto unreported kinds of usages were detected. This information is given for 37 species and compared with the already published data. As an example, *A. rosenorum* R.M. Fritsch as well as a few related species were intensively used in Tajikistan. Fresh leaves were harvested in spring since ancient times and used for the preparation of traditional dishes. It is believed that those leaves are rich sources of vitamins. Leaves were also applied for wound healing. *Allium motor* Kamelin et Levichev is used in Uzbekistan in a similar manner ("motor" has the meaning of "health"). Leaves were harvested in April to Mai and are highly esteemed as a tonic after winter time. Further on, the related species *A. komarowii* Lipsky is applied against tachycardia by elder people. In Taijikistan as well as Uzbekistan young bulbs of *A. stipitatum* Regel were pickled and often used as spicy vegetable. Additionally, *A. barsczewskii* Lipsky was often mentioned by the native population as remedy against headache, common flue and fever. Smashed bulbs were sometimes applied as surrogate for garlic. Also close relatives of common onion like *A. pskemense* B. Fedt. are much used. Whole plants as well as the bulbs are used for curing stomach problems. Moreover, *A. oschaninii* O. Fedt. is widely applied as alternative or wild surrogate for common onion.

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P 016 Agrobiodiversity of medicinal and aromatic species of Iran

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Potential productivity and long term stability of agroecosystems depends on the consistency of their biodiversity. In conventional agricultural systems, monoculture and use of high input varieties and reduction of genetic diversity have endangered the sustainability of these systems. Role of indigenous species with medicinal properties in promoting the biodiversity of traditional agricultural systems is widely recognized. Results of present study showed that 54 species of medicinal and aromatic plants with a Shannon index of 0.64 are cultivated in Iran. The ratio of cultivated medicinal and aromatic species to the total area of cultivated lands is 0.87, of which Khorasan province shows the highest ratio and diversity amongst the all provinces studied.

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P 017 Propagation and some agronomic practices of Amukkara (*Withania somnifer*a L.)

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Withania somnifera L. (Family Solanaceae, s. Amukkara; E. Indian ginseng; H. Aswaghanda) is one of the important medicinal plants widely used in traditional systems of medicine. Roots are used as ingredient in many ayurvedic preparations to cure Leprosy, nervous disorders, intestinal infections and rheumatism and to overcome all kinds of weaknesses and increase the vigour and stamina. Although favourable conditions to grow this valuable plant exists in Sri Lanka, almost 90% of local requirement (about 40,000 Kg) is annually imported from India, expending Rs. 3-4 million due to lack of systematic cultivation in the country. Therefore, there is an urgent need to develop suitable protocol to establish commercial cultivation in the country.
In the present study attempts are made to compare different nursery establishment methods, seed treatment methods, seed dormancy and found that maximum number of seedling can be obtained from the beds, sterilized by covering with transparent polythene for 10 –14 days or burning with dry rice straw techniques. Comparison of two methods of field establishment (transplanting & thinning out system) showed that uprooting for transplanting could cause damage to the taproot and hence it induces hair root system. However, this can be avoided by practicing thinning out system of cultivation. When plants do not uprooted and hence the main taproot is not disturbed.
Although numbers of pests are found in Amukkara cultivation, two pests caused serious damage (Mites and mealy bugs) to the crop. Three treatments were tested for the control of pests, i.e. Neem extracts, Dimethoate and control. All treatments were replicated 5 times. In the control treatment mites damage was about 25-32 % and increased up to 49.6% within 4 weeks, while neem and chemical treatments reduced it from 25-32 % to 9.5% and 7.1% respectively. This indicates that neem act as a natural pesticide in the control of mite attack in amukkara cultivation. Main diseases identified were damping off; little leaf disease and stem rot disease. Economical root yield could be harvested at full fruiting stage. Seed production started after 4 months after transplanting. The cost of production of roots is around US$ 560 /= ha. The best harvesting stage is 4 months after transplanting. Total root yield is around 900 kg/ha and cost per root is about US$ 0.63/= kg. Alkaloid content was higher in roots (0.53% ± 0.049). Chemical quality of both imported and locally produced roots was evaluated and there is no clear difference between two products. Therefore, it can be concluded that commercial cultivation of *Withania somnifera* L. (Amukkara) could be successfully carried out in the dry and intermediate zones to meet the exiting demand in the country.

AcknowledgementS: Conservation and sustainable use of medicinal plant project
References: Wealth Asia CD rom 1997

The effects of nitrogen supply and repeated harvests on the concentration of flavonoid glycosides and caffeic acid esters in aerial parts of stinging nettle (*Urtica dioica* L.)

P 018

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Urtica dioica L. is often used in folk medicine against various diseases. The aerial parts of *U. dioica* have in particular shown anti-inflammatory activities (1, 2). Further, it has been suggested that the immunostimulatory activity of leaf extracts could be the reason for the use in traditional anticancer treatment (3). Flavonoid glycosides and caffeic acid esters may be responsible for some of the bioactivities of *U. dioica* (2, 3). The aim of the present study was to investigate the effects of nitrogen supply and several repeated harvests on the concentration of flavonoid glycosides and phenolic acids in the aerial parts of *U. dioica*. Plants of *U. dioica* were cultivated in 2003 and 2004 with four different nitrogen levels (2003: 0, 75, 150 and 300 kg N/ha; 2004: 0, 100, 200 and 400 kg N/ha). The aerial parts were harvested at two times in 2003 (July and September) and three times in 2004 (May, July and October) and samples were stored (−24 °C) until analysis. The flavonoid glycosides quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, and kaempferol-3-*O*-rutinoside and the caffeic acid esters chlorogenic acid and caffeoyl malic acid were identified in methanol extracts by LC-MS and quantified by reversed phase HPLC. Higher nitrogen levels reduced the concentration of flavonoid glycosides significantly, in almost all harvests, whereas the effect on caffeic acid esters was only significant in the second harvest each year. The highest effect on the concentration of flavonoid glycosides were observed in May harvest 2004 with a decrease from 13.5 mg flavonoids/g dry matter (DM) at 0 kg N/ha to 2.4 mg flavonoids/g DM at 400 kg N/ha. The concentration of caffeic acid esters in the second harvest in 2003 decreased from 23.1 mg/g DM at 75 kg N/ha to 8.5 mg/g DM at 300 kg N/ha. In the second harvest of 2004 the concentration of caffeic acid esters decreased from 32.1 mg/g DM at 0 kg N/ha to 20.5 mg/g DM at 400 kg N/ha. The conclusion of the present study is that the production of *U. dioica* for medicinal purposes is a compromise between a high yield of plant material and the content of flavonoid glycosides and phenolic acids in the harvested product.

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The status of exudate species in Iran and existing challenges in their sustainable utilization

P 019

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The vast country of Iran in south-west Asia constitutes in the Northern hemisphere a peculiar geographical unit which displays a remarkable biodiversity and various ecosystems with specific biological components. The exploitation of natural resources on the Iranian plateau goes back to about 7000 years ago .The use of medicinal plants was the first method of treating diseases in ancient Iran and forms an important part of various cultures .Within medicinal plants, exudate species play an important role in the economy of the country, as Iran is known as the main producer and exporter of Gum Tragacanth within Asia-Pacific region .These species and their habitats in Iran are highly endangered by unsustainable utilization, so sustainable utilization and conservation strategies should be considered.

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P 020 Characterization of some bioactivities of verbascoside

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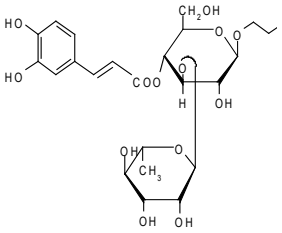

I.R.B. srl (Istituto di Ricerche Biotecnologiche) produces verbascoside in industrial fermenters from cell cultures of *Syinga vulgaris*. Verbascoside, also known as acteoside, is a well known potent natural antioxidant with a potentially wide utilization for pharmaceutical, cosmetic and nutraceutical applications. However, its use in these fields requires more detailed studies on highly purified preparations so to clearly define the full spectrum of biological activities. In fact most studies done to date, have been performed on poorly standardized material, derived form a variety of natural sources. To overcome these limitations, we have take advantage of the high level production of verbascoside from Syringa vulgaris cell cultures.

Investigations have been performed on both cell-free and cell-based models. In particular: the antioxidant activity by the luminol method was used for comparison with ascorbic acid and the flavonoid rutine, the biochemical inhibition of lipid peroxidation was utilized to compare verbascoside with a water soluble Vitamin E (trolox); the antiinflammatory activity was tested on mouse macrophages cell cultures; the ability to enhance survival of neuronal cells was performed on PC12 cell line; the antiproliferative activity was tested in vitro on melanoma cells. The results of the experiments, are resumed in the following table: (n.d.: not detected)

	anti-oxidant activity (HRP-luminol-H_2O_2) IC50 (ug/mL)	lipid peroxidation inhibiting activity IC50 (ug/mL)	antiinfiammatory activity IC50 (ug/mL)	neuroprotective effects (times/control)	antiproliferative activity on melanoma IC50 (ug/mL)
verbascoside	**0,52 ± 0,16**	**3,01 ± 0,13**	**60**	**30**	**366**
ascorbic acid	**0,53 ± 0,22**	**4,17 ± 0,17**	n.d.	**2**	**-**
rutine	**1,37 ± 0,4**	**17,16 ± 0,15**	n.d.	**3**	n.d.
trolox	n.d.	**0,32 ± 0,18**	n.d.	**8**	n.d.

It is clearly evident that verbascoside can display some interesting biological activities, such as on the antiproliferative activity on melanoma, which are not simply due to the antioxidant properties.

P 021 *In vitro* propagation, preservation and stevioside production of *Stevia rebaudiana* Bertoni

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In vitro shoots of *Stevia rebaudiana* Bertoni were cultured on MS media supplemented with 16 factorial combinations of BA and NAA (0, 0.5, 1 and 2 mg/l) under fluorescent light for 16 hours per day for 8 weeks. Multiple shoot formation averaged of 4.5 shoots/explant occurred after 3 weeks on MS medium with 1 mg/l BA. The medium with 2 mg/l NAA could induce root formation with the highest average number of 11 roots/explant. To decelerate their growth for short term preservation, in vitro shoots of this plant were culture on MS media supplemented with 3%, 6% or 9% sucrose under fluorescent light for 16 hours per day. It was shown that the MS medium with 9% sucrose result in the shortest shoots. Analysis of stevioside production in natural shoots and roots, *in vitro* shoots and roots and calli using HPTLC (High Performance Thin Layer Chromatography). It was found that the natural shoots and the *in vitro* roots contained 0.0188 and 0.0012 g/g dry weight of stevioside, respectively.

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Callus culture and cell suspension of *Pelargonium sodoides* and *P. reniforme*

P 022

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Extracts of the roots of *Pelargonium sidoides* and *P. reniforme* (Geraniaceae) are used for the treatment of upper airway infections (1-4) and contained in the pharmaceutical preparation Umckaloabo. One of the major components of the extract is umckalin, but the metabolic profile is also characterized by the presence of further tri- and tetraoxygenated coumarins. In order to produce coumarins, we started tissue cultures of both plant species. Surface disinfected leaf and root explants were cultivated on MS-medium (pH 5.9, 4% saccharose) with growth factor BAP (6-benzylaminopurine) and IAA (Indole-3-acetic acid, both 1 mg/l). Calluses grew over two weeks (diameter 4 mm) and were maintained by cutting the tissue in small pieces and plating again on new MS-agar plates in a 5 to 6 week-schedule. Callus was grown under a day/night regime (16/8 h; 3000 lux) at 24°C. Cell suspensions were initiated by transferring callus clumps into sterile 300 ml MS-medium to a 500 ml Erlenmeyer-flask, shaking at 175 rpm under same culture conditions. The cell suspension culture showed exponential growth kinetic of 5 days log-phase and 15 days stationary phase with a total cultivation time of 21 days. The presence of secondary metabolites was analyzed in root callus tissues and cell free supernatants from both species. GC-MS revealed the presence of monosaccharides, catechin and gallic acid as major constituents. In the callus cultures and in the cell suspension cultures of *P. sidoides* umckalin was detected in traces. Compounds were identified by comparison of retention times and fragmentation pattern of in house standards.

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Medicarpin production by fenugreek tissue cultures. Expression of isoflavone synthase and vestitone reductase

P 023

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The isoflavonoid medicarpin has been identified as a major phytoalexin produced in *Medicago* and *Trigonella* species [1]. Fenugreek (*Trigonella foenum graecum* L.) roots and leaflets, upon elicitation with *Rhizobium meliloti* [1] or *Helminthosporium carbonum* [2] respectively, produced medicarpin. Recently, we have identified high concentrations of medicarpin in calli, which were developed from fenugreek flowers, in the absence of any elicitor [3]. Here we report on callus development upon 20-d-incubation of fenugreek radicles, from 6 d old-seedlings. The radicles were incubated in 0.8% (w/v) agar in MS [4], 1 mg/L α-NAA, 1.5 mg/L BA, and 20 g/L sucrose at 25 °C, in darkness. From these calli, after three subcultures, were developed cell suspension cultures, which were cultured in the same medium. HPLC analysis of the 80% ethanol extracts derived from root calli or cell cultures showed similar pattern. They contained medicarpin, as one of the major constituents of the extract and also small amounts of formononetin and ononin. However, the major constituent was a glucoside which has been isolated and the identity of which is being investigated. RT-PCR analysis revealed that isoflavone synthase, the entry point enzyme to the isoflavonoid pathway, and vestitone reductase which catalyses the penultimate step of medicarpin synthesis [5], were both expressed in these calli.

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P 024 Isolation of the lignan 6-methoxypodophyllotoxin from *Linum boissieri* and first experiments concerning the biosynthesis of lignans in this species

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Lignans are phenolic compounds that are very wide spread in the plant kingdom and show a wide variety of biological activities: antitumour, anti-HIV, immunosuppressive, hipolipidemic, antifungal, phytoestrogenic and antiasthmatic activities (1,2,3). Lignans are derived from two phenlypropanoid units that are linked by a C-C bond between the side-chain carbon atoms 8 and 8'. 6-methoxypodophyllotoxin (6MPTOX) and podophyllotoxin (PTOX) belong to the class of aryltetralin lignans. PTOX is the starting compound for the production of three important clinically applied anticancer drugs etoposide, teniposide and etopophos®. Up to now PTOX is isolated from roots and rhizomes of wild growing plants of *Podophyllum hexandrum*, which is endemic in the Himalayan region and now an endangered species. In order to find alternative sources, *Linum* species were recognised to contain lignans of various types. Plant *in vitro* cultivation has several advantages over collecting plants from wild or cultivating them on fields (4). Metabolites like lignans can be produced under controlled and reproducible conditions, independent of geographical and climatic factors. It is not necessary to use herbicides or insecticides. Especially cell suspension cultures can show high growth rates combined with high accumulation of the desired metabolite in short time. In addition such cultures can be an excellent source to study the biosynthesis of secondary compounds (5). Previous work has shown that suspension and callus of the *Linum* species (Linaceae) are useful for the production and accumulation of podophyllotoxin and 6- methoxypodophyllotoxin (6). As a part of our ongoing studies on the suspension cultures of *L. boissieri,* we were able to establish a suspension culture of *Linum boissieri* which produces 6-methoxypodophyllotoxin (6MPT). As a first step to get insight into the lignan biosynthesis in *L. boissieri* cell cultures we were able to measure phenylalanine ammonia-lyase (PAL) activity in protein raw extracts. PAL is a key enzme in the early part of the general phenylpropanoid pathway leading beside others to the precursors for lignan biosynthesis.

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P 025 Carotenoid composition of yeasts of the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces* and *Sporidiobolus*

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Twenty yeast strains, each one representing a given species of the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, and *Sporidiobolus*, were investigated with the objective of evaluating their carotenoid composition. The pigments were extracted from yeast cells, quantified by HPLC-DAD and identified by APCI–MS techniques. Thirteen strains were seen to be able to produce carotenoids, from 16.5 to 184 μg g^{-1} cell DW and from 16.4 to 1993.4 μg l^{-1} culture, respectively. Some significantly ($P < 0.01$) differences have been observed. The main carotenoids produced were identified as β-carotene, γ-carotene, torulene, torularhodin, plectaniaxanthin and 2-hydroxy-plectaniaxanthin. Some minor unidentified carotenoids were also sporadically detected. The correlation matrix calculated on the basis of the carotenoid composition data matrix indicated significant ($P < 0.01$) relationships between torulene and torularhodin ($r = 0.81$), γ-carotene and torulene ($r = 0.49$), β-carotene and torulene ($r = -0.72$), β-carotene and γ-carotene ($r = 0.64$), as well as plectaniaxanthin and 2-hydroxy-plectaniaxanthin ($r = 0.96$). This study represents the first investigation on carotenoid composition of species of the genera *Rhodotorula*, *Rhodosporidium*, *Sporobolomyces*, and *Sporidiobolus* under the same experimental conditions.

Differences of phenolic responses in two *Hypericum perforatum* L cell lines elicited with *Colletotrichum gloeosporioides*

P 026

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Anthracnose, caused by *Colletotrichum gloeosporioides* (CG), is a major problem concerning cultivation of *Hypericum perforatum* (HP). Two lines of HP, Hellos (tolerant) and HPS (susceptible), were utilized as tools for studying plant defence mechanisms (namely phenolic) against CG attack. Cells cultures of both lines were faced with autoclaved CG biomass, with or without priming with salicylic acid (SA) or methyl-jasmonate (MeJ). The effect of SA and MeJ alone was also checked. Changes on soluble phenolics accumulation were analysed by HPLC-DAD-MS, during cell growth. Priming of cultures with SA or MeJ, prior to CG elicitation, leads to a high increase on xanthone accumulation both on HPS (up to 12x) and Hellos (25x). Hellos cultures, treated with CG extract only, also accumulated nearly 25x more xanthones than control samples. In contrast, HPS displayed a smaller increase on xanthones accumulated (up to 7x). Moreover, xanthone accumulation in Hellos kept at high levels throughout cell culture time, while in HPS the accumulation levels decreased 4-6 days after pathogen infection. Hellos cultures treated with SA or MeJ only, displayed an increase on xanthone accumulation of nearly 3x and 5x, respectively. However, xanthones accumulation on HPS cells is not significantly affected by SA or MeJ-treatment only. Although both cell lines display similar levels of xanthone accumulation on control samples, the higher and fast accumulation of xanthones on Hellos line may be connected to its higher resistance to CG infection, since some xanthones are known to display antimicrobial activity.

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Metabolites produced by shoots and calli of *Lavandula angustifolia* L.

P 027

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In vitro cultures of *Lavandula angustifolia* were established on two agar gelatinized modified N30K and MS media using apical buds excised from branches of in Nature growing plants. Formation of calli accompanied of shoots was induced in all hormonal variants tested independently of the formation of roots. This type of response occurred even in cultures maintained with MS medium devoid of exogenous auxin, supplemented with one cytokinin, as the only growth regulator: benzyladenine (BA); kinetin (KIN) or zeatin (ZEA). To evaluate the effect of light intensity of the 16h light / 8h dark photoperiod on morphogenesis, growth, and production of secondary metabolites the cultures on MS medium supplemented with all hormonal variants tested were subdivided in two groups: one of them was kept with a photoperiod of 8 µmol/m^2/s PAR and the other one with a photoperiod of 53 µmol/m^2/s PAR. The highest multiplication rate and the highest linear growth, accompanied however with the lowest biomass growth rate, were recorded in cultures maintained with BA kept under 8 µmol/m^2/s PAR. The increase in biomass growth occurred under both light regimens combining an auxin, indole-3-butyric acid (IBA), with BA. Having in view to study the accumulation of phenolic compounds, the calli portion was separated from shoots in all cultures maintained with the four hormonal variants and the two levels of light intensity. Both calli and shoots were submitted to extraction by maceration with acetone, the solvent evaporated and the residue redissolved in ethanol prior analysis by HPLC-DAD. Four compounds were identified and quantified in phenolic extracts from shoots: 2-O-glucosilcoumaric acid, *o*-coumaric acid, coumarin, and herniarin. However, besides these four phenolics, calli accumulated caffeic and rosmarinic acids, this last one being the major compound. The GC and GC-MS analysis of the hydrodistillates obtained from shoots revealed that these organs accumulated essential oils constituted by more than thirty compounds, 85-90% of which were identified.

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P 028 Industrial production of verbascoside in suspension cultures of *Syringa vulgaris*

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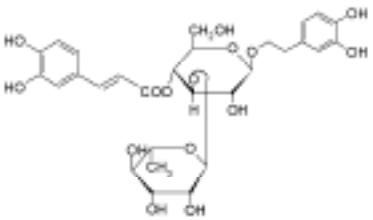
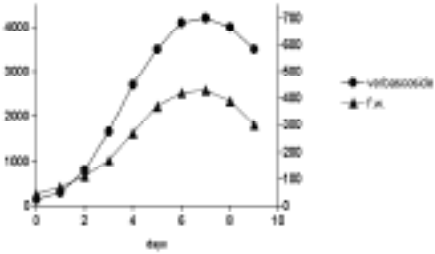
Verbascoside (VB) or acteoside (Figure 1) is one of the most powerful natural antioxidant agents and occurs widely in the plant kingdom.
The first paper dealing on the production of VB in plant cell cultures appeared more than twenty years ago. At our knowledge no report on its production in large scale by plant cell cultures has appeared till now in literature.
After several steps of clonal selection based on serial dilutions, we have achieved in isolating a high producing cell line (deposited at DSMZ – accession N° 16857). Seven day old suspension cultures of *Syringa* vulgaris carried on in non conventional fermenters of 100 liters volume, allowed to produce up to 4 g/L of pure VB.
In the crude extract recovered by solid phase extraction, VB covers more than 60% of the weight and more than 90% of all the caffeoyl derivatives produced.
A timecourse of a typical fermentation is reported (Figure 2).
In the medium sucrose (initially 20 g/L) is absent by the second day, yielding glucose and fructose completely metabolized at the fifth and the seventh day of culture, respectively.
More than one half of the nitrogen source (KNO_3 initially 2,5 g/L) at the end of the fermentation is still present in the medium, while phosphate by the second day is absent.





P 029 Antibacterial, antioxidative and cytotoxic activities of extract from in vitro grown *Ruta graveolens*

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Ruta graveolens tissue is rich in secondary metabolites with potential use in medicine e.g. furanocoumarins, alkaloids and flavonoids. The optimal conditions for R. graveolens shoots and hairy roots micropropagation were described. Fresh ground tissue (1 g) was extracted with petrol ether, chloroform and methanol using Soxhlet apparatus. Furanocoumarins and furoquinoline alkaloids were analysed quantitatively by SPE and RP-HPLC in a Hewlett-Packard model 1050 LC. Antimicrobial activity of R. graveolens extracts against several bacterial pathogens was checked using Minimal Bactericidal Concentrations test (1). Antioxidant activity has been measured with two methods: Ferric Thiocyanate Test and Thiobarbituric Acid test, which measure the amount of peroxides produced during non-enzymatic lipid peroxidation. Cytotoxic activity was determined on HeLa cell line using MTT test. The results indicate that in vitro grown R. graveolens shoots and hairy roots extracts have bactericidal activity; equivalent of about 30 mg of DW inhibit 99% of bacterial growth. R. graveolens extracts have shown cytotoxic activity on HeLa cells. IC_{50} was estimated as an equivalent of 0.06 mg of dry weight. Examined extracts have shown high antioxidant activity, similar to these of applied standard antioxidant: alpha-tocopherol and BHT. Presented data indicated that R. graveolens extracts contains biologically active compounds with antimicrobial, antioxidative and cytotoxic activities.

Acknowledgements: State Committee for Scientific Research, Grant No. PBZ-KBN-092/PO5/2003
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Antimicrobial activity of secondary metabolites from the in vitro grown *Drosera capensis*

P 030

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The *Drosera* genus is a natural source of pharmacologically important compounds used as substrates in the production of pharmaceuticlas against chronic bronchitis and asthma (1). The optimal conditions for *D. capensis* micropropagation were described as: 0.75% agar solidified ½ MS medium with 25 mg/l ascorbic acid and 2% sucrose, pH 5.6. Fresh ground tissue (1 g) was extracted via sonication in 25 ml methanol or chloroform (30 min., 20°C). The suspension was filtered, evaporated and diluted in 5 ml of methanol. The accumulation of secondary metabolites in in vitro grown *D. capensis* was determined. LC analyses were performed on Merck-Hitachi HPLC pump Model D-7000, equipped with diode array detector Model L-7450 (Darmstadt, Germany). Antimicrobial activity of *D. capensis* extracts against several human bacterial pathogens was tested. Plant extracts (equivalent of 10–80 mg FW) were evaporated to dryness and diluted in 0.2 ml M-H medium containing bacterial culture (final concentration 5 x 10^5 cfu/ml^{-1}). The mixtures were incubated overnight at 37°C and the aliquots of 0.1 ml were plated out on agar plates. After the overnight incubation at 37°C the Minimal Bactericidal Concentrations (MBC) (2) of extracts from *D. capensis* were evaluated and compared to MBC of several antibiotics. The results indicate that equivalent of 30 mg of FW of in vitro grown *D. capensis* plants have bactericidal activity.

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Levisticum officinale hairy root cultures: influence of light and light type on growth and essential oil production

P 031

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The essential oils of *Levisticum officinale* W.D.J. Koch (Apiaceae), including those isolated from the roots, are used in the cosmetic, pharmaceutical and food industries [1]. This perennial and herbaceous plant, commonly known as lovage, is widely known by its aromatic, ornamental and medicinal properties. The effect of light and light type on growth and essential oil production of lovage hairy root cultures was studied by comparison of cultures maintained under "blue-basic" (400-550nm) and "day-light" 16h light photoperiod with control cultures maintained under darkness. All cultures were maintained in SH medium [2] and kept at 24°C on orbital shakers at 80 r.p.m. Growth was evaluated by fresh weight (f.w.), dry weight (d.w.) and by the dissimilation method. The essential oil samples were isolated by distillation-extraction and analysed by GC and GC-MS. Control hairy root cultures showed a fifteen-fold d.w. biomass increase at the end of the growth period (six weeks), whereas an approximately eight-fold and ten-fold increase was obtained with "blue-basic" and "day-light" grown cultures, respectively. These differences were supported by morphological and histochemical analyses. Major changes were detected in the essential oil composition, but *Z*-falcarinol was in all cases the major oil constituent: in darkness, "day-light" and "blue-basic" grown cultures (75%, 94% and 61%, respectively).

Acknowledgements: This study was partially funded by FCT, under research contract POCTI/AGG/42961/2001.
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P 032 Nitrogen stress induction on *Levisticum officinale* hairy roots: effect on growth and essential oil production

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Levisticum officinale W.D.J. Koch (lovage) is an Umbelliferous perennial plant. For its aromatic and medicinal properties, the essential oil of lovage roots has been used for a long time [1].
Aiming at the optimization of essential oil production by lovage hairy root cultures, the effect of eight different NH_4^+:NO_3^- ratios on growth and essential oil production, under darkness and 16h light photoperiod, was studied by dry weight, fresh weight and dissimilation evaluation and by GC-MS, respectively. All cultures were kept at 24ºC on orbital shakers at 80 r.p.m.
Higher growth was attained under darkness with 10:90 (control, SH medium [2]), 50:50 and 25:75 NH_4^+:NO_3^- ratios and also with SH control medium under 16h light photoperiod. These results were supported by one-, two way ANOVA, and significance between means by Duncan's Multiple range test ($p = 0{,}05$).
Essential oil UPGMA cluster analysis showed a high degree of correlation between some of the 16 oils isolated from the different cultures, both under darkness and 16h light photoperiod, being characterized by the dominance of *Z*-falcarinol (≥41%), *n*-octanal (≥16%) or palmitic acid (≥16%).

Acknowledgements: This study was partially funded by FCT, under research contract POCTI/AGG/42961/2001.
References: 1. Bylaite E. *et al.* (1998) *J. Food Chem.* 46: 3735-3740. **2.** Schenk U.R., Hildebrandt A.C. (1972) *Can. J. Bot.* 50: 199-204.

P 033 Can flavour components of garlic be produced by mushrooms?

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The genus of onions (*Allium*) is characterized by a diverse pattern of distinct sulphur compounds, which are responsible for the remarkable aroma profiles of garlic (*A. sativum* L.), common onion (*A. cepa* L.) und leek (*A. porrum* L.). Cysteine sulphoxides of these plants were converted by the enzyme alliinase into the corresponding thiosulphinates, *e.g.*, the thiosulphinate allicin is produced from alliin in garlic. Thiosulphinates are highly instable and react to a high diversity of further compounds. Besides the onions, also mushrooms of the class of *Basidiomycetes* exhibit a strong garlic-like smell and taste. Remarkable are the shiitake mushroom [*Lentinus edodes* (Berkeley) Pegler], the garlic marasmius *Marasmius scorodonius* (Fries) Fries and the garlic parachute *Marasmius alliaceus* Jacq. Fr.. The cysteine sulphoxide lentinic acid could be already isolated from *Lentinus edodes*. Similar compounds can be assumed for the genus *Marasmius*. In the now presented investigation a laboratory model used for the study of the metabolism of the cysteine sulphoxides should be established. It was possible to find culture conditions for both *Marasmius* species, which are adaptable to a laboratory environment. The cultures exhibited a characteristic smell and showed in case of *M. scorodonius* a significant alliinase enzyme activity. *M. scorodonius* can be cultivated on agar culture mediums and is therefore available in unlimited amounts for further investigations.

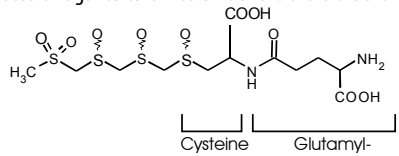

Figure. Chemical structure of lentinic acid.

Cytotoxic activity of extracts from *Linum* cell cultures

P 034

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Justicidin B is an arylnaphthalene lignan from *Linum* spp.(1) and previously known from *Justicia* spp. (Acanthaceae) and *Haplophyllum* spp. (Rutaceae). The potent bone resorption inhibitor justicidin B was used as lead compound for new antirheumatic drugs (2). The objective of this study is to determine the lignan content in the cell cultures from *Linum narbonense* L. and *Linum leonii* F.W. Schulz. (Linaceae) and to examine the cytotoxic activity of the extracts. Callus and suspension cultures of *Linum narbonense* and *Linum leonii* were developed to study the production of lignans and their cytotoxic activity. Justicidin B was determined to be the main lignan. The maximal yield of justicidin B up to 2.22 mg/g of the cell dry weight was detected in the callus cultures of *L. leonii*, followed by the callus cultures of *L. narbonense* (1.57 mg/g dwt). The cytotoxicity of the obtained extracts was measured using the MTT–dye assay extracts on human leukemic cell lines. *L. narbonense* and *L. leonii* both showed cytotoxic activity. IC_{50} values of the methanolic extracts of *L. narbonense* were found to be 0.188 mg/ml and those for *L. leonii* were 0.221 mg/ml. Because of the higher antitumoral activity, the cytotoxic effect of *L. narbonense* was additionally tested on DoHH-2 and HL-60 cell leukemic lines.

Acknoledgements: Prof. A. W. Alfermann (University of Düsseldorf, Germany) and Dr. A. Mohagheghzadeh (Shiraz University of Medical Sciences and Health Services, Iran) for the valuable discussion about identification and determination of justicidin B.
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Optimization of lignan production of in vitro cultures of *Linum tauricum* ssp. *tauricum* (Willd.) Petrova

P 035

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In continuation of our research on lignans in Linum species, Linaceae (1, 2), callus and suspension in vitro cultures of the Bulgarian endemic plant Linum tauricum ssp. tauricum (Willd.) Petrova, belonging to the Section Syllinum, were initiated in sterile conditions. In the conventional cultures of this species two main lignans - the podophyllotoxin derivatives 4′-demethyl-6-methoxypodophyllotoxin and 6-methoxypodophyllotoxin (MPTOX) were identified (HPLC and UV). The comparison was made with their content in the intact plant. The both compounds, isolated for the first time from the intact plant were identified by HPLC, UV and ^{1}H NMR. The levels in callus cultures, (grown on MS media with 0,1 mg/l 2,4-D, 0,2 mg/l IAA, 2.0 mg/l kinetin, 1,0 g/l casein) were 0,127 mg/g dw and 0,516 mg/g dw respectively, while suspension cultures, grown on liquid medium with the same composition biosynthesised the compounds only in traces. As a result of more than one year maintenance of the cultures and optimisations of growth media, a stable growth and production of both compounds was achieved. Suspension cultures synthesized 2,16 mg/g dw and 2,93 mg/g dw (4 mg/l NAA, 1 g/l casein) and callus cultures – 1,54 mg/g dw and 3,99 mg/g dw (0,1 mg/l 2,4-D, 0,2 mg/l IAA, 2,0 mg/l kinetin, 1,0 g/l casein). Experiments of influence of methyl jasmonate and red light were also carried out and growth dynamics clarified. The applied methods lead to a multiple increase of the productivity of in vitro cultures of Linum tauricum ssp. tauricum (Willd.) Petrova.

Acknoledgements: Council of Medical Sciences - Contract 7-D of Medical University Sofia
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P 036 The effect of medium composition on shikonin derivatives accumulation in suspension culture of *Arnebia euchroma*

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Plants of *Arnebia euchroma* (Royle) Johnst. (*Boraginaceae*) are distributed in Russia, China and India. They have been used as a traditional Chinese medicine for treating burns, wounds and gynecological inflammation [1]. The chemical investigation of *A. euchroma* roots demonstrated the presence of shikonin and its derivatives [2]. Shikonin possesses antibacterial, antifungal, antiinflamatory and antineoplastic activities [2, 3]. Recently the immunomodulatory and angiogenesis promoting properties of acetylshikonin (ACS) were reported [4, 5]. Also ACS and isobutyrylshikonin (IBS) shows the antimicrobial activity [6, 7]. Suspension culture was carried out in MSA medium [8] or in basal medium with reduced concentration of KNO_3 by 25% (MSA75) and 50% (MSA50). The basal MSA medium was also supplemented with L-phenylalanine (0.01, 0.1 and 1 mM). Determination of shikonin derivatives was done using HPLC-UV method. The reduction of nitrate level in the medium caused decrease in the biomass growth in comparison to full-strength basal medium, form 14-fold to 5.6-fold in the MSA50 medium. However, the highest content of ACS and IBS was detected when culturing in MSA50, 142 mg/g D.W. and 14 mg/g D.W., respectively. The addition of L-phenylalanine to the basal MSA medium had no beneficial influence on shikonin derivatives production as it was observed in callus culture of *Lithospermum erythrorhizon* [9]. Shikonin was detected only in the medium after 32 days of culture - 1.6 mg/l.

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P 037 Cytochemichal investigation of leaf secretory tissues and cell culture of *Inula viscosa* – Antifungal activity

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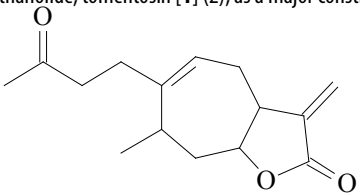
Inula viscosa is one of the four endemic species of the genus in Greece. The plant has been collected from mountain Hemettos. Leaf structures or cells with secreting activity were localized and a spectrum of products was istochemically identified within them. Leaf extracts were investigated with GC-MS techniques. Calluses produced from leaf cell cultures were also subjected to histochemical reagents and GC-MS analysis to investigate their secreting ability compared to that in leaves (1). Developed callus masses present an interesting antifungal activity. Additionally, the crude CH_2Cl_2 extract of the leaves yielded the known xanthanolide, tomentosin [**1**] (2), as a major constituents.



[1]

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Metabolites produced by shoots and cell suspensions of sage (*Salvia officinalis* L.)

P 038

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Shoots and cell suspensions and of sage (*Salvia officinalis* L.) were established using agar gelatinized and liquid MS medium respectively. Combination of benzyladenine (BA) with dichlorophenoxyacetic acid (2,4-D) afforded the highest rates of proliferation and linear growth of the shoots and the lowest biomass dry weight per shoot. The highest biomass dry weight per shoot was got using kinetin (KIN) with 2,4-D. These growth regulators (KIN with 2,4-D) were the most suitable for growth and maintenance of cell suspensions as well as for production of lipid, phenolic and essential oil compounds by these type of cultures. Lipid compounds were extracted from suspended cells with n-hexane using a Soxhlet apparatus during 48 h. Essential oil compounds from shoots and suspended cells were obtained by hydrodistillation in a Clevenger type apparatus over 1 h. Phenolics, produced by the same type of cultures, were extracted three times by maceration with acetone. The hydrodistillates and the lipid extracts were analysed by gas chromatography (GC) and gas chromatography - mass spectroscopy (GC-MS). Prior analysis, the lipid compounds were derivatized by trimethylsilylation. The phenolic extracts were analysed by HPLC-DAD. Eight saturated and four unsaturated fatty acids, a triterpenic hydrocarbon and two sterol compounds were identified in the n-hexane extract. The hydrodistillates from shoots and suspended cells were composed of more than 75 and 25 compounds, respectively, and sixteen of these ones were found out the cells, in the medium. Seventeen compounds were identified in the phenolic extracts of shoots. In suspended cells, the highest number of phenolic compounds (12) was detected at stationary phase. At the beginning of the growth cycle, phenolic extracts, composed of 4-6 compounds, showed the highest contents of 5-*O*-caffeoylquinic acid and carnosol. Great changes in the contents of the total and individual lipid compounds were also detected over the cell cycle.

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Production and analysis of bioactives in *in vitro* shoot and hairy root cultures of southern African sages

P 039

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The accumulation of polyphenolics with biological activity in the genus *Salvia* (Lamiaceae) is well established. Despite the importance of indigenous sages in traditional medicine in South Africa, biotechnological and phytochemical investigations of these plants have been limited. The sparse *Salvia* populations face threats relating to habitat loss and degradation; and, over-harvesting for informal trade. To facilitate their conservation, seedling explants of *S. runcinata*, *S. africana-lutea* and *S. chamelaegnea* were micropropagated. A 0.5 mg L^{-1} BA medium produced the greatest number of plantlets and 80% of the plantlets survived *ex vitro* transfer. Three strains of *Agrobacterium rhizogenes* (LBA9402, A4T and C58C1) were comparable for their efficiency to introduce *rol* genes when hypocotyls and primary leaves were targets. All transgenic root clones had a significantly higher growth rate than wildtype root clones; accumulating a biomass that was four times greater. In liquid culture the LBA9402 clones were the most prolific. PCR products (3.5 kb and 400 bp) were resolved after transgene DNA amplification when *rol* A1/C2 and *rol* B1/B2 primer pairs were utilized, respectively. Currently, Southern hybridization is being conducted to confirm the transgenic status of the clones. Preliminary phytochemical profiles using TLC have thus far revealed the accumulation of important bioactives such as rosmarinic acid. This is the first time that African *Salvia* species have been genetically modified to display the hairy root phenotype.

P 040 Micropropagation of the medicinal plant, *Echinacea purpurea*, in solid and liquid culture systems.

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The production of high quality phyto-pharmaceuticals is highly dependant on our ability to produce plants with a uniform, consistant chemical profile. In order to ensure that this uniformity is acheived it is necessary to remove as many sources of variability as possible. The two main factors that need to be addressed are genetic and environmantal diversity. *In vitro* propagation provides an excellent method to mass produce genetically similar plants in a relatively small amount of time and space. These plants can then be grown in controlled environments such greenhouses, growth chambers, or bioreactors to produce plant material with a well characterized, consistant chemical profile. Knowledge of *In vitro* regeneration of *E. pururea* is rather limited, mass plant production through liquid culture systems has not been established. The current study was designed to optimize an efficient liquid system for induction and expression of regeneration resulting in genetically uniform *E. Purpurea* plants . Plant regeneration requires timed exposure to a specific concentration of an inductive stimulus, subculture onto a different medium for expression of regeneration, and in some cases a third phase of maturation and germination to produce whole plants. In initial experiments, 3 different plant growth regulators viz.1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea (Thidiazuron), 2,4-Dichlorophenoxyacetic acid (2,4-D), and 3,6-Dichloro-o-anisic acid (Dicamba)) were evaluated at 0- 10 µM on solid media for their ability to induce callus formation, and eventual plant regeneration. Thidiazuron was found to produce the highest levels of growth and was capable of inducing high levels of regeneration. Tissue derived from solid media with 1.0 µM thidiazuron was used to initiate liquid cultures in the presence of 0 to 10.0 µM Thidiazuron. Large numbers of structures resembing somatic embryos were produced in the liquid with a maximum mean of 708 per gram of tissue in response to 10.0 µM thidiazuron. Plantlets were further developed and grown in a range of different solid and liquid medium growth systems to enhance the rate of plant growth and to assess the effects of changing plant growth regulators on caftaric acid, chlorogenic acid, cynarin, echinacoside,and chicoric acid. This protocol provides an ideal system to mass produce genetically uniform disease free plants; resulting in high quality, consistant plant material for commercial production and clinical trials.

P 041 *Hypericum perforatum* subsp. a*ngustifolium*: morphological, histological and phytochemical study in *in vitro* vegetative organogenesis

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Hypericum perforatum subsp.a*ngustifolium* (Guttiferae/ Clusiaceae) is well known as an antidepressive. The main constituents of *H. perforatum* extract include naphthodianthrones, phloroglucinols, flanonoids and xanthones. The high demand for these active pharmaceutical components has made it interesting to search for an alternative approach to extraction from the plant .
Calli were induced from leaf explants cultured in Murashige Skoog basal medium added with 2,4-D (1.3 mg/l), NAA (0.25 mg/l), Kin (0.25 mg/l), and sucrose (30 g/l). To induce regeneration of shoots, the calli were exposed to low temperatures and pH stress and cultured under dark or exposed to photoperiod (16/8 light/dark). Different hormonal treatments were also tested. Morphological histological observations and phytochemical analyses by HPLC/DAD and HPLC/API/ESI demonstrated that anthocyanins were present both in undifferentiated calli and in regenerated shoots at all developmental stages. Hypericins were detected only when the dark globules (secretory structures) were formed. This investigation highlighted, for the first time, the co-presence of hypericins and anthocyanins in the cultures of *Hypericum perforatum* L.

Seco-iridoid glucosides from leaves of boron deficient olive plants

P 042

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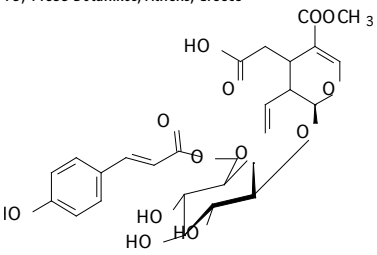

Accumulation of aromatic secondary metabolites by various stresses like nutrient deficiencies is common in higher plants (1). In particular, induction of phenylpropanoid metabolism by boron deficiency is well documented (2). Crafted olive plants (*Olea europaea* L. cv. 'Manaki') were semi-hydroponically grown for 45 days in a growth chamber. Nutrient solution was inadequate in boric acid, which resulted in the development of boron deficient plants, according to Liakopoulos and Karabourniotis (3). Investigations on the MeOH extract of the dried leaves lead to the isolation of several known compounds, namely, acteoside, isoacteoside, oleuropein, oleoside-dimethylether, as well as three new seco-iridoid glucosides. Fractionation was carried out using VLC, CC, RP-HPLC and LC-MS. The structures of the isolated compounds were established by means of NMR [1H-1H-COSY, 1H-13C-HSQC, HMBC] and MS spectral analyses. It is noteworthy that these compounds are isolated for the first time from Olea europaea and their occurrence in the plant could be explained in terms of metabolic stress.

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Cultivation of *Myrtus communis* L.: effect of nitrogen on leaves polyphenolic metabolites

P 043

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Among the species of the Mediterranean "maquis", *Myrtus communis* is traditionally used for several purposes. The typical liqueur produced in Sardinia is based on the collection of leaves and fruit from the spontaneous plants, thus increasing the genetic erosion threat. New and innovative products are recently conceived together with the implementation of a sustainable cultural system. Studies on domestication of the species and on crop management have been recently carried out in Sardinia, providing agamically propagated selections and preliminary results on the response of the plant under cultivation. In order to properly define the mineral fertilization plan, three levels of nitrogen were applied on a randomized seven-year old myrtle plantation, located in the experimental station of the DESA, University of Sassari, in Oristano (Lat. 39°54' N). Fifteen self rooted plants of the selection CPT5, divided into three blocks for each thesis were treated in march 2004 and monitored during the following growth and reproductive season. Phenological phases and vegetative growth were measured on ten shoots for each plant. Berries size and yield were strictly related to increasing nitrogen level, thus reducing the morphological heterogeneity found in the spontaneous plants. To identify the mineral requirements of the species under cultivation the amount of macro and micro elements on fruits and leaves was recorded both on open air cultivated myrtle and on pot grown one-year old plants. Each myrtle sample, wild and cultivated, was also monitored for polyphenolic composition by HPLC/DAD and HPLC/MS methods. The quali-quantitative data were calculated for the two main polyphenol classes, gallotannins and flavonoids. The mean value of total polyphenol was 70 mg/g of dried leaves and the major compounds were the hydrolysable tannins, and, among flavonoids, the galactoside, galloyl-galactoside and rhamnoside of myricetin.

P 044 Effects of Nitrogen Nutrion Seed Yield and Oil Seed Content of Naked Seed Pumpkin (*Cucurbita pepo* subsp. *pepo* convar. *pepo* var. *styriaca*)

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Naked (Styrian , Green Gold , Medicinal) pumpkin (Cucurbita pepo convar. pepo var. styriaca) is one of the valuable medicinal plants in pharmaceutical industries of developed countries. Nitrogen influenced on the growth , development and productivity of the plants. In this study , nitrogen applied at four levels (0 , 50 , 100 and 150 $Kgha^{-1}$) with three replicates in each treatments . Nitrogen fertilization were applied at three different stages of the plant growth : seed sowing time , fourth leaves and flowering stage . In order to study the effects of increasing nitrogen level on fruit and seed yield and oil seed content of the medicinal pumpkin determined . There were significant different between some treatments on fruit and seed yields , seed dry matter and oil seed content of the plants . The highest oil levels obtained at 100 and 150 $Kgha^{-1}$ of nitrogen fertilization.

P 045 Preliminary trials on effect of nitrogen nutrition on growth and on secondary metabolism of *Achillea millefolium* L. spp *collina*

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The studies on the effect of mineral nutrition on Herbs secondary metabolism and yield could lead an improvement of the production of these plants in industrialised countries, where high quality products are required. It is known that the synthesis of secondary chemicals is affected both environmental and agronomic conditions. A recent trial on Yarrow, growth in different climate localities in Alpine cultivated areas with different agronomic techniques, showed significative differences concerning growth and chemicals composition (1). Furthermore, studies on mineral nutrition of *H. perforatum* showed the relationship between nitrogen supply and Hypericin content (2). To study the effect of nitrogen nutrition on Yarrow, we made a preliminary trial on *Achillea millefolium* L. spp *Collina* growth in nutritive solutions with different nitrogen supplies in greenhouse (T 25°C, UR 60% and 18 h photoperiod). The plants growth on high level of nitrogen, compared to the ones growth on lower level, showed a significative increase of number of shoots (61%), fresh weight of shoots (311 %), dry weight of shoots (243 %) and shoots/roots ratio (204%). Differences in phytochemicals profile also emerged.

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Occurrence of amides derived from corresponding cyanogenic glycosides

P 046

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Some cyanogenic plants contain amides whose structures correspond to their cyanogenic glycosides in that the nitrile moiety is metabolized to a carboxyamide group. These amides seem to be formed during processing of the plant material; up today they could not be found in fresh plant material. The present work investigates the formation of these amides using the leaves of *Olinia ventosa* as a model, which contain the cyanogenic glucoside prunasin as well as its corresponding amide, prunasin amide (1). It was shown that the drying process strongly influences the accumulation of the amide; the influence of humidity and temperature was investigated. However, formation of the amide depends on enzymatic activity. Prunasin amide is also present in other prunasin containing species such as *Prunus laurocerasus*, *Holocalyx balansae* and *Pteridium aquilinium* when their leaves are dried under conditions investigated here. Interestingly, leaves of *Olinia ventosa* with pathologically changed areas obviously caused by a hypersensitive reaction also contain prunasin amide, in particular in their brown parts.

References: 1. Rockenbach, J. et al. (1993) Phytochem. 34: 433-436.

Substrate specificity and kinetic properties of recombinant Δ^5-3β-hydroxysteroid dehydrogenase from *Digitalis lanata* Ehrh.

P 047

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The biosynthesis of cardenolides, used in the treatment of cardiac insufficiency in man, has been studied extensively in recent years (1,2). In an early biosynthetic step Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) converts pregn-5-en-3β-ol-20-one (pregnenolone) to pregn-4-ene-3,20-dione (progesterone). The conversion involves two reactions, namely the NAD-dependent oxidation of pregnenolone to pregn-5-ene-3,20-dione and the isomerisation of this compound to progesterone (3). Δ^5-3β-HSD has already been isolated, purified and characterized from *Digitalis lanata* Ehrh. (4). Only recently, the purified recombinant protein has become available and shown to be enzymatically active (5). Here, we investigated the substrate specificity of the recombinant Δ^5-3β-HSD. In a first set of experiments steroids with 3β-hydroxy group have been tested as substrates: pregnenolone, androst-5-en-3β-ol-17-one (androstenolone), 5α-pregnan-3β-ol-20-one, 5β-pregnan-3β-ol-20-one and pregn-5-ene-3β,21-diol-20-one were well accepted, whereas cholesterol was not. Testosterone (with a 17β-hydroxy group) was also accepted and was converted to androst-4-ene-3,17-dione (=17β-dehydrogenase activity!). Steroids with 3α-hydroxy group have been checked as well. Of the two substrates tested, namely 5α-pregnan-3α-ol-20-one and 5β-pregnan-3α-ol-20-one, only the latter was accepted as substrate and converted to 5β-pregnane-3,20-dione.The recombinant Δ^5-3β-HSD was also able to catalyse the reduction, particularly of pregnanediones without a Δ^4- or Δ^5-double bond, if NADH was used as the cosubstrate. Several of the compounds mentioned were used to determine kinetic constants. The multifunctionality of the enzyme in the context of cardenolide biosynthesis will be discussed.

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P 048 Biosynthesis of acetate-derived naphthylisoquinolines and naphthoquinones

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Lianas of the Ancistrocladaceae and Dioncophyllaceae families produce structurally unique naphthylisoquinoline alkaloids. These secondary metabolites possess interesting bioactivities, e.g. against malaria, sleeping sickness and leishmaniasis. Precursor feeding studies provided evidence that naphthylisoquinolines originate from acetate (1). Members of the Ancistrocladaceae and Dioncophyllaceae also accumulate acetate-derived naphthoquinones which are taxonomically less restricted and occur in several related genera of the Caryophyllales, e.g. Drosophyllum and Plumbago.
It has been proposed that a polyketide synthase (PKS) provides the carbon skeleton for naphthylisoquinolines and naphthoquinones. To characterise the PKS biochemically, *in vitro* assays were carried out with crude protein extracts from callus cultures. In addition, a recombinant type III PKS from *Plumbago indica* was assayed for PKS activity. In both cases, however, pyrones were formed instead of naphthalene-related compounds. These results suggest that additional enzymes or co-factors may be required for the formation of the naphthalene ring.
In order to randomly identify genes encoding naphthoquinone biosynthetic enzymes, an expressed sequence tag (EST) analysis of *Drosophyllum lusitanicum* callus cultures has been carried out, and several sequences putatively involved in this pathway have been identified.

Reference: 1. Bringmann, G. and Feineis, D. (2001) J. Exp. Bot. 52: 2015-2022.

P 049 Influence of Altitude on the Amount of Antioxidative Flavonoids and Hydroxycinnamic Acids in Flowers of *Sambucus nigra* L.

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In traditional folk medicine flowers, leaves and fruits of black elder, *Sambucus nigra* L. (Caprifoliaceae), have been recommended for preventing and treating colds and regulation of bowel activity. In modern phytotherapy elder flowers act as diaphoretic and bronchospasmolytic ingredients in herbal teas, the fruits are used in herbal remedies against common cold. In Europe and America, elderberries are used as raw material as well as red colouring in fruit industry. (1) The activity of extracts of *S. nigra* is mainly attributed to its content of flavonoids and hydroxycinnamic acids, which have the capacity of acting as potent radical scavengers. From different plants (*Picea abies* Karst., *Soldanella alpina* L., *Ranunculus glacialis* L.) it is known, that their antioxidant content varies in relation to the altitude. (2,3) However, there is only limited information about the variation of antioxidative active compounds in *S. nigra* at different altitudes. For our investigation, we selected a limited area in order to have comparable climatic conditions. In June and July 2004, elder flowers were collected at 670m and 1000-1070m above sea level in cooperation with the Naturpark Sölktäler in Upper Styria (Austria). The air-dried samples were extracted in an accelerated solvent extraction procedure (ASE) with methanol 80% (v/v) (4) and analysed with RP-HPLC/PDA. Among the flavonol glycosides the major constituents rutin and isoquercitrin were quantified at 350nm, the main hydroxycinnamic acid compound chlorogenic acid was determined at 320nm wavelength. The amounts of chlorogenic acid showed no significant difference at the two altitudes. In case of flavonoids the samples at 670m yielded significantly higher amounts of rutin, the flowers collected at 1000m contained significantly higher amounts of isoquercitrin.

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Aroma precursors of Georgian wild *Allium* species

P 050

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Plants of the genus *Allium* were used by mankind since ancient times as vegetable, spice and medicinal herb. Especially *A. sativum* L. (garlic), *A. cepa* L. (common onion) and *A. ampeloprasum* L. (leek, kurrat) were cultivated and therefore intensively used. Allium species have been grown in Georgian kitchen gardens for thousands of years. On the other hand, the mountain ranges of the Caucasus host a huge diversity of wild *Allium* species, which are mostly members of the subgenera *Allium* and *Rhizirideum*. These species are subjects of the investigation presented. Bulbs and leaves were analysed by HPLC in regard to their content of cysteine sulphoxides (Figure). Theses substances are the precursors of the aroma components of onions, which are produced by catalysis of the enzyme alliinase after damage of plant tissue. The profile of aroma precursors gives some indications of a possible use as spice or medicinal plant. Remarkable profiles in terms of the relative composition of cysteine sulphoxides or in terms of the total content of these substances were found for *A. rotundum* L., sect. *Allium*, *A. albidum* Fisch. ex M. Bieb., sect. *Rhizirideum* and *A. kunthianum* Vved., sect. *Codonoprasum*. Substances of highest interest were alliin and isoalliin. A number of investigated species showed relative large amounts of cysteine sulphoxides (higher than 0.25 %).

Figure. Chemical structures of typical cysteine sulphoxides of wild *Allium* species.

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Analysis of the polyketide synthase involved in naphthoquinone formation in *Plumbago indica* L.

P 051

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Plumbago indica L. (Plumbaginaceae), is a perennial shrub, occurring mainly in the tropical regions. The roots of this plant are rich in plumbagin, an important naphthoquinone which has anticancer (1) and antimicrobial activities (2). Plumbagin was shown to be synthesized via the polyketide pathway (3,4). To investigate the biosynthetic route leading to plumbagin, *in vitro* cultures of *P. indica* were established from various explants as starting material. The plantlets derived from axillary buds were cultured on Linsmaier and Skoog's (LS) medium. Callus and roots were induced from leaf explants on Murashige and Skoog's (MS) and Gamborg's B5 medium containing phytohormones. The adventitious roots were cultured on MS liquid medium containing 30 g/l sucrose. In ethanolic extracts of the tissue cultures plumbagin was detected. A recombinant PKS (*Pin*PKS) isolated from *P. indica* synthesized pyrones but not plumbagin. To identify additional enzymes that can complement the PKS reaction and promote naphthoquinone formation, coupled assays were performed with recombinant *Pin*PKS and crude extracts from plantlets, callus or roots, and a novel enzymatic product was formed.

Acknowledgements: This work was supported by the Royal Golden Jubilee Ph.D. program, Thailand Research Fund (RGJ-TRF) and the German Academic Exchange Service (DAAD, Deutscher Akademischer Austauschdienst).
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P 052 Medicarpin production by *Trigonella foenum-graecum* L. in response to biotic elicitors. Expression of isoflavone synthase and vestitone reductase.

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Medicarpin, an isoflavonoid pterocarpan, has been identified as a major phytoalexin produced from several leguminous plants such as alfalfa, sweet clover and fenugreek (*Trigonella foenum-graecum* L.) in response to biotic stress [1]. Leaves, cotyledons, stems and roots were collected from 15 days old fenugreek plants, extracted with 80% ethanol and prepared for RP-HPLC analysis [2]. Idendification of the compounds was based on co-chromatography with authentic samples. From these tissues, only roots contained high amounts of medicarpin. Two compounds, which were present in high amounts in leaves, but did not correspond to any of the authentic samples used, were isolated with semi-preparative RP-HPLC and identified as flavonol glucosides using modern spectroscopic techniques (1-D and 2-D NMR). In order to clarify the role of medicarpin in the plant response to biotic stress, a suspension of *Pseudomonas syringae* pv. tomato (Pst) [3] was injected via the stomata into the lower surface of the first leaf of 15 days old plants. RP-HPLC analysis showed that leaves infected with Pst, produced high amounts of medicarpin, which reached its highest level after 36h of treatment. RT-PCR analysis revealed that isoflavone synthase, which catalyses the first step to isoflavone biosynthesis, and vestitone reductase, which catalyses the final step of medicarpin synthesis [4], were both expressed in these leaves during the 36h of infection.

Acknowledgments: A grant to CGS in the the framework of "HERAKLEITOS", which has been co-funded by 75% from E.E. and 25% from Ministry of Education and Religious Affairs is gratefully acknowledged.

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P 053 Investigations on the cardinal temperature for germination of *Plantago ovata* and *Plantago psyllium*

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Plantago ovata and *Plantago psyllium* (Plantaginaceae) are important species for their seed and seed husk mucilage which has divers applications in medicine and industry. In a laboratory study the effect of different temperatures on seed germination of these species was investigated. Cardinal temperatures for germination were estimated at 5, 7,10, 15, 20, 25, 30 and 35 ˚C and germination parameters for each species were calculated. The effect of temperature on rate and percentage of germination in both species was significant. The highest germination rates were in 20 and 25 ˚C for *P.ovata* and *P. psyllium*, respectively. Maximum germination for *P.ovata* (100%) and *P. psyllium* (99%) obtained in 15 and 25 ˚C ,respectively. Optimum termal range for seed germination of *P. ovata* was (10 - 20˚C) and for *P. psyllium* was (15 - 25˚C). Change in temperature beyond these range, led to significant decrease in germination. The minimum, optimum and maximum temperatures were determined using a triangular regression model for each species (Fig. 1).

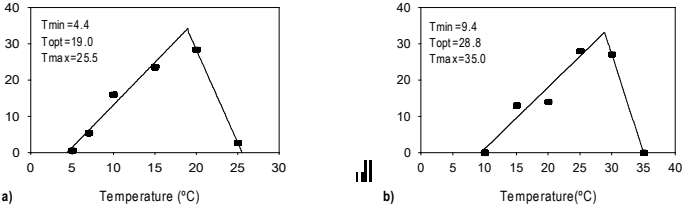

Fig.1. Relationship between temperature and germination rate(germinated seed per day) in: a) *P.ovata* , b) *P.psyllium*

Observations on the erratic occurrence of maytansinoids in plants and microorganasms

P 054

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Maytansinoids occur within the orders *Celastrales, Euphorbiales and Rhamnales* but also in taxonomically unrelated taxa such as a gram positive bacterium, *Actinosynnema pretiosum* (*Streptomycetaceae)* and even in mosses e.g. *Claopodium crispifolium* (HOOK) Ren. & Card. Maytansine is a maytansinoid ansa antibiotic with a strong antineoplastic activity (1).
The disjunct occurrence of maytansinoids suggests that a microbial organism may be responsible for the occurrence of maytansine in higher plants.
We have investigated Actinomycetes from the rhizosphere of a maytansine, containing plant, *Putterlickia verrucosa*, and isolated a hitherto unknown bacterium which was characterised and named *Kitasatospora putterlickiae*. This bacterium harbours genes required for maytansinoid biosynthesis.

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Gene expression profile in TNF-α stimulated 293 cells after treatment with parthenolide

P 055

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Sesquiterpene lactones are known for their anti-inflammatory activity, which has been proven in various assays on DNA, mRNA and protein level (1-3). Here we report on the change in the gene expression profile in TNF-α stimulated 293 cells after treatment with parthenolide using a cDNA microarray analysis. Twenty-one of 7028 genes were found to be up- and 18 down-regulated. They encode for chemoattractants, immune system proteins, glycoproteins, metabolism, serine proteinases, and transcription factors. New targets were identified for SLs beyond the NF-κB pathway. Confirmatory analyses were carried out using quantitative real-time RT-PCR (TaqMan®).

Acknowledgments: We thank Marinella Klein (Renal Division, Department of Medicine, University Hospital Freiburg, Germany) for technical assistance with the microarray analysis and Daniel Faller from the Freiburg Center for Data Analysis and Statistical Modeling for statistical analysis of the microarray data.
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P 056 Molecular cloning and expression of a recombinant 3β-hydroxysteriod dehydrogenase from *Digitalis lanata* Ehrh.

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Digitalis lanata Ehrh. is an important medical plant and the cardiac glycosides (cardenolides), mainly synthesised and accumulated in the rosette leaves (1), are used in the treatment of cardiac insufficiency in man. The biosynthesis of cardenolides has been studied extensively, whereas molecular data are available only for a few cardenolide biosynthesis specific enzymes and their corresponding genes, such as cardenolide-16'-O-glucohydrolase (2) or lanatoside-15'-O-acetylesterase (3). We have isolated a full-length cDNA clone from *Digitalis lanata* leaves that encodes a 3β-hydroxysteroid dehydrogenase (3β-HSD), showing high homology with the deduced and directly determined amino acid sequence of 3β-HSD, reported by Finsterbusch et al. (4). The reading frame of 3β-HSD is 780 nucleotides corresponding to 260 amino acids. In *Digitalis* plants the transformation of pregnenolone to progesterone, which is a precursor in cardenolide biosynthetic pathway, is catalysed by 3β-HSD. Overexpression was achieved using the pQE vector-system. A Sph1/Kpn1 fragment was cloned into the pQE-30 vector and transformed into the bacterial host strain M15[pREP4]. The recombinant His-tagged gene product was purified under native conditions on a Ni-nitrilotriacetic acid (Ni-NTA) matrix and its size was determined by SDS-Page to be about 29 kDa. The purified recombinant protein was enzymatically active, as proven in a standard enzyme assay, using pregnenolone and NAD as a substrate and cosubstrate, respectively. The protein exhibits strong 3β-hydroxysteroid dehydrogenase activity with pregnenolone. The crystallisation of the recombinant 3β-HSD is in progress. Biochemical data will be presented elsewhere (5).

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P 057 Plant propagation through somatic embryogenesis of *Stemona tuberosa* Lour., an Asian antitussive traditional herb

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Stemona tuberosa Lour. (Stemonaceae) has been used as traditional medicinal herb in China and many countries in East Asia. The conventional propagation for supplying the drug market is not economic for commercial production due to several limitations. In the present investigation, a protocol for the large scale production of *Stemona tuberosa* Lour. through somatic embryogenesis and subsequent plant regeneration was established. Compact callus was induced by culturing shoots on semisolid Murashige and Skoog (MS) medium supplemented with 20 μM TDZ. Friable callus developed from compact callus on MS medium supplemented with 5 μM 2,4-D, 5 μM BAP, 5 μM TDZ and 5 μM IBA. Somatic embryos were obtained by subculturing friable callus on MS medium supplemented with 3 μM 2,4-D. Embryo germination and plant formation could be achieved after transfer to solid MS media supplemented with 0-1 μM of auxins (2,4-D or IAA) and 0-3 μM of cytokinins (BAP and kinetin) in the dark for 2 weeks and further 2 weeks under light conditions. Plantlets could be successfully established and grown in the greenhouse.

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The anti-malarial artesunate and the epidermal growth factor receptor tyrosine kinase inhibitor OSI-774 for the combination treatment of glioblastoma cells

P 058

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Question: Artesunate (ART) and OSI-774 each possess profound cytotoxic activity. We studied cellular and molecular determinants of the combination treatment of both drugs in glioblastoma multiforme (GBM) cell lines.
Methods: Growth inhibition and colony forming assays, comparative genomic hybridization, and hierarchical cluster analysis were performed.
Results: Supra-additive cytotoxicity was found in U-87MG cells transduced with deletion mutant epidermal growth factor receptor (*EGFR)*, while additive effects were present in cells transduced with wild-type *EGFR*, kinase-deficient *EGFR*, or mock vectors. Among other non-transduced GBM cell lines, two reacted in a supra-additive and seven in an additive manner. Sub-additive or antagonistic effects were not observed. Genomic gains and losses of genetic material in the non-transduced cell lines were correlated with the IC_{50} values for ART and OSI-774 and subjected to cluster analysis. A certain profile of genomic imbalances predicted drug sensitivity.
Conclusion: The combination treatment of ART and OSI-774 resulted in increased cytotoxicity of cell lines compared to each drug alone. Several genes located at sites of genomic imbalances may serve as candidate resistance genes of GBM cells towards ART and OSI-774.

Seed Transmittance of an Epibiotic Clavicipitalean Fungus Occuring on a Dicotyledoneous Plant (*Ipomoea asarifolia)*

P 059

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Ergoline alkaloids are present in Clavicipitalean fungi and Convolvulaceae (1, 2). Various observations and the disjunct occurence of ergoline alkaloids in nature suggest that fungi are responsible for the occurence of ergoline alkaloids in Convolvulaceae plants (3). Indeed we found a plant associated fungus on *Ipomoea asarifolia* Roem. & Schult. Contruction of phylogenetic trees based on 18S rDNA and ITS sequencing showed that this fungus belongs to the family Clavicipitaceae. This fungus is seed transmitted as is common among Clavicipitalean fungi living on Poaceae plants. Our data suggest that ergoline alkaloids occurring in Convolvulaceae plants are generated by a plant associated Clavicipitalean fungus.

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P 060 The possible role of fungi in the accumulation of ergoline alkaloids in *Ipomoea asarifolia* (Convolvulaceae)

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Ergoline alkaloids are constituents of Clavicipitaceous fungi living on Poaceae plants. These ergoline alkaloids are also present in various Convolvulaceae plants including *Ipomoea asarifolia* Roem. et Schult. Currently, there are three hypotheses for the existence of these natural products in unrelated taxa. Firstly, during evolution genes for the biosynthesis of alkaloids may have been transferred from one taxon to another taxon in a horizontal (lateral) gene transfer (1). Secondly, this biosynthetic pathway may have been repeatedly invented. Thirdly, the alkaloids could be synthesized by an associated fungus.
Here we report that the treatment of *Ipomoae* plants with fungicides results in concomitant elimination of ergoline alkaloids and epiphytic fungi associated with the secretory glands on the upper leaf surface (2).
From these epiphytic fungi we could amplify segments of a putative dimethylallyltryptophan synthase (DMATS) gene, which encoded the first pathway-specific enzyme of ergot alkaloid biosynthesis.
These results support the suggestion that the occurrence of ergoline alkaloids depends on the presence of a plant associated fungus.

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Cytotoxicity of kava extracts and kavalactones in primary cultured rat hepatocytes and human HepG2 cells

P 061

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Kava (*Piper methysticum*) and kavalactones were recently suspected to be the cause of drug induced hepatitis. Our in-vitro screening was aimed on the detection of cytotoxic effects of differently produced kava extracts and isolated kavalactones in liver cells.

Methods: Kava root powder, an ethanolic full extract, an acetonic special extract and the six major kavalactones (kavain, dihydrokavain, methysticin, dihydromethysticin, yangonin and desmethoxyyangonin) were tested in primary cultured rat hepatocytes and human HepG2 cells using cytotoxicity assays including the MTT test.

Results: The tested kava extracts were essentially non-toxic in the relevant dosage range. Kavalactones showed a differential behaviour with concentration-dependent toxicity only detectable in primary cultured rat hepatocytes, but not in human HepG2 cells (EC_{50} >200 µg/ml). In rat hepatocytes, only kavain and methysticin (EC_{50} 45 respectively 63 µg/ml) displayed noteworthy effects.

Conclusions: In this *in vitro* model, neither the tested kava extracts nor the individual kavalactones displayed relevant liver cell toxicity. The results argue against the suspicion of potentially severe hepatotoxicity of kava products.

Cytotoxic activity and Comparative Molecular Field Analysis of cycloartanes of *Parthenium argentatum*

P 062

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Argentatin B, a cycloartane triterpene, is a component of the resin of *Parthenium argentatum* Gray, species intensively studied as a renewable native source of natural rubber (1). Recently, we have reported that argentatin B inhibits the cell growth of several cancer human cell lines. On human lymphocyte it showed cytostatic activity without genotoxic effects (2). As a strategy to elucidate structural requirements involved in the inhibitory activity of argentatin B, now we report the inhibitory activity of a series of argentatin B derivatives on K562 cell line as well as a Comparative Molecular Field Analysis (CoMFA) of these derivatives.

According with our results, a low electronic density on both ring A and hydroxyisopropyl moiety, as well as a bulk moiety at C-2, enhance the inhibitory activity of argentatin B on K562 cell line.

Acknowledgements: Partial financial support from DGAPA (IN-224802) is acknowledged.

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P 063 *In vitro* cytotoxicity against human cancer cells of plant called Hua-Khao-Yen

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Interviews of the selected traditional doctors in Southern Thailand revealed that they used Hua-Khao-Yen as the ingredients in their remedies for cancer which was accounted for about sixty percent of the list of herbal drugs used for cancer treatment (1). From the intensive interview with 23 experienced Thai traditional doctors and excursions into the forest with them to collect Hua-Khao-Yen, it was found that Hua-Khao-Yen were the rhizomes of 5 species, i.e. *Dioscorea membranacea* Pierre (Dioscoreaceae), *D. burmanica* Prain ex Burkill, *Smilax corbularia* Kunth (Smilacaceae), *S. glabra* Roxb. and *Pygmeopremna herbacea* Prain et Burkill (Verbenaceae). The extraction procedures used to prepare water and ethanolic extracts of the 5 plants (Hua-Khao-Yen) in the present study were similar to those practiced by Thai traditional doctors. Two extracts from each plant were tested against cervical cancer cell lines (Hela cell) and liver cancer cell lines (HepG2) by SRB assay (2) and the cytotoxic plants were tested mechanism of cell death (apoptosis) using with tunnel assay. The ethanolic extract of *D. membranacea* and *D. birmanica* showed cytotoxic activity against hela cell (IC_{50}= 29.2 and 34.2 μg/ml respectively) and HepG2 (IC_{50} = 16.2 and 33.3μg/ml respectively). The ratio of apoptosis of the ethanolic extract of *D. membranacea* against hela cell at concentration 10 and 50 μg/ml were 4.1% and 6.8% respectively , *D. birmanica* extracts were 3.9 and 6.5% respectively. The water extracts of all plants exhibited no cytotoxic activity against Hela and HepG2. These results can supported using these plants for cancer treatment.

Acknowledgement Prince of Songkhla University for the financial support

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P 064 Sesquiterpenes from *Zinowiewia costaricensis* as Chemosensitizing Agents of the Multidrug Resistance Phenotype in Cancer

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In an intensive study of South American medicinal plants, herein we report the isolation, structure elucidation and biological activity of eleven new and three known dihydro-β-agarofuran sesquiterpenes from the leaves of *Zinowiewia costaricensis*. Their structures were determined by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear and heteronuclear correlation experiments. The absolute configurations of the new compounds were determined by CD studies, chemical correlations or biogenetic grounds. All compounds have been tested on human MDR1-transfected NIH-3T3 cells, in order to determine their ability to revert the multidrug resistance phenotype due to P-glycoprotein overexpression. Six compounds from this series showed similar effectiveness to the classical P-glycoprotein modulator verapamil when reversing resistance to daunorubicin, but it is up to sixteen times greater than that of verapamil when reversing resistance to vinblastine.

Acknowledgements: Spanish Grants SAF-2003-04200-CO2-01, and BQU2003-09558-CO2-01. C. R. M. also thanks the Banco Santander for financial support.

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Sesquiterpenes from *Maytenus canariensis* and their Inhibitory Effects on Epstein-Barr Virus Early Antigen Activation in Raji Cells

P 065

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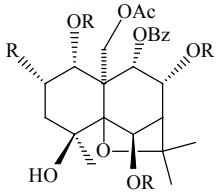
In a continuation of our work on Celastraceae species (1), we report herein on the isolation of eight sesquiterpenoids with a dihydro-ß-agarofuran skeleton. Their structures were elucidated on the basis of spectral analysis, inclunding homonuclear and heteronuclear correlation NMR experiments.



The compounds have been tested for their antitumour-promoting effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA), as a test for potential cancer chemopreventive activity (2). Five of them showed strong inhibitory activities in this assay.

Acknowledgements: Gobierno Autónomo de Canarias-IUBO for a fellowship. DGES (BQU2003-09558-C02-01) project for financial support.

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Chemical Composition and Cytotoxic Activity of the Essential Oils of five *Eryngium* species from Crete

P 066

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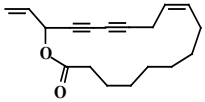

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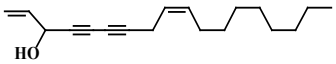

2

Volatile oils of different plant families have demonstrated an activity against tumor cells (1, 2). In the course of our research attempting to identify biological active constituents of oils obtained from Greek plant species, we decided to investigate the essential oils of five species of the genus *Eryngium* growing in the island of Crete. The essential oils were obtained from basal leaves and the stems with inflorescence by hydrostillation and analyzed by GC and GC-MS. Falcarinol, muurol-9-en-15-al, germacrene-D, β-selinene and valencene were some of the major constituents of the oils. In addition, the new natural product campestrone [1] was isolated from oil of *E. campestre*. All essential oils exhibited considerable cytotoxicity against leukaemia cells (HL-60) with IC_{50}s ranging from 0.11 μg/ml up to 5 μg/ml. Falcarinol [2] is mainly responsible for the cytotoxic activities of the oils.

References: 1. Saenz, M.T. et al. (1996) Il Farmaco 51: 539-540. 2. Forey, L. et al. (1999) J. Essent. Oil Res. 11: 522-526.

P 067 In vitro cytotoxicity of extracts from Hungarian Asteraceae

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Aqueous and organic extracts of plants of Asteraceae were screened for cytotoxic activity against human cell lines, by MTT assay. Plants were collected on several distinct of Hungary, in their flowering period. The extracts were prepared with methanol using ultrasonic bad, and were participated against n-hexane and chloroform. The residual plant materials were dried and extracted with boiling water. Several organs of fifty species, 400 extracts in total were examined against three human cell lines, HeLa (cervix epithelial adenocarcinoma), A431 (skin epidermoid carcinoma) and MCF7 (breast epithelial adenocarcinoma). Out of all investigated fractions, 41 exhibited cytotoxic activity against HeLa, 38 against MFC7, and 21 against A431. Some of them were active in all of three cell lines, while some of them showed specific activity against one and other cell lines. The 25 species showed activity belong to six tribes of the family. 40 chloroform soluble parts of the methanolic extracts demonstrated considerable inhibition on the proliferation of the tumor cell lines. Hexane fractions of 14 plants showed only moderate activity. In this experiment water-methanol fractions of the methanolic extracts and aqueous extracts did not exhibited cytotoxic activity. Species showed considerable activity are currently under further investigation with the objective of isolation and identification of the active principle responsible for the activity.

Acknowledgement: The authors thank to Tamás Rédei (Institute of Ecology and Botany of the Hungarian Academy of Sciences) for the identification and collection of plant material.

P 068 Constituents and antiproliferative activity of the Malaysian plant *Hydnophytum formicarium*

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Hydnophytum formicarium Jack (Rubiaceae) is an epiphyte plant native to Malaysia and Indonesia. This plant displays an interesting symbiotic relationship with ants, and serves as home of ant colonies (1). Chemical and pharmacological investigations have not been reported so far on H. formicarium.

In this study we describe the isolation and structure elucidation of compounds from the tuber of H. formicarium and assay of the antiproliferative activity of its extracts and compounds. The plant material was extracted with MeOH, H_2O and petroleum ether, and the extracts were tested on various human cancer cell lines (MCF-7, Caov-3 and NCIH-23). The results demonstrated that the MeOH extract inhibit the proliferation of MCF-7 and Caov-3 cells, so this extract was fractionated by guidance of antiproliferative assay. From the active fractions 7,3',5'-trihydroxyflavanone, 4-hydroxysalicylic acid methyl ester, resorcine, 4-hydroxybenzoic acid methyl ester, β-sitosterine and stigmasterine were isolated by means of CC, VLC, PLC and RP-HPLC.

The isolated compounds showed cell growth inhibitory activity except of the 4-hydroxybenzoic acid methyl ester. The most active compound was 7,3',5'-trihydroxyflavanone with $IC_{50} = 8.5$ μg/ml (MCF-7). Not all the tested compounds show cytotoxic effect against normal cell lines (MDBK, Chang Liver and Vero)..

Acknowledgement: The authors thank the financial support of Szeged Foundation for Cancer Research (Szeged, Hungary), and the Human Resources and Administration Department, University Sains Malaysia (Malaysia).

References: 1. Plummer, N. (2000) Cactus and Succulent Journal 72: 142–147

Effect of terpenic and phenolic compounds on apoptosis induction on mouse lymphoma cells

P 069

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Apoptosis, or programmed cell death, is an essential physiologic process that plays a key role in tissue and organ development during embryogenesis as well as in adult tissues during cell turn-over and in the immune response. However, apoptosis is also involved in a wide range of pathologic conditions namely cancer. Therefore, in recent years, the induction of apoptosis has become an approach for the discovery of antitumor drugs. In fact, it has been demonstrated that apoptosis plays a central role in the cell death induced by some chemotherapeutic agents and may be the primary mechanism for their anti-cancer activity. Thereby, the deregulation of the apoptotic pathway can increase drug resistance in cells and may be an important factor for the ineffectiveness of anticancer therapy. The stages of apoptosis include a reduction in cell volume, chromatin condensation and activation of caspase enzyme cascade which leads to fragmentation of DNA, resulting in the production of apoptotic bodies. Apoptosis is also accompanied by a loss of membrane phospholipid asymmetry, resulting in the exposure of phosphatidylserine at the surface of the cell due to translocation from the inner membrane. Expression of phosphatidylserine at the cell surface plays an important role in the recognition and removal of apoptotic cells by macrophages. Because apoptosis is a major modality by which tumor cells can be eliminated, new drugs to modulate the expression of molecules involved in the apoptotic pathway and to induce apoptosis also in multidrug resistant tumor cell lines are of great importance in cancer chemotherapy (1). In this study we have investigated the ability of fourteen terpenic and five phenolic compounds isolated from *Euphorbia lagascae* to induce apoptosis on multidrug resistant (MDR1) L5718 mouse lymphoma cell line. For evaluation of apoptosis, the cells were stained with fluorescein isothiocyanate (FITC)-labeled annexin-V and propidium iodide and the effect was measured by flow cytometry (2). 12H-benzo(α)-phenothiazine was used as a positive control as apoptosis inducer. Five of the tested compounds can be considered as moderate inducers of apoptosis as compared with the positive control.

Acknowledgements: The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant.
References: 1. Simoni, D. et al (2005) J. Med. Chem. 48: 723-36. **2.** Koopmann, G. et al (1994) Blood 84: 1415-20

Evaluation of three new macrocyclic lathyrane diterpenes from *Euphorbia lagascae* on the reversal of multidrug resistance on mouse lymphoma cells

P 070

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Multidrug resistance (MDR) is considered to be one of the major difficulty in cancer treatment. This phenomenon is characterized by cross-resistance to many different anticancer drugs which do not share a common chemical structure, pharmacological target or metabolization pathway. MDR is often associated with the overexpression of P-glycoprotein (Pgp), that acts as an energy dependent efflux pump and can prevent the accumulation of drugs by expelling them from the cell membrane before they are able to interact with their cellular targets. A promising strategy to go beyond drug resistance is to develop MDR modulators (chemosensitizers) that can inhibit Pgp. These compounds could be either obtained from natural products or by chemical synthesis (1, 2). The aim of the present study was to evaluate the ability of three new macrocyclic lathyrane diterpenes named latilagascenes A-C, and two known policyclic diterpenes to reverse MDR on mouse lymphoma cells. These compounds were isolated from the methanolic extract of *Euphorbia lagascae* by chromatographic methods, and their structures were deduced from their physical and spectral data (IR, MS, 1D NMR, ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments). MDR reversion was investigated in a rhodamine 123 exclusion test using L5178 mouse lymphoma cell line transfected with pHa MDR1/A retrovirus, and verapamil was applied as positive control. Latilagascenes were shown to enhance drug retention by inhibiting the efflux pump activity mediated by P-glycoprotein, two of them being much more active than the positive control in a dose dependent form. The polycyclic diterpenes were found to be inactive. These results indicate that macrocyclic lathyrane diterpenes can be considered as very promising lead compounds for the reversal of multidrug resistance.

Acknowledgements: The authors thank Dr. Teresa Vasconcelos (ISA, University of Lisbon, Portugal) for identification of the plant.
References: 1. Hendrich, A. et al (2003) Bioch Biophys Res Com 304: 260-65. **2.** Fu, L. et al (2001) Anticancer Res 21: 2273-80

P 071 Differential inducibility of CYP1A mRNA expression by green tea extract in two intestinal cell lines

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Green tea, one of the most popular beverages worldwide, has gained increasing scientific interest over the past decade due to possible beneficial/protective effects on life-style related diseases. Beside antioxidative activities of green tea, focus has been laid on its anticarcinogenic effects. Accordingly, protection against polyaromatic hydrocarbon (PAH)-induced cancers by green tea has been demonstrated in different animal models. The CYP1A enzymes are responsible for the metabolism of various chemical carcinogens, including PAHs, found in the environment and diet. Therefore, the CYP1A enzymes represent a first line of defense in the gut.
We investigated the influence of the green tea extract EFLA®942 (GTE) on the mRNA expression levels of CYP1A isoenzymes in the human gastrointestinal cell lines Caco-2 and LS-180. Therefore, mRNA expression levels of CYP1A1 or CYP1A2 were determined by quantitative RT-PCR.
In LS-180 cells GTE, but not EGCG, significantly induced CYP1A2 mRNA expression, whereas neither GTE nor EGCG modulated CYP1A1 mRNA expression. In Caco-2 cells CYP1A1 as well as CYP1A2 mRNA expression was significantly increased in a dose-dependent manner by GTE or EGCG. Our observations suggest differences in the inducibility of CYP1A1 mRNA expression by GTE between the two intestinal cell lines LS-180 and Caco-2.

P 072 The effect of *Cousinia shulabadensis* ethanol extract on the matrix metalloproteinase activity

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Matrix metalloprotainases (MMPs) play an important role in several pathologic processes such as malignancy in which they facilitate invasion and metastasis. Here, in this study, we investigated the cytotoxicity effect of *Cousinia shulabadensis* extract as well as its impact on MMPs activity using a model of cell line (Fibrosarcoma-Wehi164)(1). In the in vitro cytotoxicity assay, cells in the exponential phase of growth were incubated 24h at 37 C, 5% CO2 with a serial dilution of extract. Cell proliferation was evaluated by a modified Crystal Violet colorimetric assay. To assess anti-invasiveness potentials, a modified zymoanalysis method was used to measure MMP-2 and MMP-9 activities in the conditioned-media. A densitometric pattern of zymography was then expressed as percentage of "Relative Activity ".Our data on cytotoxcity analysis shows a direct dose-response fashion with Cousinia shulabadensis; the higher concentration, the higher cytotoxicity. The extract showed a moderate toxicity on the cell line population. In the anti-invasive assay, the extract exerted a sudden decrease on MMPs activity in doses between 20 to40 µg/mL.

References: 1. Saadat F. (2003) PJMS. 19:13-17.

Isolation of Tumour Modulatory Compounds in Chinese Herbal Remedies Through Activity-Guided Fractionation

P 073

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Chinese herbal remedies (CHRs) range from single to multiple herb preparations and the efficacy of these remedies is traditionally attributed to the combined effects of their constituents. It is therefore important to investigate not only the effects of CHRs as a whole but also the effects of their constituents. Our previous studies have shown the CHRs *Oldenlandia diffusa*, *Polygonum multiflorum* and Long Dan Xie Gan Wan to be toxic to the HL60 leukaemic cell line, whilst having little effect on primary blood lymphocytes. Using activity-guided fractionation, the aim of our present study was to isolate compounds within these CHRs that exert a toxic effect on the HL60 cell line. Extracts were split into fractions using HPLC, analysis of the toxicity of fractions was carried out using the trypan blue exclusion assay, and analysis of the acitve fractions was conducted using GC-MS and LC-MS. Results showed a single fraction of *Polygonum multiflorum* exerted levels of toxicity equivalent to that of the CHR in its entirity, killing all HL60 cells after 48 hours of exposure, indicating that a single compound within *Polygonum multiflorum* may be responsible for its anticancer activity on the HL60 cell line *in vitro*. For *Oldenlandia diffusa* and Long Dan Xie Gan wan multiple fractions exhibited significant toxic effects on the HL60 cell line, however no single fraction exhibited levels of toxicity equivalent to the CHRs in their entirity, thus indicating that multiple compounds may be responsible for the anticancer activities of both *Oldenlandia diffusa* and Long Dan Xie Gan Wan *in vitro*.

Apoptotic activity of flavonoids isolated from *Astragalus verrucosus* Moris

P 074

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Astragalus verrucosus Moris is a very rare perennial plant, belonging to *Fabaceae* family, widespread in Italy only in a restricted area of South-western Sardinia (**1**). Genus *Astragalus* is well-known in Chinese folk medicine, where its roots were used for increasing resistance against viral infections, for re-balancing immunologic system as well as for its liver tonic action (**2**). In this work a screening on the methanolic extracts were performed by LC-DAD-ESI-MS. Seven flavonoids (apigenin, apigenin 7-O-β-D-(6*p*-cumaroil)-glucoside, quercetin, rutin, daidzein, genistein and genistin) were isolated and identified by NMR and MS techniques. The potential cytotoxic activities of these compounds were evaluated on two tumor cell lines: HCT116 (human colon cancer) and MCF7 (human breast cancer). Daidzein and genistein were not tested on this cellular lines before. They showed apoptotic activity, at high concentrations, on both tumor cell lines. Genistin and apigenin 7-O-β-D-(6*p*-cumaroil)-glucoside didn't show any significant apoptotic effects. The tested glycosidic flavonoids showed a lower activity in comparison with their corrispondent aglycons.

References: 1.Pignatti, S. (1982) Flora d'Italia. Ed. Agricole. Italy. **2**.Pistelli, L. (2002) Studies in Natural Products Chemistry. Elsevier. Amsterdam.

P 075 Chemopreventive activity of natural compounds on Nitric Oxide donors induced Mouse Skin Carcino genesis

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The present study was carried out to examine the chemopreventive activity of natural compounds(antioxidants) polyphenols, curcumin and Tabebuia avellandae ext. on the nitric oxide(NO) donors induced carcinogenesis.
We previously reported that antioxidants show the scavenging activity against NO generation in cultured cells system. On the fundamental such results, recent studies have demonstrated that these antioxidants were observed the inhibitory effect against peroxynitrite(PN), NO donors induced tumor initiating activity using mouse skin. Female SENCAR mouse (6 weeks of age) were treated topically with single dose of PN solution,
followed by TPA twice a weekly for 20 weeks. Tumor incidence were 100% with 5 to 6 per mouse at end of experiment. Antioxidants were orally fed with drinking water for only 2 weeks, before and after initiation and following promoting treatment with drinking water only. In our observation, antioxidants treated group cause about 60 % reduction in the average number of tumors per mouse after 20 weeks of experiment, respectively. Western immunoblot analysis of cell signaling protein showed that H-Ras and MEK expression in mouse skin were markedly increased by PN, NO donor and these protein levels were slightly decreased by antioxidants treatment. These results were in agreement with inhibitory effects observed in parallel studies
with SENCAR mouse. These data suggested that natural compounds are promising candidates as chemopreventive agents for infectious and inflammatory induced carcinogenesis.

P 076 Chemopreventive activity of naturally occurring Compounds against advanced Glycation endproducts induced Carcinogenesis

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Advanced glycation end products(AGE) is generally recognized as several complications including cancer and incresed the volume in diabetes mellitus patients. In course of our human related carcinogens studies for cancer chemoprevention, we were investigated the AGE(HSA and glucose mixture) sample for carcinogenic activity and were found the significant tumor initiating potency on two-stage mouse skin test. SENCAR mice were initiated with single dose of 100 µg AGE and promoted with 1µg TPA twice a week for 20 weeks. Recently, we also found some naturally occurring compounds (EPA, ursolic acid and betanin etc.) showed the inhibitory effect against AGE induced carcinogenesis. For this study, from 1 week before to 1 week after initiation treatment by anti-oxidants, they delayed the formation of papillomas and reduced the number of papillomas per mouse.These findings are important for the interpretation of intervention studies of anti-oxidants in rodent and for the design of clinical.

Anacardic acids and cardanols from the bark of *Amphipterygium adstringens* Schiede ex Schlecth.

P 077

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The bark of *Amphipterygium adstringens*, commonly known as cuachalalate, is widely used in Mexico for the treatment of cholelithiasis, fevers, gastritis, gastric ulcer and cancer of gastrointestinal tract (1). Now we wish to report the isolation of 6-nonadecyl and 6-heneicosenyl salicylic acids, 3-nonadecylphenol and phenol ester from the n-hexane extract of *A. adstringens*. The 6-nonadecyl salicylic acid and the 3-nonadecylphenol exhibit cytotoxic activity against HCT-15 (colon), U251 (CNS), K-562 (leukemia) and PC-3 (prostate) human cancer cell lines. Previous investigations showed that the long chain phenols such cardanols were identified as the antitumor principles of *Ginkgo biloba* (2), these alkyl aromatic compounds exhibitive inhibitory activities against phosphatylinositol-specific phospholipase C, an enzyme associated with tumor progression (3). Although the mode of action of the alkyl aromatic compounds present in *A. adstringens* need to be elucidate. The cytotoxic compounds present in cuachalalate could be valid the use of this species as anticancer remedy in the Mexican traditional medicine.

References: 1. Olivera-Ortega, A. et al. 1999. J. Ethnopharmacol. 68: 109-113. **2.** Itokawa, H. et al. 1987. Chem. Pharm. Bull. 35:3016-20. **3.** Lee, J. et al. 1998. J. Nat. Prod. 61:867-871.

Cytotoxic activity of , 3β,6α-diol-cholest-8-ene, 14α-methyl-3β,6α-diol-cholest-8-ene and 3β, 16β, 22α, 28-tetrol-olean-12-ene

P 078

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Previous studies indicated that some sterols and triterpenes isolated from plants showed cytotoxic activities (1). The aim of this work is to evaluated the cytotoxic effects of 3β,6α-diol-cholest-8-ene (**1**), 14α-methyl-3β,6α-diol-cholest-8-ene (**2**) and 3β, 16β, 22 α, 28-tetrol-olean-12-ene (**3**), all isolated from the medicinal plant *M. geometrizans* (Cactaceae) on the HCT-15 (colon), MCF-7 (breast), U-250 (CNS), PC-3 (prostate) and K-256 (leukemia) human tumor cell lines and MT-2 (normal lymphocytes). The evaluation was carried out using the SRB assay (2). The results showed that, **1** and **2** showed potent cytotoxic activity against all cell lines at 50 μM, while their acetylated derivates were inactive. On the other hand, **3** was inactive in all the cell lines tested. However, its tetra-acetate derivative (**4**) showed potent cytotoxic activity against U-251, K-562 and MCF-7 tumor cell lines. Although **1** and **2** were toxic to normal lymphocytes, **4** did not showed cytotoxic activity against this cell line. This result suggest a different susceptibility of the cell lines tested to **1**, **2** and **4**.

References: 1. Akihisa T., Yasukawa K. (2001) Studies in Natural Products Chemistry. Elsevier Science B. V. U.K . **2**. Monks A., et al. (1991) J. Nat. Cancer Inst. 83: 757-766.

P 079 Bioactive Lanostanes and Friedolanostanes from the bark of *Garcinia speciosa*

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The chloroform extract of the bark of *Garcinia speciosa* Wall. collected from Southern Thailand, furnished four 17, 14-friedolanostanes and five lanostanes. The friedolanostanes were the previously known methyl ester of (24*E*)-3α, 23α-dihydroxy-17, 14-friedolanostan-8, 14, 24-trien-26-oic acid and the methyl esters of three hitherto unknown acids, 3α-hydroxy-16α, 23α-epoxy-17, 14-friedolanostan-8, 14, 24-trien-26-oic acid, 3α, 23α-dihydroxy-8α, 9α-epoxy-17, 14-friedolanostan-15-oxo-24-en-26-oic acid and 3α, 23α-dihydroxy-17, 14-friedolanostane-15-oxo-8(14), 24-dien-26-oic acid. New lanostanes were the methyl ester of 3β-hydroxy-23-oxo-9, 16-lanostadien-26-oic acid and 3β, 9α-dihydroxylanost-24-en-26-al. The structures of the compounds were established by ^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, NOESY and HRMS. In the case of the lanostanes the previously unassigned C-25 stereochemistry (1) was shown to be 25*R* by X-ray analysis. The configuration at C-23 of the friedolanostanes was established as 23 *R*. The compounds were evaluated for their effect on the *in vitro* growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) and were found to be moderately active on the three cancer cell lines.

Acknowledgements: FCT (Unidade de I&D nº 226/94), FEDER, POCTI (QCA III), NCI (USA.)

Reference: 1. Rukachaisirikul, V. et al. (2000) Phytochemistry 55 : 1775.

P 080 The Cytotoxicity of Naphthazarine Derivatives from *Onosma arenaria*

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The cytotoxicity of two isolated compounds from *Onosma arenaria* was studied. The MTT test (1) was used to evaluate the cytotoxicity on leukemia K562 cells and non-malignant periferal blood mononuclear cells (PBMC), while the KBR test (2) was used to test the cytotoxicity against HeLa cells.

Plant material was collected on mountain Piperi (Podgorica) in July 2002. The powdered, air dried roots were extracted on room temperature with C_6H_{12}, CH_2Cl_2 and MeOH. PTLC of cyclohexane extract led to isolation of two compounds which were identified on the bases of their NMR spectral data as β-hydroxyisovalerylalkannin (**1**) and acetylalkannin (**2**).

Both tested compounds exhibited a pronounced cytotoxic activity against HeLa (**1:** IC_{50}=2.30±1.01 μM; **2:** IC_{50}=0.55±0.16 μM) and K562 cells (**1:** IC_{30}=0.61±0.36 μM; **2:** IC_{50}=0.34±0.07 μM). Also, there was not the selectivity in toxicity of tested compounds against malignant and healthy cell lines (PBMC) before and after the activation with phytohaemaglutinin (-/+PHA). Compound **1** was highly cytotoxic against PBMC with an IC_{50}=2.12±0.83 μM (-PHA) and IC_{50}=2.19±0.36 μM (+PHA), as well compound **2** (IC_{50}=0.39±0.68 μM,-PHA; IC_{50}=1.55±0.17 μM, + PHA).

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The Use of Shiitake Mushroom polysaccharides in Cancer Treatment

P 081

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The number of mushrooms on Earth is estimated at 140,000 yet may be only 10 % are known. Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. However the components responsible for such action have not been clearly defined. One of the most popular mushrooms in the world is the Shiitake mushroom. In the present study *Lentinus edodes (Shiitake)* mycelia were grown in submerged culture and the polysaccharides were extracted from culture broth by precipitation with ethanol. The structure of polysaccharide was elucidated using NMR spectra, which indicated that the polysaccharide is highly branched glucan containing mainly 1,3 and 1,6 linkage. These results are in agreement with that reported by Mizuno (2000). The results showed that the polysaccharides possess anticancer activity against human esophageal cancer cell line. The results also showed that the polysaccharides enhance the immunoresponses of the human body thereby increasing resistance to cancer disease. Mushroom polysaccharides consider to be immunopotentiators or biological response modifiers. Mycelia formed by growing pure cultures in submerged conditions are of constant composition, and submerged culture is the best technique for obtaining consistent and safe mushroom products.

Fig (1). Cytotoxic activity of polysaccharide against esophageal cancer cell line.

Acknowledgements: National Research Center, University of Cape Town, and Prof. Dr Etidal W. Jwanny.
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A novel polyacetylene inhibits angiogenesis and induces apoptosis in human endothelial cells

P 082

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Preclinical studies have shown that anti-angiogenic therapy, targeting tumor endothelium, offers great promise for the treatment of cancer. In the present study we identified a novel polyacetylene compound, namely 1,2-dihydroxy-5(*E*)-tridecene-7,9,11-triyne, from a folk medicine *Bidens pilosa* Linn. (*Compositae*). The possible molecular mechanism underlying the anti-angiogenic bioactivites of the new polyacetylene was investigated using various biochemical and cellular bioassays in primary human umbilical vein endothelium cells (HUVEC). We demonstrate here that 1,2-dihydroxy-5(*E*)-tridecene-7,9,11-triyne (at 2.5 μg/ml) indeed possesses significant anti-angiogenic effect manifested by the inhibition of HUVECs proliferation, migration, and their ability to form tube-like structures in collagen gel. Moreover, the compound at a dose of 10 μg/ml was able to induce HUVECs to undergo apoptosis via attenuation of a number of cell cycle mediators (*i.e.*, p21(Cip1), P27(Kip), and cyclin A), activation of caspase 7 and cleavage of poly(ADP-ribose) polymerase (PARP). This study suggests that the novel polyacetylene compound may have the potential to be developed as an anti-cancer agent through its anti-angiogenic and/or apoptotic bioactivities.

P 083 Cytotoxic Activity of Lupane Type Triterpenes from *Glochidion sphaerogynum* and *Glochidion eriocarpum*

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The lupane type triterpenes lupenone (**1**), 3-epilupeol (**2**), lup-20(29)-en-3,23-diol (**3**), glochidone (**4**), glochidonol (**5**), glochidiol (**6**) and lup-20(29)-en-1,3-diol (**7**) were isolated from the roots and stem wood of *Glochidion sphaerogynum* and *Glochidion eriocarpum*. Triterpenes **2**-**7** were evaluated for their effect on the *in vitro* growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) using the protein-binding sulforhodamine B method (1). Lupanes **3**, **5**, **6** exhibited strong inhibitory effect against all three cell lines while 3-epilupeol (**2**) was moderately active. The triterpene **7** was also moderately active in MCF-7 but devoided of activity in both NCI-H460 and SF-268 cell lines. Surprisingly, glochidone (**4**) was found to be inactive in the three cell lines tested. Using the TUNEL assay to evaluate the capacity of thesetriterpenes to induce apoptosis in the MCF-7 cells, it was found that glochidonol (**5**) and glochidiol (**6**) exerted their antiproliferative activity through the involvement of apoptosis while triterpene **3** did not.

Acknowledgements: FCT (Unidade de I&D nº 226/94), FEDER, POCTI (QCA III), Khon Kaen University, NCI (USA).

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P 084 Cytotoxic activity of phenolic triterpenoids from *Celastraceae*. Structure- Activity Relationship

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In our search for anticancer agents from natural sources, we have assayed the cytotoxic activity of 16 phenols, eight of which were isolated from different *Maytenus* species. HeLa (human carcinoma of the cervix), Hep-2 (human carcinoma of larynx) and Vero (african green monkey kidney) cell lines were used to evaluate the cytotoxic activity of these compounds.

Most of the compounds showed some grade of cytotoxicity, at log and lag phase of growth cycle, ($IC_{50} < 20$ µgr/ml) against the two cancer cell lines. Five phenols (31 %) were active against HeLa cells and three (19 %) against Hep-2 cells ($IC_{50} \leq 5$ µgr/ml). Among these compounds Pristimerol showed the strongest activity against HeLa (IC_{50}=0.84 µgr/ml) and Hep-2 cells (IC_{50}=2.2 µgr/ml).

A structure cytotoxic relationship is also reported here and, as a summary, we can conclude that phenolic triterpenes with unconjugated double bond in B ring is important for the activity.

Structure/activity relationship of isoquinoline alkaloids against HeLa tumour cell line and *Pseudomonas aeruginosa*

P 085

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Berberine, coptisine, palmatine, chelerythrine and sanguinarine are isoquinoline alkaloids responsible for the activity of plants traditionally used for their medicinal properties, such as *Chelidonium majus*, *Enanthia chlorantha*, *Berberis aristata* and *Sanguinaria canadensis* (1,2). In the present work an attempt was made to correlate structural characteristics of these alkaloids with their biological activity. Cytotoxicity against tumour cells (HeLa, American Type Culture Collection) was assessed using the colorimetric MTT reduction assay. All the alkaloids tested inhibited the growth of the tumour cells, but to a different extent. EC_{50} values were 35.5, 24.0 and 20.8 μg/ml for palmatine, berberine and chelerythrine, respectively, whereas the values for coptisine and sanguinarine were < 2.5 and 2.1 μg/ml. This difference in activity correlated with the structural difference between both palmatine/berberine and coptisine, and between chelerythrine and sanguinarine: the dramatic increase in cytotoxic effect is associated with the existence of two closed rings adjacent to rings A and D in the alkaloid structure (in coptisine and sanguinarine); cytotoxic effect of the alkaloids with two (berberine) or four methoxy groups (palmatine) was significantly lower. The increase in cytotoxicity was also correlated with an increase in octanol/water partition coefficient logP. Some of the cytotoxicity may be due to the inhibition of mitochondrial NADH and succinate dehydrogenase, although this does not explain the differences found between the alkaloids with and without methoxy groups, which had a similar inhibitory effect on these enzymes (3). Activity against *Pseudomonas aeruginosa* followed a different pattern more difficult to explain based on structural features.

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Comet assay and apoptosis induction by three methanolic plant extracts

P 086

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Three Peruvian plants with ethnobotanical interest (1, 2, 3) were selected for a study of their ability to induce tumor cell dead. Extracts of *Gentianella alborosea* (Gilg) Fabris (Gentianaceae), *Polypodium leucotomos* L. (Polypodiaceae), *Uncaria tomentosa* (Willd.) D. C. (Rutaceae), were made. 200 g of each dried and powdered plant was sequentially macerated with ether, chloroform and methanol. Previous studies reveal that methanolic extracts of all plants had the highest cytotoxic activity. Therefore, the apoptosis induction of all methanolic extracts, on HeLa (human uterus tumor cell line) were tested (4). Dried methanol plant extracts was dissolved in phosphate saline buffer (PBS). PBS was used as negative control and doxorrubicin as positive control. After incubation (48 h), the cells were fixed with methanol and stained with hematoxilin-eosin (Sigma kit) for the observation of nuclear morphology. Proportion of apoptotic nuclei was calculated and expressed as percentage from the total nuclei. In order to know the way that extracts induce cell dead the DNA Comet assay was assessed (5). This test could testify if these extracts induce apoptosis damaging DNA. Single cell gel electrophoresis or 'Comet assay' is a rapid and very sensitive fluorescent microscopic method to examine DNA damage and repair at individual cell level. *G. alborosea* induce apoptosis of HeLa cells. However this activity is not related with a severe DNA fragmentation. The comet assay with *Gentianella* extracts was noticeable, no tails were observed in cells incubated with this extract. *Polypodium* extracts did not show apoptotic effect, and also did not affect DNA. At last, *Uncaria* extract did not induce tumor cell dead, even it damage DNA.

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P 087 The apoptotic, anti-angiogenic and immunotropic properties of convallamaroside, the steroidal saponin isolated from rhizomes and roots of *Convallaria majalis* L.

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Saponins exhibit various biological properties, they act as cytotoxics, stimulate the resistance system, inducing apoptosis in tumor cells, and inhibiting tumor - induced angiogenesis. So far, there have been no reports on the biological activity associated with the exposure to saponins of *C. majalis*. The main compound in the group of saponins of rhizomes and roots of *C. majalis*, isolated by using column chromatography (1), is a furostane glycoside having three saccharide chains, which was found to be 1-0-[α-L-rhamnoside-(1→2)-β-D- guinovoside]-3-0-[β-D-glucoside -(1→4)-α-L-rhamnoside] -27-o-[β-D-glucoside]-5β,20β(H)furost-25-en-1β,3β,22α,27-tetraol (2). The aim of this study is to evaluate the effects of the convallamaroside on the neovascular reaction induced in syngeneic or semi-syngeneic mice grafted intradermally with the L-1 sarcoma or L-1210 leukemia mouse cells (TIA test), and also on two parameters of immune response, antibody production (anti-SRBC), and graft-versus -host reaction (LIA test). In the in vitro study using HL-60 cells (promielocytic leukemia) we assessed the ability of convallamaroside to induce apoptosis. Results: Convallamaroside in doses 50 µg and 100 µg showed a significant inhibitory effect on a number of new vessels induced in mice by sarcoma L-1 ($p < 0.001$), and also significantly prevented tumor neovascularization in mice grafted intradermally with L1210 mouse leukemia cells. Convallamaroside stimulated the local graft-versus-host reaction and had no effect on the production of anti-SRBC antibodies in mice. Flow cytometry studies revealed that convallamaroside concentrations above 80µM significantly induce apoptosis of HL-60 cells.

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P 088 Bioactive Triterpenes from the Bark of *Cleistanthus gracilis*

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Cleistanthus (Euphorbiaceae) is a genus of about 140 species, native to Africa and India through to Australia. So far, only *Cleistanthus collinus* Roxb and *C. patulus* have been investigated chemically. Besides the cytotoxic lignans cleistanthins A and B, other lignans such as (+) sesamin, taiwanin, paulownin have also been isolated from the heartwood of *C. collinus* and *C. patulus*. (1, 2)
In the continuation of our search for natural cytotoxic compounds, we have investigated the chemical constituents of *Cleistanthus gracilis*, collected in Thailand. Isolated from the chloroform extract of its stem wood were β-sitosterol (**1**), stigmasterol (**2**), lupeol (**3**), lup-20(29)-en-3α, 23-diol (**4**) as well as 3-oxo-friedelin (**5**). The triterpene **4** was evaluated for its effect on the *in vitro* growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) using the protein-binding sulforhodamine B method and was found to be moderately active in MCF-7 but devoid of activity in both NCI-H460 and SF-268 cell lines.

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Antitumor activity of sphaerophorin and pannarin (lichen metabolites)

P 089

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Cancer is the largest single cause of death in both men and women. Therefore, the research of more effective and less toxic drugs has become necessary. In an ongoing effort to identify new natural anticancer compounds, our laboratory has focused its search on a poorly investigated lichen metabolites, sphaerophorin (depside) and pannarin (depsidone). To this end, we treated DU-145 (androgen-insensitive prostate cancer cells), a cell line resembling the last stage of prostate carcinoma, with different concentrations (6-100 μM) of these compounds for 72 h. Sphaerophorin was isolated from *Sphaerophorus globosus*, and pannarin was isolated from different species of the genus *Psoroma* (*Psoroma reticulatum*, *P. pulchrum*, *P. palladium*). After extraction the lichen compounds were isolated by chromatography using Si gel column, and identified by spectroscopic techniques as previously described (1, 2). Cell viability, by tetrazolium salts assay (MTT) (3) and membrane breakdown, by lactic dehydrogenase (LDH) release (4), were measured. The possible induction of oxidative stress was evidenced by performing a fluorescent analysis of intracellular reactive oxygen species (ROS) production (4). In addition, DNA damage determined by COMET assay was examined (3). Our results clearly indicate that sphaerophorin and pannarin induce a significant cell growth inhibition ($p<0.001$), correlated to membrane breakdown only at higher concentration (100 μM) ($p<0.001$), interfering with ROS production ($p<0.001$). With respect to genomic DNA damage, the results obtained show a significant increase of TDNA and TMOM values when compared with the untreated control ($p<0.001$). These *in vitro* results show for the first time an antiproliferative effect of sphaerophorin and pannarin on human prostate cancer cells, and encourage further investigations on the chemotherapeutic properties of this class of molecules.

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Influence of various herbal preparations on the tamoxifen-induced suppression of breast cancer cell growth in vitro

P 090

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Alternative therapies used for menopause symptom relief include herbal preparations of *Actaea racemosa* L. (= *Cimicifuga racemosa* (L.) Nutt. (CR), *Trifolium pratense* L. (Red clover) and *Glycine max* (L.) Merr. (Soy). Despite the varying degrees of scientific evidence supporting the efficacy of these herbs, there is an increase in use particularly in women with breast cancer experiencing naturally occurring or tamoxifen (Tam) related menopausal symptoms. Considering their demands, it is important to carefully evaluate the potential of these herbal preparations to exert estrogen-agonistic effects on estrogen-dependent breast cancer or to interfere with the antineoplastic action of tamoxifen. In the present study we investigated the effects of commercially available herbal menopause preparations containing Red clover, Soy and CR on the proliferation of estrogen receptor- positive human breast cancer cells (MCF-7) in vitro. The experiments were performed in a low-estrogen environment designed to mimic a postmenopausal state as well as in the presence of Tam and estradiol in order to evaluate Tam interference.
The soy and red clover-containing preparations significantly stimulated MCF-7 cell proliferation and antagonized the Tam-induced suppression of tumor cell growth. The isopropanolic CR-extract (Remifemin®), however, did not enhance but even decreased the proliferation of the breast cancer cells and augmented the Tam effect. Also supporting recent clinical data CR showed the best safety-profile in this study, because it did neither stimulate cancer cell proliferation nor antagonized the tumor-inhibiting effect of tamoxifen.

P 091 Phytodolor® and its components (*Populus tremula*, *Fraxinus excelsior*, and *Solidago virgaurea*) suppress COX-2 and TNFα gene expression in activated human monocytes

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The treatment of rheumatic diseases by Phytodolor® is supported by clinical studies (1). Aim of the present study was to characterize the effect of this herbal fixed combination and that of its components, extracts of *Populus tremula*, *Fraxinus excelsior*, and *Solidago virgaurea*, on pro-inflammatorily activated human monocytes in comparison to standard medications in this indication, such as aspirin, diclofenac and rofecoxib, as reference substances. Monocytes from buffy coats of healthy subjects were pre-incubated for 90 min in serum-free RPMI 1640 medium with the test substances, or one of the reference substances. Thereafter they were activated with interferon-gamma (INF-γ; 50 U/ml; 45 min) and lipopolysaccharide (LPS; 1 µg/ml) for 48 hours. The percentage of apoptotic monocytes and the cyclooxygenase 2 (COX-2) and tumor necrosis factor-alpha (TNFα) gene expressions (RT-PCR and real-time PCR) were measured. The pro-inflammatory activation of monocytes by IFN-gamma/LPS increased their survival rate significantly. Phytodolor® and *Populus tremula* extract inhibited this pro-inflammatory activation by reducing the increased survival rate significantly and concentration dependently, while *Fraxinus excelsior* and *Solidago virgaurea* extracts as well as aspirin, diclofenac or rofecoxib were less effective. Phytodolor® and its components reduced the IFN-gamma/LPS increased gene expressions of COX-2 and tumor necrosis factor-alpha, in part, concentration dependently and significantly similar to aspirin, diclofenac or rofecoxib. In summary, Phytodolor® and its components, extracts of *Populus tremula*, *Fraxinus excelsior*, and *Solidago virgaurea* are suggested to contribute not only to the inhibition of inflammatory processes, but also via this pathomechanism to pain relief in rheumatic diseases.

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P 092 Modulation of cellular protein nitration by phenolics from *Phagnalon*

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The presence of 3-nitrotyrosine (3-NT), an indicator of the formation of reactive nitrogen species, is of interest in some chronic inflammatory and neurodegenerative diseases such as rheumatoid arthritis or multiple sclerosis. There are two main pathways by which tyrosine nitration is produced. The first one is mediated by peroxynitrite ($ONOO^-$), which is formed from the reaction of superoxide and nitric oxide and produces nitration via nitrogen dioxide ($NO_2^{\bullet}$) or a CO_2 adduct. In the second pathway, myeloperoxidase (MPO) produces tyrosine nitration, either via oxidation of nitrite (NO_2^-) in the presence of H_2O_2 to form $NO_2^{\bullet}$ or via the reaction of NO_2^- with the main MPO product, hypochlorous acid (HOCl), to form nitrosyl chloride (NO_2Cl) as a nitrating species (1). In this study we have used the Western blotting technique with a 3-NT antibody to evaluate the effect of a prenylhydroquinone glucoside (**1**) and two dicaffeoylquinic derivatives (**2**,**3**) isolated from *Phagnalon rupestre* (Asteraceae) (2) on both types of protein-bound tyrosine nitration pathways. To do so we have used murine fibroblasts stimulated with $ONOO^-$ itself, and phorbol 12-myristate 13-acetate (PMA)-stimulated human leukocytes, which contain high quantities of MPO. All the compounds tested, as well as epigallocatechin gallate (EGCG), which was used as a reference, are very active in both systems, with an IC_{50} ranging from 5-15 µM. The activity in the fibroblasts may be due to their ability to scavenge $ONOO^-$. For its part, compound **1** undergoes its own nitration, whereas caffeoyl derivates are easily oxidized (3). As 3,5-di-*O*-caffeoylquinic acid methyl ester (**2**) was only a moderate inhibitor of MPO activity (4), this interaction should have only a minor role in the inhibition of tyrosine nitration in PMA-stimulated neutrophils.

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Biflavonoids from *Decussocarpus rospigliosii* as PDE inhibitors

P 093

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In the framework of our research on bioactive biflavonoids (1), we isolated three *3'-8'' biapigenin derivatives* from aerial parts of *Decussocarpus rospigliosii (*Pilg.) De Laub*. (Podocarpaceae) and evaluated their inhibition properties over five cyclic nucleotide phosphodiesterase (*PDE) isozymes (2). The three biflavonoids inhibited PDE1, PDE2 and PDE4 in the micromolar range. Podocarpusflavone A, bearing only one methoxy group in position C4''', showed the greatest selectivity toward PDE2. Podocarpusflavone B, with two methoxy groups (C7,C4''') inhibited preferably PDE2 and PDE4 while its isomer, amentoflavone 7,7''-dimethyl ether (C7,C7''), was a good PDE4 inhibitor but quite inactive toward PDE3 and PDE5.

Biflavonoids	IC_{50} (μM)				
	PDE1	PDE2	PDE3	PDE4	PDE5
podocarpusflavone A	2.2	**0.5**	5.7	6.2	3.1
amentoflavone 7,7''-dimethyl ether	6.6	2.2	> 50	**1.3**	> 50
podocarpusflavone B	3.1	**1.8**	31	**1.4**	7.6

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In vitro Anti-Inflammatory Activity of Lupane Triterpenoids from *Maytenus* species

P 094

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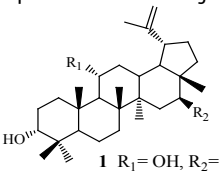
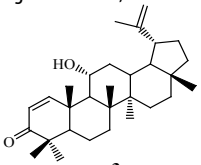
Over the last 30 years or so, a large number of secondary metabolites exhibiting a wide range of bioactivity have been extracted from species of the genus *Maytenus* (1). Lupane triterpenoids are pentacyclic compounds, which are reported to have wide range of biological activities, such as anti-inflammatory activity (2).



1 R_1= OH, R_2= H
2 R_1= H, R_2= OH



3

Three new lupane triterpenes, in addition to sixteen known ones, were isolated from *Maytenus* species. These compounds and four derivatives have been tested for potential anti-inflammatory activity. Some of them exhibited potent inhibitory effects on NO and prostaglandin E_2 production in mouse macrophages.

Acknowledgements: Gobierno de Canarias for a fellowship and DGES (BQU2003-09558-C02-01) project for financial support.
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P 095 Inhibition of cyclooxygenase isoforms by sesquiterpenes from a *Petasites hybridus* rhizome extract

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Recently we found direct inhibition of cyclooxygenase-2 by several commercial *Petasites hybridus* rhizome extracts with different contents of petasins in an *in vitro* assay. (1) By activity-guided fractionation several sesquiterpenes of the eremophilane type could be isolated and identified by NMR. 8β-H-eremophilanolide, 8β-hydroxy-eremophilanolide, 2-Mta-8β-H-eremophilanolide, 2-Ang-8α-H-eremophilanolide, 2-Ang-8β-H-eremophilanolide and a mixture of S-petasin and neo-S-petasin (**1**) were identified as known compounds. 2-Tig-8β-H-eremophilanolide, 8β-H-9β-hydroxy-petasitolide-A, 2-methacroyl-8α-H-eremophilanolide, 2-methacroyl-8β-H-eremophilanolide, 2-Sen-8α-H-eremophilanolide in a mixture with 2-Ang-8α-H-eremophilanolide (**2**) and 2-Tig-8α-H-eremophilanolide (**3**) were identified as new compounds. **1** showed an IC_{50} value of 69.9 µg/ml for COX-1 and 8.7 µg/ml for COX-2. Also **2** and **3** were strong COX-2 inhibitors with IC_{50} values of 13.3 µg/ml and 7.1 µg/ml, respectively. All other compounds neither inhibited COX-1 nor COX-2 at 10 µM or10 µg/ml, respectively. The results show that other compounds than petasins contribute to the anti-inflammatory activity of *Petasites* extracts.

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P 096 Antiinflammatory potential of hyperforin

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In a previous investigation, hyperforin – the major lypophylic constituent present in *Hypericum perforatum* L. (Guttiferae) – was tested as a stable dicyclohexylammonium salt (Hyp-DCHA) for cytotoxicity, inhibition of matrix proteases and of tumor invasion and metastasis, emerging as a promising compound for prevention and inhibition of tumor expansion and metastatic invasion (**1**). Since growing evidence points to chronic inflammation as a risk factor for cancer development (**2**), we have tested the effect of Hyp-DCHA on a series of proteolytic enzymes released mainly by the polymorpho-nuclear leukocytes (PMNs leukocyte elastase (LE), cathespin G (cath.G) and proteinase-3 (PR-3),). Hyp-DCHA inhibited mostly the activity of LE, in a dose-dependent and non-competitive manner, with an IC_{50} of 3 µM and a Ki <5 µM. While Hyp-DCHA could inhibit also cath.G, with an IC_{50} around 20 µM, it was uneffective on PR-3. Hyp-DCHA did not affect PMN's viability up to 10 µM, but at lower concentration was able to hinder their chemotactic (through gelatin) as well as chemoinvasive (through Matrigel) response in the Boyden chamber assay (IC_{50} <1 µM) **(3)**. These results indicate that this lipophilic compound from *Hypericum* remarkably contributes to down-modulation of PMN recruitment at the site of inflammation, and support the use of oily solvents in the traditional medicine for the treatment of mainly cutaneous inflammation. The systemic use of the stable salt (Hyp-DCHA) may also prove useful in preventing and treating other internal inflammatory conditions **(3)**.

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Inhibition of topoisomerase II in the HL-60 human promyelocytic leukemia cell line by plumbagin

P 097

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DNA topoisomerase II is a critical cellular target of several clinically important anticancer agents. Plumbagin, a cytotoxic naphthoquinone present in plants from the Plumbaginaceae and Droseraceae families, has been reported to inhibit the activity of topoisomerase II in cell-free studies (1). The objective of this research was to determine whether plumbagin isolated from Drosera binata inhibits topoisomerase II activity in the HL-60 cell line. The mode of topoisomerase inhibition has previously been demonstrated to be through the stabilization of DNA-topoII cleavable complexes (1). The stabilization of the cleavable complex blocks the religation stage leading to the formation of double strand breaks. In order to determine whether plumbagin induces DNA strand breaks resulting from the stabilization of the cleavable complex, the comet assay was employed. The comet assay reveals cellular DNA damage in cells in the form of a "comet" image, where the extent of DNA damage corresponds with the overall span of the comet tail (2). Plumagin induced DNA damage in HL-60 cells in a dose-dependant manner (in the concentration range: 0.1 – 1 μg/ml), as determined by the comet assay. The clinically used topoisomerase II inhibitor, etoposide (dose range: 0.1 – 1 μg/ml), induced a similar dose-dependant increase in DNA cleavage. In the HL-60/MX2 cell line with reduced topoisomerase II activity, the level of DNA damage was 80% lower than in the HL-60 cell line. The results of this research indicate the involvement of topoisomerase II-mediated DNA damage in the HL-60 cell line.

Acknowledgements: State Committee for Scientific Research, Grant No. PBZ-KBN-092/P05/2003
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Immunotropic activity of taxol derivatives in vitro

P 098

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We demonstrated previously that three derivatives of taxol: *N*-benzoyl-(2'R,3'S)-3'-phenylisoserine, methyl (*N*-benzoyl-(2'R,3'S)-3'-phenylisoserinate), and 10-deacetylo-baccatin III (the different „parts" of taxol moiety) showed antiviral activity *in vitro*. 10-deacetylo-baccatin III was also active *in vivoa*. Taxol strongly affects various parameters of immune system. The aim of the present study was to investigate immunotropic activities of three derivatives of taxol *in vitro*.
The influence of the compounds on selected immunological parameters was evaluated. Their effect on lymphocyte T and B proliferation was assessed by measuring the activity of tritiated thymidine incorporated into cellular DNA, with a scintillation counter. The influence on NK cells activity was evaluated using FACSCalibur flow cytometer. The ELISA method was used to assess the effect of the compounds on synthesis of IL-2, TNF-α and IFN-γ cytokines by blood mononuclear cells.
One of the selected compounds - methyl (*N*-benzoyl-(2'R,3'S)-3'-phenylisoserinate) significantly inhibited lymphocyte T proliferation in both tested concentrations. No effect of this compound on the other immunological parameters, as well as the immunotopic activity of the other taxol derivatives, were found.
Taxol derivatives, especially methyl (*N*-benzoyl-(2'R,3'S)-3'-phenylisoserinate) may constitute a potential source of the compounds with immunotropic properties.

P 099 Studies on natural phenolics as inhibitors of elastase

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Human neutrophil elastase (HNE) is an enzyme which plays an important role in inflammation. In continuation of our research on natural compounds as inhibitors of HNE or of its release we here investigated nine natural phenolics in a HNE release assay which can also be used for the measurement of direct inhibition. Neutrophils were isolated from fresh blood from healthy adult volunteers. Epicatechin and epicatechin-(4α-8)-epicatechin only inhibited HNE release. A maximal reduction of 27 % and 44 %, respectively, was observed at concentration of 200 µMol. Resveratrol and genistein already known as inhibitors of HNE release (1-2), were studied again. Under our conditions lower IC_{50} valuesa than published were obtained in all cases. A direct inhibition of HNE could be excluded. Tyrosol, hydroxytyrosol and triacetoxystilbene also failed to directly inhibit HNE. Preliminary studies revealed that these phenolics inhibit HNE release. The missing direct inhibitory activity of hydroxytyrosol towards elastase demonstrated that besides *o*-dihydroxy groups a specific lipophilic shape is also a structural prerequisite for a strong inhibition at the active site. The two larger molecules, agrimoniin and pedunculagin, did not influence HNE release, but inhibited HNE with IC_{50} values of 0.9 and 3.0 µM. The molecule with the highest molecular weight was the most potent inhibitor. To get insights in which way the active compounds inhibit directly elastase ligand docking calculations were carried out with agrimoniin and pedunculagin. These large tannins inhibit HNE in an unspecific manner. Presumably, they cannot bind to the active site because of their size, but cover the active site or inactivate the enzyme because of the high amount of phenolic groups.

Acknowledgements: We are grateful to Prof. Rimpler for providing us with test compounds.
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P 100 Evaluation of the anti-inflammatory properties of *Solanum dulcamara* extracts using human neutrophil elastase

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Dulcamarae stipites, derived from European bittersweet (*Solanum dulcamara* L., Solanaceae), have a long tradition in European folk medicine as anti-rheumatic and anti-asthmatic remedy . Today, dermatological preparations are used to treat chronic skin diseases, e.g. atopic eczema. As a member of the genus *Solanum*, *S. dulcamara* is known to contain steroidal glycoalkaloids, e.g. solasonine as well as steroidal saponins and thus may cause intoxications (1). Recent studies concerning the anti-inflammatory properties of this plant revealed a COX 1-inhibitory activity and inhibition of the PAF-induced exocytosis (2,3).
In order to further evaluate possible modes of action of this plant remedy, we evaluated the ability of different extracts to inhibit the serin proteinase human neutrophil elastase, which plays an important role in the inflammatory response of different tissues. A crude methanolic extract exhibited a significant inhibition of 60% at a concentration of 10 µg/ml. A subsequent assay conducted on the CH_2Cl_2, EtOAc, and n-Butanol fractions of this extract revealed that most of the activity was concentrated in the CH_2Cl_2 fraction (95% inhibition at 5 µg/ml), whereas the n-Butanol fraction was devoid of activity. Further fractionation of the active extract led to the isolation of different caffeic acid derivatives, e.g. *trans*-feruloyltyramine.

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Aspects of the chemistry of active compounds from *Magnolia*

P 101

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A large variety of lignans and neolignans with biological activities are known from various *Magnolia* species both from Eastern Asia and North America (1). The genus *Magnolia* plays a major role in Eastern Asian medicinal systems like the Traditional Chinese Medicine and the Japanese Kampo medicine. Among the main lignan constituents of the medicinally used *Magnolia* species are honokiol and magnolol which are known to possess e.g. anti-inflammatory activities. These activities have been demonstrated in tests involving cyclooxygenase-2 where inhibitory activities (IC_{50}) of 1.7 and 2.0 µg/mL for honokiol and magnolol, respectively, have been found (2). The findings corroborate the traditional usage of magnoliaceous plants in the treatment of e.g. arthritis, rheuma and fever.
To get insights into the structure-activity-relationship, derivatives of these lead compounds have been synthesized. These derivatizations included changements of the polarity, the chain length, saturation and others and led predominantly to new and hitherto undescribed compounds. The purification of the compounds has been done using chromatographic methods like HPLC. Structural elucidation of these lignan derivatives has been achieved by means of NMR and MS.

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Health-promoting potential of *Onopordum illyricum* L. (Asteraceae)

P 102

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The development of dietary agents for chemoprevention is a highly attractive anticancer strategy, because of the-long-standing exposure of humans to compounds of this type, their relative lack of toxicity, and the existence of encouraging epidemiological clues. Polyphenols present in the diet, for example, are receiving increasing interest as chemopreventive agents because of the epidemiological association between nutrients rich in polyphenols and the prevention of diseases like cancer and stroke, (1,2,3). In Sardinia, *Onopordum illyricum* L. ("cardo maggiore", a type of thistle) is used as a vegetable, young scapes and capitula being eaten raw in salad, as well as a medicinal plant because of alleged antipyretic or in skin soothing properties (4). Total extracts of different parts of the plant have been analysed by HPLC-DAD-ESI/MS in comparison with standards isolated from the natural source and showed a different phytochemical profile, both qualitatively and quantitatively. The sesquiterpene onopordopicrin represents a useful marker for the lipophilic fraction, while caffeoylquinic acids are the main constituents of the polar fraction.
We have studied the effects of these dietary compounds on TNFα-induced NF-κB activation in luciferase-based assays. NF-κB is a family of transcription factors involved in the control of a variety of cellular processes, such as immune and inflammatory responses, development, cellular growth, apoptosis and cancer. Indeed, epidemiological studies have shown a correlation between NF-κB inhibition and cancer prevention.

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P 103 Withanolides from *Withania frutescens* (Solanaceae) and their biological activity as modulators of the transcription factor nuclear factor-kappa B (NF-κB).

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Withania frutescens (Solanaceae) has been studied in a bioassay-guided fractionation following activity as inhibitor of NF-κB. Two compounds of the withanolide class were isolated and identified using a range of chromatographic techniques and spectroscopic analysis (NMR, MS). They were identified as 5, 6-epoxy-1-oxowitha-2, 14, 24-trienolide (**1**) [1], and withaferin A (**2**) [1]. Both compounds were isolated from the chloroform extract, which was active as inhibitor of the NF-κB in IL-6/Luc assay (to 36% of the positive control) and no cytotoxicity was detected. Each compound was tested against the transcriptional control of NF-κB based upon the luciferase gene being controlled by the IL-6 promoter. Compound **1** was found to reduce NF-κB activity to 27% at 25 μM in HeLa cells after 7 h of exposure. Compound **2** was active at 25 μg/ml reducing to 16% NF-κB activity. This project has shown the benefit of international collaboration in medicinal plant research utilising a specific target of the inflammatory cascade.

Acknowledgments: European Union Framework 5 (cell factory program QLK3-2000-00 463) for funding the Anti-inflammatory Natural Products (AINP) project. Consejo Nacional de Ciencia y Tecnología (CONACyT, México), and the Secretaría de Educación Pública (SEP, México) for funding the PhD and giving a supplementary studentship to NJH.
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P 104 Use of NF-kB Reporter Gene Assay System to Screen Anti-inflammatory and Immuno-modulatory Herbal Extracts/Phytocompounds

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In drug discovery, medicinal herbs were considered as important sources for the future of medicine. Many traditional medicinal herbs are reputed to confer medical efficacies including anti-inflammation and immuno-modulatory activities. But most of them were not evaluated and studied systematically. Recent researches indicated that NF- kB transcription factor is a key mediator in the immune system. The objective of this project was to use a quantitative assay system to screen the bioactivity of traditional medicinal herbs and phytocompounds, based on NF-kB-inducible ELAM-1 composite promoter transgene system. The NF-kB activities were measured by a cell-based assay using a luminescence detection method. The whole plants of medicinal herbs were extracted with water, then fractionated into the water fraction (W), the 70% ethanol fraction (E), the methanol fraction (M), and the hexane fraction (H) according to their polarity. Our results showed that the W fraction of most of the herbal extracts confer high NF-kB activities, whereas M fractions and H fractions of most of the herbal extracts down-regulate NF-kB activities. This M and H fractions can also inhibit the effect on up-regulation of NF-kB activities induced by LPS on B16 mouse melanoma cell. The crude extracts and phytocompounds with up- or down-regulated NF-kB activities may be hence considered as useful assay for the future medicine herbal research of anti-inflammation and immuno-modulatory activties.

The influence of dicaffeoyl-quinic derivatives on protein tyrosine nitration

P 105

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One of the major manifestations of cellular toxicity associated with the genesis of nitrogen monoxide (NO) is mediated by its combination with a superoxide anion to give peroxynitrite ($ONOO^-$), a strong oxidant and nitrating species. Recent research has clearly established that some plant phenolics, e.g. flavonoids, cinnamates and gallic derivatives, act as scavengers of $ONOO^-$, thus protecting biomolecules from degradation. In this context, the present communication reports on the effects on protein-bound tyrosine nitration of two dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid methyl ester and its carboxyl-free derivative) isolated from *Phagnalon rupestre* (Asteraceae) (1). To this end, we have applied different experimental designs to follow the nitration of bovine seroalbumin (BSA) by $ONOO^-$ or nitrite/heme/H_2O_2 (2) in both the absence and the presence of bicarbonate (3). Both compounds produced a significant reduction on the nitrite/heme/H_2O_2-induced BSA nitration (IC_{50} 22.9 and 23.3 μM, in the absence of bicarbonate, versus 26.7 and 27.8 μM, respectively, in its presence). Epigallocatechin gallate (EGCG), which was used as a reference, showed IC_{50} values of 36.5 and 33.3 μM, respectively. Their effects were found to be similar on $ONOO^-$-induced BSA nitration (IC_{50} 10.3 and 11.1 μM, respectively). However, the reactivity of $ONOO^-$ was significantly modified by bicarbonate, as evidenced by the fact that the methyl ester derivative alone showed half of the potency, while the carboxyl-free quinic derivative was inactive ($IC_{50} > 100$ μM). In contrast, the activity of EGCG was affected only slightly by bicarbonate (IC_{50} 35.6 μM versus 46.2 μM). The fact that both caffeoyl conjugates showed considerable ability to protect against protein-bound tyrosine nitration improves their pharmacological profile, which had previously been established for free tyrosine (4).

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

P 106

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Tyrosinase, also known as polyphenol oxidase (PPO) (1), is a copper containing enzyme found in microorganisms, plants and animals. Tyrosinase catalyzes the oxidation of phenolic substrates to o-quinones, which are then polymerized to brown, red, or black pigments. It is related to melanization in animals, but is also responsible for the browning in fruits and vegetables.
In a previous paper, we reported the isolation and identification of secondary metabolites from the methanol extracts from the aerial parts of *Marrubium velutinum* (2). In a continuation of our chemical investigations on Marrubium species of the Greek flora, M. cylleneum has been studied. The structures of the isolated compounds were established by means of NMR [^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HSQC, HMBC, HMQC-TOCSY, NOESY] and MS spectral analyses. The assay was performed according to the procedure of Kubo et al. (3) with slight modifications. 42 secondary metabolites have been tested. Phenylethanoid glycosides have been proved the most active. It is the first report on the tyrosinase inhibitory activity of this class of compounds.

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P 107 Hypoglicemic activitiy of *Senecio* species

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Disorders of carbohydrates uptake may cause severe health problems such us diabetes and obesity (1-2). This study deals with the investigation of the a-amylase inhibitory property of extracts of S. *samnitum* Huet., S. *ambiguus* subsp *ambiguus* (Biv.) DC. and *S. vulgaris* L. aerial parts. α-amylase is endoglucanases that catalyze the hydrolysis of internal alpha-1,4-glucosidic linkages in starch and other related polysaccharides (3). Dried aerial parts of *S. samnitum, S. ambiguus and S. vulgaris* were extracted with MeOH. The extracts was further acidified with 2.5% aq H_2SO_4 and then partitioned with *n*-hexane, CH_2Cl_2, EtOAc and the extracts taken to dryness under reduced pressure. The remaining solution was stirred with powdered Zn at 25°C overnight and then filtered and basified. The alkaline solution was extracted with chloroform (4). The principle of the assay is that in the presence of α-amylase starch is converted into maltose. The generation of maltose can be quantified by reaction with 3,5 dinitrosalicylic acid solution in alkaline conditions. The reduction of this compound to 3-amino-5-nitrosalicylic acid by maltose corresponding to colour change from orange-yellow to red is detectable at 540 nm. The BuOH extract of *S. samnitum* showed at 100 μg/ml a inhibition of 88.27%. Same results was obtained for BuOH extract of *S. vulgaris* , which gave 86.92% of inhibition at 100 μg/ml. The most active fraction of *S. ambiguus* was *n*-hexane that showed a inhibition of 84.07% at 500 μg/ml.

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P 108 Impact of computer-assisted methods on the discovery of non-alkaloid acetylcholinesterase inhibitors from *Cichorium intybus*

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Due to the direct correlation of cholinergic deficit and the severity of dementia, Alzheimer's disease is preferentially treated with acetylcholinesterase (AChE) inhibitors to supplement the acetylcholine level. In this study we focussed on non-alkaloid AChE inhibitors from natural sources in order to discover new lead structures. In the course of an extract screening of native plants using an enzyme assay with Ellman's reagent (1), the dichloromethane extract of chicory roots (*Cichorium intybus* L.) showed significant inhibitory effect on AChE. At a concentration of 1 mg/ml an inhibition of 70% was obtained. Based on a 3D multiconformational database consisting of secondary metabolites from *C. intybus*, virtual screening experiments were conducted. Some low molecular weight sesquiterpenoids showed distinct interactions with the pharmacophore model (Fig.1), which was generated based on the co-crystal structure of AChE and galanthamine (2; PDB entry 1QTI). In order to verify the computer-aided strategy, an activity-guided fractionation of the chicory root extract was performed. This resulted in the isolation of two sesquiterpene lactones, 8-deoxylactucin and lactucopicrin which showed pronounced and dose-dependent inhibitory activities on AChE with IC_{50} of 80,2 μg/ml (CI_{95} 63,5-105,5 μg/ml) and 61,7 μg/ml (CI_{95} 42,2-77,4 μg/ml), respectively. A comparison of the two different approaches used for the discovery of the anti-cholinesterase compounds from *C. intybus* revealed the potential of the computer-assisted methods.

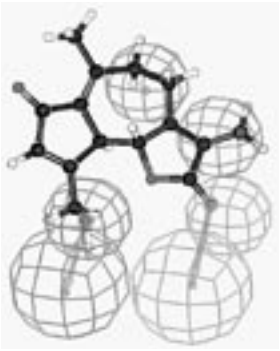

Fig.1: Preferred mapping of 8-deoxylactucin into the AChE-pharmacophore model

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Induction of neutral endopeptidase (NEP) activity of SK-N-SH cells by natural products

P 109

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Deposition of amyloid β-peptide (Aβ) as senile plaques in brain is one of the neuropathological hallmarks of Alzheimer's disease (AD), which is the most prevalent progressive neurodegenerative disease leading to dementia (1). NEP is one of the major Aβ degrading enzyme in the brain (2). Since NEP enzyme levels are the lowest in those AD brain areas that are most vulnerable to senile plaque development compared with other brain regions and in peripheral organs, it is speculated that up-regulation of NEP in the brain may prevent AD development by increasing Aβ clearance. In order to examine the influence of different polyphenols and other natural products, which are present in the green tea extract we used the neuroblastoma cell line SK-N-SH and studied the changes in the NEP activity after long-term treatment with this substances (3). The assay of NEP activity was determined by measuring the fluorescence of the released AMC from Suc-L-Ala-L-Ala-L-Phe-7-amido-3-methylcoumarin (SAAP-AMC) as substrate (4). We determined the influence of the tested substances in single experiments as well as in combination experiments on the NEP activity. We could detect that caffeine more than theophylline and theanine led to upregulating of NEP activity and inhibition of cell proliferation. We could also find that the combination of epicatechin and epigallocatechin with caffeine or theophylline induced the cellular NEP activity and did not influence the proliferation indicating that the induction of NEP activity was independent from the inhibition of cellular proliferation. It is suggested that the enhancement of the cellular NEP activity might be correlated with an elevated level of cyclic adenosine monophosphate (cAMP) (5).

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Inhibition of Angiotensin-Converting Enzyme of *Salsola kali* L. and *Salsola soda* L.

P 110

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Inhibition of Angiotensin Converting Enzyme (ACE) is currently considered to be a useful therapeutic approach in the treatment of high blood pressure (1). Inhibition of ACE will be an effective screening method in the serch for new antihypertensive agents (2). This study deals with the investigation of the ACE inhibitory properties of extracts of S*alsola kali* L. and *Salsola soda* L. aerial parts. Dried aerial parts of *S. kali* (212.92 g) and *S. soda* (291.63 g) were extracted with MeOH at room temperature. The combined MeOH solution were concentrated under reduced pressure. A solid residue was precipitated and separated by filtration from *S. kali* extract upon cooling. Both extracts were dissolved in a MeOH/H_2O (9:1) mixture and partitioned with *n*-hexane, dichloromethane, ethyl acetate and ethyl acetate after basification. The evaluation of activity of extracts was investigated by the method based on the ACE-catalyzed cleavage of the chromophore-fluorophore labelled substrate dansyltriglycine into dansylglycine, which is quantitatively measured by HPLC (3). Solutions of inhibitors were made by dissolving 1 mg of extract test in 1 ml HEPES assay buffer, to obtain the final concentration of 330 µg/ml. The most effective fraction of *S. soda* is the EtOAc extract, which gave 55.87% inhibition at 330 µg/ml. Also the *S. kali* EtOAc extract exhibited ACE inhibition activity with a value of 36.21% at the same concentration.

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P 111 In vitro binding of an isopropanolic extract of black cohosh to selected central nervous receptors

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We investigated the binding of an isopropanolic extract (iCR) of *Actaea (=Cimicifuga) racemosa L. Nutt.* (black cohosh) to 21 subtypes of 3 classes of central nervous system receptors (dopamine, serotonin, and GABA) in order to identify possible pharmacological targets. The observed clinical effects of iCR (Remifemin®) in the management of menopausal symptoms should be elucidated. The *in vitro* tests were based on the displacement of radiolabelled natural ligands. A dose dependent decrease in receptor-bound radioactivity corresponding to the displacement of natural ligands by black cohosh was measured with a scintillation counter. iCR showed greatest affinities (IC50 < 15 mg herbal dry matter/ml) towards the 5-HT1A, 5-HT1D, 5-HT7, and GABAA receptors: An IC50 of 3 mg/mL at the 5-HT1A receptor, an IC50 of 77 mg/mL at the 5-HT1B receptor, an IC50 of 15 mg/mL at the 5-HT1D receptor, and an IC50 of 4 mg/mL at the 5-HT7 receptor. The IC50 at the GABAA receptor was in the same range, i.e. 3 mg/L, while the IC50 at the D4.4 receptor was at a remarkable higher, though still detectable concentration, i.e. 368 mg/mL. Of special interest is the finding that within the wide concentration range (10 ng/ml - 1000 mg/mL) tested, iCR did not influence serotonin transport, nor did it interfere with serotonin secretion or release. The high affinity of iCR to selected central nervous receptors adds to its renowned pharmacological activities. It also suggests an association between CNS receptor-mediated effects and an efficacious treatment of menopausal symptoms.

P 112 Screening of Danish medicinal plants for CNS activity

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Plants used in Danish folk medicine for treatment and prevention of epilepsy and convulsions, plants used for sedation and plants used to enhance memory, were identified (1).
Extracts from 42 plant species, used for treatment or prevention of convulsions, were tested for affinity to the $GABA_A$-benzodiazepine receptor, the assay was carried out according to (2). Extracts from 21 plant species used for sedative purposes, were tested for affinity to the serotonin transporter (SERT) according to (3) and extracts from 13 plant species used for memory enhancement were screened for acetylcholinesterase (AchE) inhibiting activity using a microtiterplate assay (4).
The most active extracts in the $GABA_A$-bezodiazepine receptor assay were the ethanolic extracts of the leaves of *Primula elatior* and *P. veris*, and of the aerial parts of *Tanacetum parthenium* showing 8 ± 2%, 10 ± 2% and 2 ± 0.7% binding respectively, in a concentration of 0.45 mg ml^{-1}. In the SERT assay ethanolic extract of the aerial parts of *Borago officinale* showed 25 ± 3% binding, and in the AchE assay, aqueous and methanolic extract of herbs and bulbs of *Corydalis cava*, C. *intermedia* and C.. *solida* showed from 57% to 97% inhibition in a concentration of 0.1 mg ml^{-1}. Using a thin-layer chromatography assay (4) it was shown that the activity to a great extend was due to alkaloids.

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Meso-dihydroguaiaretic acid attenuates the neurotoxic effect of staurosporine in primary rat cortical cells

P 113

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The effects of *meso*-dihydroguauiaretic acid (MDGA), a lignan isolated from the bark of *Machiuls thunbergii*, on staurosporine-induced neuronal apoptosis and potential mechanisms were investigated in primary cultured rat cortical cells. Pretreatment of the cells with MDGA (0.1 – 10.0 μM) 1h prior to 150 nM staurosporine exposure for 18h, markedly elevated cell survival. Incubation with MDGA also reduced the cytosolic condensation caused by staurosporine. MDGA diminished the Ca^{2+} influx that accompanies staurosporine-induced apoptosis, and inhibited the subsequent overproduction of cellular peroxide and peroxynitrite to the level of normal cells. MDGA also significantly inhibited caspase 3/7 activities and cytochrome C release. Collectively, these results suggested that MDGA significantly inhibited apoptosis induced by staurosporine through the inhibition of cellular Ca^{2+} influx, cellular oxidation, caspase 3/7 activities and cytochrome C release.

Acknowledgements: This work was supported by the Korea Research Foundation Grant (KRF-2003-105-E00216)
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Anti-UV properties of lipidic extracts of three selected marine microalgae

P 114

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Today, marine microalgal biomass and their extracts have been incorporated in several cosmetics for skin-care and sun-protecting purposes as well as in some pharmaceutical applications for the therapy of oxidation associated diseases, like inflammations. Some of the bioactive substances are included in the lipidic fraction of microalgae.
In the present study we evaluated the potential role of the lipidic extracts from the miroalgae *Porphyridium cruentum*, *Phaeodactylum tricornutum* and *Nanochloropsis* sp., grown in photobioreactors at Necton (Algarve, Portugal). Algal biomass was extracted with hexane:methanol (1:1). The extracts were chemically characterized by TLC and GC-MS and the major fatty acids were identified.
The potential role of these extracts in protecting rat skin fibroblast cells from UV-damage was evaluated. Fibroblast cells were irradiated with UV-light (254 nm) for 5 min, a condition that caused significantly cell death after 24 hours (around 40%). When the cells were irradiated in the presence of lipidic extracts of *P. cruentum* (0.53 mg/ml), *P. tricornutum* (0.36 mg/ml) and *Nanochloropsis* sp. (0.57 mg/ml), no cell death was observed. The extracts exhibited poor antiradical properties, as cheeked using DPPH.
Taking in account the results, the extracts shown to protect cells from UV-damage but this faculty seems not linked to their antioxidant (namely radical scavenging) properties.

Acknowledgments: Dr. Vitor Verdelho (Necton SA, Belamandil, Algarve, Portugal) for the kind gifts of microalgal biomass.

P 115 Effects Of Laurel (*Laurus nobilis* L.) Extracts And CCl_4 Derivatives On Production Of $OH^{\bullet}$ Radicals

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Laurel is one of the most well known medicinal plants used in history. In traditional medicine, ether oil from laurel leaves is used as carminative, excito-aromatic and in perfumes industry. The fruits were used as spice, and today they are the source of oil. Oil is usually used in the mixture with other medicaments. The aim of this research was to investigate effects of the laurel and their combination with CCl_4, on the production of $OH^{\bullet}$ radicals. Crude methanol extracts of macerated laurel leaves and poppies were obtained in extraction with 70% methanol. After evaporation to dryness, dry matter was dissolved in water and extracted with ether, chloroform, ethyl-acetate, and n-butanol, thus leaving water solution as well. 10% (v/v) solutions of extracts in 50% ethanol were prepared. The effects of these extracts on production of $OH^{\bullet}$ radicals was determined by monitoring the chemical degradation of deoxyribose(1). Reaction is initiated by hydroxyl radicals obtained in Fenton's reaction(2), which yields products that react with thyobarbituric acid (TBA test). Obtained products, among which malonyl-dialdehyde is the most important, are determined by spectrophotometric metod according to Buege-Aust (3). Pure preparations of CCl_4 was used in different amounts. All extracts of laurel leaves decreased the production of $OH^{\bullet}$ radicals, and in combination with CCl_4 significant increase is achieved with EtOAc extract. Only EtOAc extracts of poppies caused reduction in production of $OH^{\bullet}$ radicals, while n-BuOH extract significantly increased it. In combination with CCl_4, EtOAc and H_2O extracts decreased the production of $OH^{\bullet}$ radicals, and $CHCl_3$ and n-BuOH eextracts increased the $OH^{\bullet}$ radical production in combination with high concentrations of CCl_4. It can be concluded that different results obtained in application of different extracts on the production of $OH^{\bullet}$ radicals are probably the consequence of different content of secondary biomolecules and total flavonoids in the given extracts.

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P 116 Extracts and isolated phenolics from Solidago canadensis L. and their effect on glutathione-S-transferase (GST)

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An infusion of S.canadensis is used to treat inflammatory conditions of the urinary tract and is known to have antioxidant activity, thought to be due to the saponins and flavonoids.
As well as their inherent antioxidant effects, it was thought of interest to investigate the effect of extracts and constituents of S. canadensis on GST, one of the principal detoxification enzymes produced by cells. In vitro studies using HepG2 cells showed that a crude extract and fractions containing flavonoids caused upregulation of GST using 1-chloro-2,4-dinitrobenzene as a substrate (1). Rutin, the major flavonoid present, and its analogues quercetrin and quercetin were tested and it was seen that the diglycoside gave a greater GST induction than the monoglycoside while the aglycone had a slight inhibitory activity. This explained the activity of the extract which contained mainly glycosides. It therfore appears that the flavonoids present boost the antioxidant activities of the cells as well as exerting their own antioxidant effects.

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Possible influence of ginkgotoxin on human pyridoxine-5′-phosphate oxidase (PNPO)

P 117

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Ginkgotoxin (4′-O-Methylpyridoxine, MPN) is present in the leaves and seeds of the Ginkgo tree (Ginkgo biloba l., Ginkgoaceae) (1,2). Ingestion of seeds may lead to epileptic seizures and death (3).
The symptoms of intoxication can be alleviated by vitamin B6 indicating that ginkgotoxin interfers with vitamin B6 function.
In an attempt to clarify the mechanism of intoxication we have overexpressed the human pyridoxine-5′-phosphate oxidase and tested its activity in the presence of ginkgotoxin.
However, no impact of the toxin on the enzyme was observed.

Acknowledgements: This project is part of the Graduiertenkolleg 677 "Structure and Molecular Interaction as a Basis for Drug Action" and is financially supported by Deutsche Forschungsgemeinschaft (DFG).
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Inhibition of pyridoxal kinase by ginkgotoxin

P 118

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In China and Japan the toxicity of *Ginkgo biloba* seeds -if over consumed-has been known for a long time. Symptoms of these poisonings (gin-nan sitotoxism) are convulsions, loss of consciousness and death (1, 2). The responsible neurotoxin, 4′-O-methylpyridoxine (ginkgotoxin), was first isolated in 1985 from *Ginkgo biloba* seeds (3). Mainly the seeds of *Ginkgo biloba* were shown to accumulate ginkgotoxin, but also the leaves, which are the source of extracts used in pharmacotherapy, contain the toxin (4, 5).
The toxicity of ginkgotoxin can be alleviated by vitamin B_6. It is therefore likely that ginkgotoxin, a structural analogue of vitamin B_6, interferes with vitamin B_6 dependent enzymes in the human brain. Here we show that human pyridoxal kinase (PKH) phosphorylates not only pyridoxal (vitamin B_6) but also phosphorylates ginkgotoxin with a significant lower K_m-value when compared to pyridoxal (4.95×10^{-6} M versus 5.87×10^{-5} M). In the presence of ginkgotoxin the formation of pyridoxal phosphate is suppressed. The inhibition of PKH by ginkgotoxin may lead to a deficiency of vitamin B_6 in the human brain and to seizures. Human pyridoxal kinase was overexpressed in *E. coli* and purified to homogeneity by affinity chromatography and gel filtration.

Acknowledgements: This project is part of the Graduiertenkolleg 677/1 "Structure and Molecular Interaction as a Basis for Drug Action" and is financially supported by Deutsche Forschungsgemeinschaft (DFG).
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P 119 Effects of STW 5 (Iberogast®) on secretion in the human colon

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Clinical studies have shown the efficacy of the phytomedicinal combination STW 5 (Iberogast®) in irritable bowel syndrome (IBS, 1). As a potential mechanism of action its influence on intestinal motility and afferent hypersensitivity has been discussed. While in pharmacological studies of gastric secretion it was shown that STW 5 has an inhibiting effect on acid secretion but a stimulating effect on mucin secretion (2), studies of its effect on intestinal secretion, which is of potential relevance for its effect in IBS, are missing.
We studied the effect of STW 5 on secretion in muscle stripped mucosa/submucosa preparations of the human colon using the Ussing chamber technique. STW 5 significantly enhanced short circuit currents. The increased secretion was not abolished by the neurotoxin tetrodotoxin (1µM). Results suggest that STW 5 activates secretomotorneurones in the human colon leading to enhanced chloride secretion.
These results allow the conclusion, that the influence on intestinal secretion is a potential mechanism of action of STW 5 (Iberogast®) in the treatment of patients with irritable bowel syndrome. It can be supposed that especially patients with obstipation type of this disease can have a clinical benefit from this mechanism of action.

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P 120 STW 5 (Iberogast®) and its components have powerful region specific effects on gastric motility

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Functional dyspepsia (FD) is among the most common functional gut disorders associated with severe motility disorders consisting of impaired gastric accommodation and antral hypomotility. Phytomedicine, in particular STW 5 (Iberogast®) is successfully used for treatment of FD, as is shown also by controlled and randomized clinical studies (1), yet its mode of action in gastric motility is not fully understood (2). We therefore studied the effect of STW 5 on muscle preparations from guinea-pig gastric fundus, corpus and antrum *in vitro*. STW 5 evoked a concentration dependent sustained relaxation of the gastric fundus and corpus caused by a 19.2 ± 4.6% (lowest concentration) to 59.4 ± 6.0% (highest concentration) reduction ($p<0.001$) in muscle tone. Contrary to its inhibitory effect in the fundus and corpus, STW 5 evoked a significant increase in antral motility by 20.4 ± 5.2% to 75.4 ± 11.7% ($p<0.001$). These results from guinea pig gastric muscle were confirmed in human gastric muscle preparations. We hypothesized that the dual effects may be due to specific action of STW 5 ingredients. We therefore tested the individual effects of all extracts present in STW 5. This revealed that Matricariae fructus, Angelicae and Liquiritae radix mimicked the relaxation in the fundus and corpus while all extracts including in addition Menthae pip. folium, Chelidonii herba, Carvi fructus, *Iberis amara* and Melissae folium mimicked the excitatory response in the antrum. All effects of STW 5 and of the individual extracts were observed at concentrations that are well below those administered by a single therapeutic dose of Iberogast®. Our results indicate that the STW 5 evoked effects are a result of the region specific effects of its individual extracts that relax fundus and corpus and stimulate the antrum. These two mechanisms may be crucial for its beneficial effects in FD-patients who will profit from the improved accommodation and the enhanced antral activity. This helps to normalize gastric reservoire functions as well as gastric emptying.

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β-Secretase (BACE1) Inhibitors from Sanguisorbae Radix

P 121

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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, β-secretase (BACE1) inhibitors were isolated from the ethyl acetate soluble fraction of Sanguisorbae Radix (the dried roots of *Sanguisorba officinalis* L.). They were identified as 1,2,3-trigalloyl-4,6-hexahydroxydiphenoyl-β-D-glucopyranoside (Tellimagrandin II, **1**) and 1,2,3,4,6-pentagalloyl-β-D-glucopyranoside (**2**) and were shown to non-competitively inhibit β-secretase (BACE1) with the IC_{50} values of $3.10x10^{-6}$ M and $3.76x10^{-6}$ M, respectively. The *Ki* values of **1** and **2** were $6.84x10^{-6}$ M and $5.13x10^{-6}$ 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.

β-Secretase (BACE1)-Inhibiting Stilbenoids from Smilax Rhizoma

P 122

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Alzheimer's disease is a neurodegenerative disorder clinically characterized by the deposition of amyloid protein (amyloid plaques) in the parenchyma of the amygdale, hippocampus, and neocortex. In Alzheimer's disease, the major component of the amyloid plague is the β-amyloid protein (Aβ), which is a 39-43 amino acid peptide composed of a portion of the transmembrane domain and the extracellular domain of the amyloid precursor protein (APP). APP is cleaved by three types of proteases, which are designated α-, β-, and γ-secretase. To initiate Aβ formation, β-secretase cleaves the APP to form the *N*-terminus of Aβ at the Asp+1 residue of the Aβ sequence. Following β-secretase cleavage, C99 is the substrate of the second protease, γ-secretase, which cleaves the APP to generate the *C*-terminus of Aβ, and the mature peptide is secreted from the cell. Among the secretases, BACE1 is at present the most attractive target for the inhibition of amyloid production since there is strong evidence that it is the major β-secretase in neurons and the absence of BACE1 results in the inhibition of the production of amyloid and C99 stubs or β-CTF (*C*-Terminal Fragment) without any major side-effects. Therefore, the β-secretase (BACE1) inhibitors could be a promising target for developing anti-dementia drugs. In the course of searching for BACE1 inhibitors from natural products, the ethyl acetate soluble fraction of Smilax Rhizoma (the dried rhizomes of *Smilax china* L.) showed potent inhibitory activity. The active compounds were identified as a *trans*/*cis*-resveratrol mixture, oxyresveratrol, veraphenol, and *cis*-scirpusin A. They were shown to non-competitively inhibit BACE1 with the *Ki* values of $5.4x10^{-6}$ M, $5.4 x10^{-6}$ M, $3.4 x10^{-6}$ M, and $5.4 x10^{-6}$ M and IC_{50} values of $1.5x10^{-5}$ M, $7.6x10^{-6}$ M, $4.2x10^{-6}$ M, and $1.0x10^{-5}$ M, respectively. The active compounds 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 123 Coumarins from *Peucedanum ostruthium* as inhibitors of acetylcholinesterase

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Different plant extracts have been screened by TLC bioautography (1) in an effort to discover new acetylcholinesterase (AChE) inhibitors. The CH_2Cl_2 extract of *Peucedanum ostruthium* (L.) Koch (Apiaceae) roots was found to contain several compounds inhibiting AChE activity. Active constituents were isolated by bio-guided fractionation using almost exclusively centrifugal partition chromatography (CPC). Several coumarins (ostruthin, imperatorin, ostruthol, and oxypeucedanin hydrate) and a chromone derivative (peucenin) were found to be potent inhibitors of AChE. In this bioassay, ostruthol, the most active of the tested coumarins, was about ten-fold more active than the commercial AChE inhibitor galanthamine, and as strong as huperzine A, from *Huperzia serrata* (Lycopodiaceae). Thus these coumarins are non-alkaloidal metabolites which strongly inhibit AChE activity, as are xanthones from *Gentiana campestris* (Gentianaceae) (2).

Acknowledgements: The Swiss National Science Foundation (grant no. 200020-100083 to K. Hostettmann) is gratefully acknowledged for financial support.
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P 124 Acetylcholinesterase inhibition by Portuguese medicinal plants

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Alzheimer is a disease most common in the elderly people that must result from the malfunctioning of different biochemical pathways. The fact is that are several routes to tackle this problem, although the one that has been used with more success is the «cholinergic hypothesis». The drugs approved for the Alzheimer therapy act by counteracting the acethylcoline deficit, that is, they try to enhance the acetylcholine level in the brain (1). The molecular basis of the Alzheimer drugs used till present is precisely their action as acetylcholine esterase inhibitors. Some of the drugs approved for therapeutic use show hepatotoxicity, consequently there has been a continuous search for new drugs. In the present work three different extracts from plants collected in Beira Interior (east of Portugal), during the summer of 2004, used in folk medicine, *Melissa officinalis, Paronychia argentea, Pimpinella officinalis, Lavandula officinalis, Hypericum perforatum, Mentha rotundifolia* and *Lavandula stoechas*, were searched for acethylcholinesterase (E.C.3.1.1.7.) inhibitory activity. Essential oils and ethanolic extracts were obtained by standard methods known in the art. Aqueous extracts were obtained boiling 5g of dried plant material in 100 mL of distilled water for 20 minutes. Solution was filtered and aliquots of 1 mL were frozen and used when necessary for the enzymatic tests. The enzymatic activity was measured using an adaptation of the Ellman *et al* method (2). All extracts exhibit some inhibitory capacity. The poorest results were obtained with the three extracts of *Lavandula officinalis.* The best results (in brackets is presented the % of inhibition) were obtained with the ethanolic extracts of *Melissa officinalis* and *Paronychia argentea* (35% and 48% respectively), the essential oil *Paronychia argentea* (58%), and the aqueous extracts of *Lavandula stoechas*, *Mentha rotundifolia* and *Hypericum perforatum* (68%, 69% and 80% respectively).

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The phytoestrogen genistein activates human osteoblast survival via genomic TGFβ signalling

P 125

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We characterized gene expression profiles using high-density oligonucleotide arrays in a time-dependent treatment of human primary osteoblast-like cells induced by either synthetic genistein (Bonistein™), the soy phytoestrogen genistein, or 17β-estradiol (E2). Comprehensive statistical analysis revealed the expression of 149 differentially regulated genes including members of several different pathways, such as transforming growth factor (TGF)β, Ras/extracellular signal-regulated kinase (ERK) signalling, apoptosis/survival factors, phosphat-idylinositol-3 kinase (PI3K)/Akt and anchorage-dependent signalling, as well as members of the ubiquitin/proteasome degradation pathway. A subset of these genes was confirmed by quantitative real-time RT-PCR. We observed that osteoblast survival is tightly regulated by genistein similarly to the action of estrogens that play a fundamental role in bone micro homeostasis. In addition to rapid nongenomic activation of the Ras/ERK pathway induced by E2, we identified a secondary, more prolonged, stimulation of ERK phosphorylation via a genomic autocrine/paracrine TGFβ1 loop paralleled with a strong increase in the type I TGFβ receptor, activin receptor-like kinase 1 (ALK-1), mRNA levels. In conclusion, we have demonstrated that genistein, like E2, can promote cell survival signalling in cultured human osteoblast cells. However, in contrast to E2, genistein does not activate rapid nongenomic ERK phosphorylation, but rather signals the genomic activation of a TGFβ pathway that activates prolonged ERK and Bad phosphorylation. Conversely, both substances were able to activate the nongenomic PI3-kinase/Akt pathway. Therefore, dietary supplementation with Bonistein™ might be helpful in the prevention of age-related osteopenic disorders.

Pharmacological investigation at $5HT_3$ receptors on active principles in fractionated volatile oil of ginger (*Zingiber officinale*)

P 126

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The antiemetic properties of ginger are well known and have been proved in different in-vitro and in-vivo studies. [6]-, [8]- and [10]-gingerol and [6]-shogaol were shown to interact with the $5HT_3$ ion channel complex exhibiting partly their antiemetic properties(1). These constituents can not be found in the volatile oil of ginger although it too has apparent antiemetic properties. To identify the active compounds in the essential oil it was fractionated by flash-chromatography on a silica column. The resulting five fractions of differing polarity were examined by GC-MS. Fractions 1 to 5 and the identified compounds of fraction 1 and 4 were tested. Fraction 1 consists of alkanes such as monoterpenoids and sesquiterpenoids, fraction 4 contains hydroxylated and acid derivates of terpenoids. [^{14}C]Guanidinium influx into N1E-115 cells was used to investigate a potential mode of action via $5HT_3$ gated ion channels. Fraction 4 showed the highest ability to inhibit serotonin-induced [^{14}C]guanidinium influx through $5HT_3$ receptor channels in comparison to the remaining four fractions. Among the identified substances of fraction 1 α-phellandrene and ß-pinene were potent inhibitors of serotonin-induced [^{14}C]guanidinium influx at concentrations in a submillimolar range whereas tropisetrone inhibits [^{14}C]guanidinium influx at submicromolar concentrations. The further aim of our studies is to propose a drug extract with accumulated compounds exhibiting a high pharmacological potency in its antiemetic effect.

Acknowledgments: The support by Lichtwer Pharma, Berlin, is greatly acknowledged.
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P 127 Inhibition of MAO-A and MAO-B by flavonoids, a xanthone and a furanone from *Hypericum hircinum*

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As apart of the ongoing project "Formulazione e attività biologica di fitocomplessi in sistemi micro e nanoparticellari" (PRIN 2004), we examined *Hypericum hircinum* (Hypericaceae), a plant used in Italian folk medicine as herbal remedy against cough (1). Extracts of the leaves from *H. hircinum* have been shown to possess anti-microbial activity (2). In this research we first assayed the crude methanol extract of the leaves of *H. hircinum* in order to have a preliminary indication of its inhibition activity against MAO. For this purpose we used different volumes of the extract, ranging between 10-50 μL and the higher activity was observed with 50 μL (50% of inhibition). Fractionation of the extract by VLC, subsequent CC, and HPLC (RP-18) resulted in the isolation of quercetin, quercetin-3'-O-β-D-glucopyranoside, eriodictyol, eriodictyol-7-O-β-D-glucopyranoside, 1,6-dihydroxy,5,7-dimethoxyxanthone, and (4R)-4-hydroxy-5,5-dimethyldihydrofuran-2-one. The structures of the compounds were elucidated by one and two dimensional NMR techniques and Mass spectrometry (EI, ESI). To our knowledge, all the known compounds, except quercetin, have been isolated for the first time from this plant while flavanones were found for the first time in the genus Hypericum. All the isolated compounds were tested on MAO A and B. The most active compound was quercetin showing a selective inhibition against MAO A (IC_{50} MAO A =10 nM and IC_{50} MAO B =20 μM) with a Selective Index, $SI_{B/A} = 2000$.

Aknowledgements: This work was supported by a grant from MIUR (PRIN 2004).

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P 128 Effects of STW 5 (Iberogast®) on H_2O_2-induced contractions of ileum of mice in vitro

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In our study we investigate the effect of STW 5 (Iberogast®) and its components on H_2O_2-induced contraction of the ileum of mice which might be part of inflammation reactions in bowel. STW 5 is a fixed combination of nine hydroethanolic herbal extracts and indicated for functional gastrointestinal disturbances including motility dysfunction. Longitudinal smooth muscle strips of the ileum are mounted in a perfused organ bath and spontanous peristaltic activity and tonus are recorded. First the effect of H_2O_2 (0. 5 mM) 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. As a result we found that H_2O_2 induced initially a tonic contraction with a constant amplitude, but subsequent the amplitude decreased strongly. Herbal drugs influenced H_2O_2-induced contraction in different ways: peppermint and camomile inhibited amplitude and tonus, STW 5, melissa and angelica root exhibited a strong blocking of the tonic contraction, whereas liquorice root, caraway and milk thistle showed a reduction in the amplitude.

In parallel experiments we could provide evidence that H_2O_2 is able to increase significantly free radical production in gut tissue which is known to modify peristaltic motility during inflammation. In earlier studies of Germann et al. (1), strong antioxidative properties have been demonstrated for STW 5 and its different herbal components. Therefore we assume that at least some of the therapeutic effects of STW 5 (Iberogast®) also with respect to the peristialtic motility are due to the antioxidative activity of its constituent extracts.

Acknowledgements: Supported by Alfred Teufel-Stiftung, Nagold, Germany

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Investigations on quality of saffron from *Crocus sativus L.* by means of GC-MS and capillary electrophoresis; influence of saffron extracts on human keratinocytes

P 129

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Saffron, prepared from *Crocus sativus* L. (Iridaceae) belongs to the most expensive spices; also medical applications within dermatology and the use as abortivum and as psychotropic agent is described. Because of the high price on the world market saffron is known to be used in improper quality. The present study was performed in order to investigate the current status of quality of saffron available mainly on the german market. For that 20 commercial samples were investigated concerning macroscopic occurrence, microscopic properties and TLC purity. For detailed investigation of volatile oil a GC-MS method was developed for quantification of the main constituents safranal, isophorone and ketoisophorone. For determination of dyes a capillary electrophoresis method was developed and optimized for simultaneous determination of crocins (crocetin glycosyl esters). Data obtained showed that about 10% of samples investigated did not contain saffron. Several samples did not contain volatile oil components in sufficient amounts. Crocin-derivative contents varied highly between the different samples. No correlation was obtained between the contents of essential oil components and crocins.
Ethanolic and aqueous extracts of one saffron sample were tested concerning the influence on human keratinocytes, indicating that the extracts exhibited slight cytotoxic potential (LDH release) and reduced cell proliferation. Differentiation behaviour was not influenced.

Phytochemical and functional investigations on *Myrhothamnus flabellifolius* Welw.: volatile oil composition, arbutin content, proanthocyanidins and effects on human keratinocytes

P 130

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Myrothamnus flabellifolius (Myrothamnaceae), a species belonging to the vast areas from southern africa, evolves the remarkable ability to survive regular periods of extrem dehydration to an air-dry-state (resurrection plant). A high tannin content is described in literature and the leaves and twigs are used in many traditional preparations. Within phytochemical investigations on this plant the volatile oil was isolated be steam distillation. GC-MS analysis indicated the presence of a complex mixture with *trans*-pinocarveol (48%) and pinocarvon (34%) beside minor amounts of limonene and cineol. Extracts obtained higher amounts of arbutin, while methylarbutin was absent. A crude aqueous acetone extract of *Myrothamnus* contained – as determined by TLC – both, condensed and hydrolysable tannins. Concerning the condensed tannins low molecular flavan-3-ols such as catechin and epicatechin seem to be present as well as higher oligomeric proanthocyanidins. An aqueous-ethanolic extract stimulated the *in vitro* proliferation of human keratinocytes at 100 μg/ml as determined by BrdU-incorporation ELISA.

P 131 Antiviral effects of some aqueous extracts from plants of the Lamiaceae family on herpes simplex virus type 1

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The antiviral effects of some aqueous extracts from plants of the Lamiaceae family [*Melissa officinalis* (Lemon balm), *Mentha x piperita* (Peppermint), *Salvia officinalis* (Sage) and *Thymus vulgaris* (Thyme)] against herpes simplex virus type 1 (HSV-1) were examined. The inhibitory activity against herpes simplex virus was tested using a plaque reduction assay. 50% toxicity concentrations (TC_{50}) of the extracts *in vitro* on RC-37 cells were determined at 0.2% (*Melissa, Thymus*) and 0.45 % (*Mentha, Salvia*). All extracts exhibited high levels of antiviral activity against HSV-1 in viral suspension tests. In order to determine the mode of antiviral action, the extracts were added to the cells or viruses at different times during infection. HSV-1 was significantly inhibited when it was pretreated with one of the extracts prior to adsorption. At noncytotoxic concentrations of the extracts, plaque formation was significantly reduced by >93%. In time-response studies over a period of 2 hours, a clearly time-dependent activity was demonstrated. Already after 20 minutes of incubation of herpes simplex viruses with the extracts, an antiviral activity of about 70-80% was shown. And after 1 hour incubation >90% plaque reduction was reached. These results indicate that the extracts affect the virus before adsorption, very quickly after the contact. But there is no effect on the virus after penetration into the host cell. Thus the extracts are capable to exert a direct antiviral effect on HSV-1. Considering the hydrophilic nature and the low cytotoxicity of the extracts, they might be suitable for topical therapeutic use as antiviral agents in recurrent herpes infections.

P 132 Exometabolites of the cyanobacterium *Nostoc insulare*

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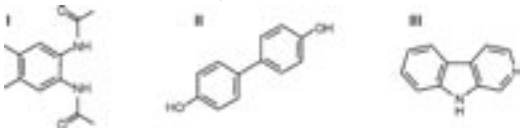
Cyanobacteria are a well known source for products of pharmaceutical or commercial interest, such as fatty acids, vitamins or pigments. Some species produce toxic compounds, also (1, 2, 3). Besides such metabolites, accumulated in the microalgal biomass, cyanobacteria frequently excrete other organic compounds into their environment, which are mostly unknown but possibly of pharmaceutical, toxicological or commercial interest, too. Against this background, exometabolites of the cyanobacterium *Nostoc insulare* were investigated in detail. Three compounds were identified, namely N,N'-(4-5-dimethyl-1,2-phenylene)bis-acetamide (**I**), 4,4'-dihydroxy-biphenyl (**II**) and 9H-pyrido(3,4-b)indole (**III**). Compounds **I** and **II** were unknown as metabolits of cyanobacteria until now, whereas compound **III** was discovered earlier in our study group as an exometabolite of the cyanobacterium *Nodularia harveyana* (4). The concentrations of the three substances determined for several culture media of *Nostoc insulare* amounted up to 387.0±4.5 µg/l for **I**, up to 1251.9±50.1 µg/l for **II** and up to 15.8±0.5 µg/l for **III**. Compounds **II** and **III** possessed cytotoxic activity against various bacteria, cyanobacteria and one yeast (*Candida albicans*), whereas compound **I** was not active against these test organisms. The usability as pharmaceutical or commercial products as well as the toxicological relevance of these cyanobacterial exometabolites is discussed.



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Antigenotoxic effects of quercetin and ursolic acid on HepG2 cells

P 133

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Quercetin (Q) and ursolic acid (UA) are among the plant compounds that possess hepatoprotective activities (1, 2). They are also referred as antigenotoxic in various *in vitro* models (3, 4). However, despite the high free radical scavenging activity of the flavonoid Q (3), the triterpenoid UA is virtually inactive as free radical scavenger at reasonable concentrations (4, 5). The aim of this study was to investigate the potential chemoprotective effects of different Q and UA concentrations in the *tert*-butyl hydroperoxide (*t*-BHP)-induced oxidative DNA damage in a human hepatoma cell line (HepG2). Three different types of treatments were used: simultaneous treatment of Q or UA with the *t*-BHP (200μM for 1hr), 24hr of pre-treatment with Q or UA before exposure to the toxic (*t*-BHP 200μM for 1hr) and the latter followed by a 2hr recovery period. DNA damage was assessed by the alkaline single cell gel electrophoresis (comet) assay and analysed by visual scoring of the cells in a fluorescent microscope (6). We observed that *t*-BHP induced DNA damage in a concentration-dependent manner. Co-incubation of Q (12.5 - 50μM) with *t*-BHP significantly protected DNA from oxidative damage. Pre-incubation of HepG2 cells with Q 50μM or UA 25μM for 24hr also significantly decreased the extent of DNA damage induced by *t*-BHP by 27% and 19%, respectively. The rate of DNA repair when allowed to recover for 2hr did, however, not change significantly from control (*t*-BHP 200μM for 1 hr, with pre-treatment with vehicle for 24hr) in cells pre-treated with Q or UA. Our results suggest that, although Q can be protective to DNA through their direct scavenging activity and modulation of *t*-BHP toxicity, UA seems to modulate *t*-BHP toxicity. Additionally, we observed an antiproliferative effect of Q (25 and 50μM for 24hr) in HepG2 cells using the colorimetric MTT assay.

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Investigations on *Ferula halophila* Peşmen (Umbelliferae)

P 134

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We aimed to put forward the phytochemical and microbiological investigations on *Ferula halophila*, an endemic[1] plant for Turkey and also a species found in the endemic and vulnerable category; a category in which plants having a potential of extinction according to the Red Data Book of Turkish Plants[2].
Aerial and underground parts were investigated for their main active compounds. For this purpose these materials were extracted with methanol and then chloroform and ethyl acetate fractions were prepared. From the chloroform fraction Columbianetin, Osthol, Isoimperatorin were determined as major compounds and Edultin as minor compound; and from the ethyl acetate fraction, Isopimpinellin, was determined as major compounds by means of TLC and HPLC.
Extracts of aerial and underground parts, prepared with different solvents were investigated for their antibcaterial and antifungal activities against *Staphylococcus a*ureus (ATCC 25923), *S. aureus* (MRSA), *Bacillus subtilis* (ATCC 25923), *Bacillus cereus* (RSKK 1122), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231). Though aerial and underground parts had no activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Candida albicans*; they were active against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, the latter was found to be more active.

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P 135 Antimicrobial effect and mineral composition of *Erica* species (Ericaceae) native to Turkey

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Erica species are widely native in Turkey. These species are *E. arborea* L., *E. manipuliflora* Salisb., *E. bocquetti* (Peşmen) P. F. Stevens and *E. sicula* Guss (1). In this study, in vitro antimicrobial activity of water, methanol, chloroform, ethyl acetate and buthanol extracts, prepared from dried and powdered aerial parts of the four *Erica* species were investigated by Disc Diffusion Method against microorganisms which cause urinary system infections especially (2). These microorganisms are: *Staphylococcus aur eus* (ATCC 25923), methicillin-resistant *Staphylococcus aureus* (MRSA, clinical isolate), methicillin-resistant *Staphylococcus epidermidis* (MRSE, clinical isolate), *Streptococcus faecalis* (ATCC 29212), *S. agalactiae* (ATCC), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Proteus mirabilis* (clinical isolate), and *Candida albicans* (ATCC 10231). Chloroform, ethyl acetate and buthanol extracts; obtained from the fractionation of methanol extract were also tested. Plant materials were digested in Microwave Acid Digestion System.The important minerals of these extracts (Zn, Cu, Fe, Ca, Mg, Pb, Cd, Mo, Mn, As) were measured by AAS and Graphite Furnace System. Ethyl acetate extracts of the four species of *Erica* showed activity against *S. aureus* (ATCC 25923 and MRSA), *Proteus mirabilis* and *S. epidermidis*. Ethyl acetate extracts of all *Erica* species were not found effective against *Escherichia coli*, *Pseudomonas aeruginosa* and *C. albicans.* These four *Erica* species have low amount Cd, Mo and As (3).

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P 136 Antimicrobial of *Punica granatum* fruit peel extracts

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Antimicrobial activity of *Punica granatum* fruit peel extracts against some pathogenic microbes was investigated using disc diffusion method (1). Both ethyl acetate and methanol extracts significantly showed some antimicrobial activities against *Staphylococcus aureus*, *S. epidermidis*, *Trichophyton rubrum*, *T. mentagrophytes* and *Microsporum gypseum*. Using agar dilution method (1) to investigate minimum inhibitory concentration of the extracts found that the ethyl acetate extract exhibited antimicrobial activity stronger than that of the methanol extract, especially against *S. aureus*, *T. mentagrophytes* and *M. gypseum* (Table 1).

Table 1 Antimicrobial activity of *Punica granatum* fruit peel extracts

Microbes	Minimum inhibitory concentration (mg/ml)	
	Ethyl acetate extract	Methanol extract
S. aureus ATCC 25923	0.5	2.0
S. epidermidis ATCC 12289	1.0	1.0
T. rubrum	2.0	2.0
T. metagrophytes	1.0	2.0
M.gypseum	2.0	4.0

Acknowledgements: Faculty of Pharmaceutical Sciences, Prince of Songkla University
Reference: 1. Lorian, V. (1996) Antibiotics in laboratory medicine 4th ed. Williams & Wilkins. Baltimore.

Biological Investigation of different extracts from *Dendrosicyos socotranus*

P 137

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D. socotranus grows on the coastal plains and hills in Socotra island. It is the only tree in the Curbitaceae family. Two cucurbitacines were isolated from the stem bark[1]. Dried powdered leaves were extracted in a Soxhlet apparatus successively with petroleum ether, dichlormethane, methanol, and cold water. Evaporation of the solvents was followed by drying in vacuo to provide crude extracts. The dried crude extracts were tested for antibacterial activity by using the modified disc diffusion method[2]; for their cytotoxic effects on FL-cells by using the neutral red assay[3], and for their effects in the isolated rabbit ileum assay[4]. Phytochemical evaluation of plant extracts was performed using TLC. No antibacterial activity against (*S. aureus*1 (ATCC 29213), *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) has been shown for the extracts till now. DM- and PE- extracts showed IC_{50} of 110 and 500 µg.

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Chemical composition and antimicrobial activity of essential oil of *Physocaulis nodosus* (L.) W.D.J.Koš.

P 138

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Aerial parts of *Physocaulis nodosus* were collected in July, 2004 in Montenegro on two different localities: Hajla mountain (1460 m), sample **1**, and Trnovački Durmitor (1360m), sample **2**. Air-dried aerial parts were subjected to hydrodistillation for 3h using a Clevenger-type apparatus. Light yellow essential oils were obtained in 0.62% and 0.38% yield, respectively. The essential oil was analyzed by GC and GC/MS. The GC analysis was carried out on a GC HP 5890 II apparatus, equipped with FID and GC/MS on a GC-HP 5890 II, with MS detector HP 5971 A. Identification of the individual oil components was done by comparison of retention times with standard substances and by matching mass spectral data with MS libraries using a computer search and literature (1,2). Antimicrobial activity was determined by agar dilution method on Mueller-Hinton (for bacteria) and Sabouraud dextrose agar (for funghi). The oil was screened against: *Micrococcus lysodeiktus* ATCC 4698, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli ATCC 25922*, *Candida albicans* ATCC 24433, *Fusarium sporotrichoides* and *Aspergilus niger* and the inhibition zone was measured. The essential oils differed in qualitative and quantitative composition. Both oils were richer in monoterpene fractions than in sesquiterpene hydrocarbons. Among 21 compounds in sample 1, sixteen were identified, representing 93.02% of total oil composition. Dominant compounds were sabinene (36.36%), limonene (27.68%), β-pinene (9.76%) and terpinen-4-ol (3.78%). Among 16 compounds in sample 2, fourteen were identified representing 91.50% of total oil composition. Dominant compounds were sabinene (27.24%), p-cymene (16.35), terpinen-4-ol (15.78), p-cymen-7-ol (12.04%). Essential oil of Sample 1 was tested for antimicrobial activity. Antibacterial activity was noticed against *M. lysodeictis, S.aureu* and *B.subtilis*. The essential oil did not show any antifungal activity.

References: 1. Adams, R.P. (1989) *Identification of Essential Oils by Ion Trap Mass Spectroscopy*. Academic Press, San Diego, CA. **2**. Sandra, Bicchi, C. (1987) *Capillary Gas Chromatography in Essential Oil Analysis*, Dr A. Huethig Verlag, Heidelberg.

P 139 Antimalarial and Antileishmanial Activity of Plant Extracts from Crete

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Malaria and Leishmania are two of the most common parasitic diseases infecting a large human population in five continents. Discovering untapped natural sources of novel antiprotozoal compounds from nature remains a major challenge and a source of novelty in the era of combinatorial chemistry and genomics.
In order to evaluate the potency of plants from the Greek island, Crete, different parts of 65 plants were extracted and 279 extracts were investigated for their antiprotozoal activity. They were biologically evaluated for their activity against Plasmodium falciparum, D6 and W2 strains, and Leishmania donovani promastigotes. In addition their cytotoxicity was tested on VERO cell line.
A total of twenty two plant extracts showed activity against P. falciparum strains, and sixty three extracts against L. donovani. More precisely the dichloromethane and methanolic extracts of Berberis cretica, as well as the mathanolic extracts of Cytinus hypocystis ssp. hypocystis and C. hypocystis ssp. orientalis had the most significant activity against both strains of P. falciparum (IC50<10 μg/ml). The dichloromethane extract of Eryngium amorginum, Eryngium ternatum, Origanum dictamnus, Origaum microphyllum, showed the mot significant activity against Leishmania donovani (IC50<10 μg/ml), while none of the extracts had cytotoxic activity.

P 140 Chemical Composition and Antibacterial Activity of Essential Oils of *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart

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The essential oils of four lines of Origanum vulgare L. subsp. hirtum (Link) Ietswaart cultivated in Hungary were analysed by GC and GC-MS methods. The contents and chemical compositions of the essential oils were compared. The twenty identified constituents accounted 97.1-97.7 % of the oils. These oils were found to contain carvacrol, γ-terpinene and p-cymene as main constituents. The antimicrobial activities of the various oils were tested on Gram-positive and Gram-negative bacterial strains, and two Saccharomyces cerevisiae and two Candida albicans strains. The most active compounds were tested on these strains by an agar diffusion method. No difference in sensitivity was found between of Escherichia coli and Staphylococcus epidermidis and the yeast strains tested, but there were marked differences in sensitivity between the proton pump-deficient mutant of E. coli and its wild type as regards the growth inhibition and MIC values. The proton pump deficient E. coli cells were non sensitive than the wild type to the antibacterial effects of volatile oils studied.

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Antiviral activity of different extracts and pterocarpans from *Psoralea bituminosa* L.

P 141

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Psoralea bituminosa L. (= *Bituminaria bituminosa* L.) (Leguminosae) is a plant widespread in the whole Mediterranean area. It has been used in Italian traditional medicine as vulnerary and for its expectorant activity (**1**).
In a previous phytochemical study on *P. bituminosa* two pterocarpans with cytotoxic activity, bitucarpin A and erybraedin C, were isolated from the aerial parts (**2**, **3**).
Fresh and dried aerial part samples of *P. bituminosa* collected at Elba Island were extracted by different techniques and solvents and analysed by LC-ESI-MS and HPLC-UV. All different extracts and bitucarpin A and erybraedin C, isolated and identified as main constituents of the less polar extracts, were tested on the Herpes Simplex Virus type 2 (HSV-2). The chloroformic extracts showed the most significant antiviral activity.

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Action of the antifungal triterpenoid saponin Phytolaccoside B on the production of chitin of fungal cell walls

P 142

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Phytolaccoside B, the most active antifungal compound isolated from *Phytolacca tetramera*,(1) produced shortening and highly branched bulbous hyphal tips, suggesting that it could act inhibiting the synthesis of one of the polymers of the fungal cell wall.(2) Nevertheless, it did not inhibit (1,3)β-glucan synthase and enhances 85% the activity of chitin synthase, enzymes that catalyze the synthesis of the major polymers of the wall. This hyperproduction of chitin was corroborated by using Calcofluor which showed an enhanced fluorescence in tips and septa under fluorescence microscopy. (3) The quantification of chitin in cells treated with sub-inhibitory amounts of Phytolaccoside B showed a 30 % higher content of acetyl glucosamine (constitutive units of chitin) of treated cells respective of control cells. In addition, the observation of the *Neurospora crassa* cell-walls with Transmission electron Microscopy (MET) showed a two times higher thickness (400 nm) of treated than of control cell-walls (200 nm). The activation of the enzyme chitin-synthase of fungal cells by Phytolaccoside B and subsequent abnormal deposit of chitin could be one of the mechanisms of antifugal action of Phytolaccoside B. According with Roncero, (3) an abnormally high deposit of chitin produce the arrest of fungal cells' growth.

Acknowledgements: ANPCyT PICT R 260; CYTED Proyecto X.7
References: 1. Escalante, A. et al.(2002), J. Ethnopharm. 82: 29-34. **2.** Onishi, J. et al. (2000), Antimicrob Agents Chemother. 44: 368-377. **3.** Roncero, C. et al. (1985) J. Bacteriol. 163: 1180-1185.

P 143 Spore germination Inhibition of *A.niger* by *Ganoderma sp*. (Basidiomycetes)

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The increasing demand in finding novel antimicrobial agents, especially antifungal agents capable of overcoming deep stated mycoses and resistance induction has diverted scientists attention toward natural products (1). Micoorganisms are one of the most important sources of bioactive compounds especially those with antimicrobial activity.
Many basidiomycetes (mushrooms), because biotechnological techniques can be applied, are being considered not only as food material, but also as materials for development of medically useful compounds.*Ganoderma sp* as a basidiomycete is found on decaying logs and tree stups, The fruiting body is used medicinally. Active constituents in this mushroom are sterols, coumarin, manitol, polysaccharide and triterpenoids called ganoderic acid. In this research antifungal activity of different extract of the fungus ranging from non polar to polar were examined. Inhibition of spore germination test was used. The results obtained from the experiments showed that the ethyl acetate extract of the fungus totally inhibited spore germination of *A. niger* at the concentration of 10mg/ml, meanwhile chloroform extract inhibited spore germination at the level of 70%. Hexane extract was also shown ver low activity. It inhibited spore germination at the level of 20%.

References: 1. Kerr KG and Lacy RW. (1995). Why do we still get epidemics. Past prespective and future trend. Society for General Microbiology. Cambridge

P 144 Antifungal Activity of Thiophenes from *Echinops ritro* L.

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In a program aimed at identifying natural fungicides as alternatives to conventional synthetic agrochemicals, more than 240 crude extracts of plants from Greece were screened for biological activity in preliminary screens. Growth inhibition of *Colletotrichum acutatum*, *C. fragariae*, and *C. acutatum* for all extracts was determined using direct bioautography [1] leading us to select extracts from *Echinops ritro* for further studies.
Bio-guided fractionation of the dichloromethane extract of *E. ritro* lead to the isolation of eight thiophene derivatives.The structure determination was performed usind 1D and 2D NMR experiments. In order to evaluate the activity of the pure compounds the microtiter assay was used [2]. In a 96-well the sensitivity of all the isolated compounds in comparison with known fungicidal standards against *B. cinerea, C. acutatum, C. fragariae, C. gloeosporioides, F. oxysporum, Phomopsis,* and *P. Obscurans* was determined. Some of the pure compounds in a concetration of 3µM, excibited significant activity, similar to that of the fungicidal standards.

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Antimicrobial activity of the extracts of *Lavandula hybrida* Reverchon

P 145

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Recent studies have been carried out on chemical composition and biological activities of lavandin essential oil (Lavandula hybrida Reverchon) (1, 2). Our research was performed with three-year old crops of Lavandula hybrida Rev. from the continental growing region that had significantly different ecological conditions in comparison to its native mediterranean habitat in Croatia. The leaves were collected in spring (March) and inflorescence with stalks in full blooming stage (July). In order to evaluate the antimicrobial activity we prepared fluid ethanolic extracts of each plant part (inflorescence, stalks and leaves) as well as different evaporated extracts of inflorescence using hexan, chloroform, acetone and methanol. The extracts were tested against ten microorganisms (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 20212, *Sarcina lutea* ATCC 9341, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076, *Escherichia coli* ATCC 25922, *Proteus mirabilis* MFBF, *Klebsiella oxytoca* MFBF Z1, *Klebsiella pneumoniae* MFBF 649.) using agar-well diffusion method and dilution method. In both testing system fluid ethanolic extract of inflorescence exerted the most prominent activity; minimum bactericidal concentration ranging from 0.12% to 6.5 % (v/v) while those of the leave extract and stalk extract were 2.5-17.5% and 6-15% respectively. Different extracts of lavandin inflorescence were found to posess significant antimicrobial activity with minimum bactericidal concentrations $\leq$ 0.4 % (w/v). The most susceptible microorganism was *Pseudomonas aeruginosa* (MBC 0.06-0.1 %) while *Enterococcus faecalis* was the most resistant strain to the majority of lavandin extracts.

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Antimicrobial activities and essential oils composition of *Nigella arvensis* and *Nigella orientalis*

P 146

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Nigella arvensis L. and *N. orientalis* L. (Ranunculaceae) are annual herbaceous plants occurring in Mediterranean and West Asian region. Their seeds are locally used as condiments (1). The hexane, chloroform and methanol extracts as well as volatile oils obtained from seeds of both species were tested against *Bacillus cereus, Bacillus subtilis, Bacteroides fragilis, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes* by broth microdilution method (2). Significant antimicrobial activity was registered for the hexane and chloroform extracts of both species tested. The chloroform extract of *N. arvensis* was the most active, inhibiting predominantly Gram–positive bacteria with minimum inhibitory concentrations ranging from 0.25 to 1 mg/ml. Steam distilled essential oil from the seeds of *N. orientalis* was analyzed by GC-MS and contained predominantly sesquiterpenes, main constituent being β–elemene (69%). The oil of *N. arvensis* was rich in monoterpenes such as carvacryl methyl ether (26%), β–pinene (21%) and n–alkanes. None of these volatile oils exhibited significant antimicrobial activity.

Acknowledgements: This research was supported by research project FRVS 1692/2005
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P 147 Composition and antimicrobial activity of the essential oils from the fruits of *Athamanta turbith* ssp. hungarica and *A. turbith* ssp. *haynaldii*

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Athamanta turbith (L.) Brot. (Umbelliferae) inhabits limestone rock crevices in SE Europe region. *A. turbith* ssp. *hungarica* (Borbás) Tutin is distributed in gorges of S Carpathians and NE Serbia, while *A. turbith* ssp. *haynaldii* (Borbás & Uechtr.) Tutin is endemic Dinaric plant (1). In this study, essential oils of mature fruits of *A. turbith* ssp. *hungarica* (sample 1) and *A. turbith* ssp. *haynaldii* (sample 2) were analysed. The essential oils were isolated from the powdered fruits by hydrodistillation, according to the procedure of the European Pharmacopoeia 4 (2), using n-hexane as a collecting solvent. Essential oil yields were 7.1% and 7.7% (w/w), respectively. The chemical analysis of the oils was performed using GC-FID and GC-MS. Thirty-eight compounds (99.7% of total amount) and thirteen compounds (98.9% of total amount) were identified in sample 1 and sample 2, respectively. The major component in both oils was myristicin 58.6% (sample 1) and 75.9% (sample 2). The content of sesquiterpenes was 36.9% in sample 1 and 16.3% in sample 2, while monoterpenes were present in smaller quantity 4.2% and 6.7%, respectively. The microbial growth inhibitory properties of isolated oils were determined using the agar diffusion method (3) against Gram(+) bacteria *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, Gram(–) bacteria *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* NCIMB 9111, *Escherichia coli* ATCC 25922, and a yeast *Candida albicans* ATCC 24433. Essential oil of *A. turbith* ssp. *hungarica* exhibited the best inhibitory effect against *K. pneumoniae, S. aureus* and *P. aeruginosa,* while the oil of *A. turbith* ssp. *haynaldii* showed the best antimicrobial activity against *E. coli*, *S. aureus* and *C. albicans.*

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P 148 *In vitro* inhibitory activities of essential oils from *Thymus magnus* against antibiotic-resistant bacteria

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Thymus species are well-known sources of antimicrobial essential oils and vary tremendously in composition depending on the plant source. *T. magnus* (Labiatae) contain high percentages of thymol. They are native to Korea and they have been used as diaphoretics and carminative in traditional medicine. To develop a new natural antibiatics against antibiotic-resistant bacteria the in vitro inhibiting activities of the essential oils from *T. magnus* as well as their main constituents were evaluated against *Streptococcus pneumoniae, Staphylococcus aureus, Salmonella enteritidis,* and *S. typhimurium,* which exhibit resistant to the corresponding antibiotics in therapy. The methods used in this study were broth dilution methods, disk diffusion tests and checkerboard titer tests. The essential oil fraction of *T. magnus* and its main components exhibited significant inhibitory activities against the antibiotic-susceptible as well as resistant strains of S. *pneumoniae*, *S. aureus, S.enteritidis,* and *S. typhimurium.* The oil fraction and its main component, thymol, displayed different patterns of activity against the tested species as exemplified by the differential minimum inhibiting concentration (MIC) values. The disk diffusion test showed that the activities were dose dependent. Furthermore, the results of the checkerboard titer test confirmed the synergism between norfloxacin and *T. magnus* oil or thymol, especially against norfloxacin resistant strains of *S. aureus.*

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In vitro antistaphylococcal activity of *Leuzea carthamoides*

P 149

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Since the first appearance of methicillin-resistant *Staphylococcus aureus* in 1961, much effort has been spent to find a proper therapeutic agent against this extremely flexible microorganism. However, until now it has been able to overcome all antibiotics developed during the past 50 years (1). In the course of our investigation of antimicrobial activity of Siberian medicinal plant *Leuzea carthamoides* DC (Compositae) (2) we observed pronounced activity of dichloromethane extract from aerial parts against *S. aureus*. The extract was fractionated by column chromatography on silica gel using a set of eluents (petroleum ether, toluene, dichloromethane, ethyl acetate, methanol, water) into sixteen fractions, which were tested *in vitro* against 19 *S. aureus* strains using broth microdilution method (3). The minimum inhibitory concentrations (MICs) of the active fractions ranged from 64 to 1024 µg/ml. An ethyl acetate fraction (EA 1) with the widest range of activity inhibited all of the strains with MIC range 128-512 µg/ml. This fraction exhibited potent activity against strains, which showed associated resistance to oxacillin, ciprofloxacin and erythromycin.

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Investigation of chemical constituents and the Antimicrobial activity of *Otostegia fruticosa* Forssk.

P 150

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The volatile oil of fresh aerial parts of *Otostegia fruticosa* (family Labiatae) growing in Egypt was extracted using steam distillation technique (0.05 %) and analyzed by G C/ MS. The results revealed the presence of 13 Compounds in which Caryophylline Oxide is the major one (60.86 %) . The study of lipid fraction resulted in the identification of fatty alcohol is, unsaponifiable constituents and the fatty acids. Three Known flavonoids were isolated and identified as : 7,4′ dimethoxy–6– hydroxy apigenin, 6,5′ – dihydroxydiosmetin and chryseoriol – 7-O- glucoside in addition to ferulic acid .The structure of compounds was established using UV, MS and H & C^{13} nmr analyses. The antimicrobial activity of different extracts with different concentrations (pet. ether, CHC, EtOAc, 70% aq. MeOH) and ferulic acid against some selected micro-organisms (Gr. + ve, Gr. –ve baeteria and fungui) was studied .The results showed that,the fatty acid fraction exhibited the inhibitory and bactericidal activity against both *St. aureus* ,*B.subtilis and E. coli* at conc. 750ug/ml.while ferulic acid showed antifungal activity against *A.niger.*

References : **1.** Rizk , A.M. " The plytochemistry of the flora of Qatar " (1986), published by King print of Richmond, UK., p. 98. **2.** AL-Musayieb, N.M; Abbas, F.A. ; Ahmed ; M.S ; Mossa, J.S. and EL- Feraly , F.S. (2000) phytochem. 54 (8) , 771-775.

P 151 Cytological changes in *Bacillus subtilis* by phenolic compounds

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In our search for antimicrobial agents from natural sources, we have isolated a series of 6-oxophenolic triterpenoids (zeylasteral and demethylzeylasteral) from *Maytenus blepharodes* Lundell, with antimicrobial activity (**1**).
These compounds have clearly bactericide effects against *B. subtilis* and cause morphological changes in the colony counts in agar plates. Optical, fluorescence and transmission electron microscopy have been used to determine the effects of these drugs on cells. Significant differences were observed in the structure, size and elemental composition in *B. subtilis* after drugs exposure.
Optical microscopy reveals filamentous multiseptates cells when they were treated with these phenolic compounds. Furthermore, the electron micrograph showed that treatment with both compounds at MIC for 1 h produced elongated filaments, variability in wall thickness and cell wall and cytoplasmic membrane breaks. Thus, the predisposition to lysis, the morphological changes seen by microscopy, suggest that the phenolic compounds could be compromise the cell wall synthesis.

References: 1. León, L. et al. (2005) Planta medica. 71.

P 152 Comparison of the antibacterial activity of Azorean and Lithuanian honeys

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Numerous studies demonstrated that honey possesses antimicrobial activity by destroying or inhibiting the growth of pathogenic vegetative microorganisms (1, 2, 3). It was reported that antibacterial activity of different honeys is variable and depends, partly, on the floral origin of honey (1, 2). We had opportunity to compare inhibition properties of honeys from two remote geographic locations with different climate and flora. This study was aimed at the evaluation of antibacterial properties of different origin Azorean (Portugal) and Lithuanian honeys and some beebread products. The inhibitory action of 34 honey and 4 beebread samples from Lithuania was tested against *Staphylococcus aureus* and *Staphylococcus epidermidis* by the agar well-diffusion method (1). The antimicrobial activity of 10 Azorean honeys was tested against six strains of *S. aureus* in the same way. Total antibacterial activity was evaluated by measuring the clear zone around the well, and expressed in phenol concentration possessing equivalent activity. Honey samples were tested after dilution to 50, 25 and 10 %. The solutions containing 10 % of honey (both countries) did not have any effect on the growth of bacteria; some Lithuanian honey samples had no inhibitory activity at the all concentrations used. The contribution of catalase and neutralization to the antimicrobial activity of honey was also assessed. It was found that the antibacterial activity of Lithuanian honeys was dependent on hydrogen peroxide formation, while such dependence was not observed for the Lithuanian beebread and Azorean honey samples. The total antibacterial activity of tested Azorean honey solutions was equivalent to 5.8-29.3 % phenol, while that of Lithuanian honeys 0.59-6.97 %. Floral source of honey and bacteria culture, as well as bacteria strain, were other factors related to the antibacterial activity. To prove possible contribution of phytochemicals, which may be transferred to honey other methods should be applied.

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Evaluation of *Zanthoxylum limonella* essential oil and ethanolic fruit extract for their biological activities

P 153

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Zanthoxylum limonella (Dennst.) Alston is a small forest tree of Northern Thailand, India and Laos. Its fruits have stimulant, astringent, aromatic, digestive properties and are used in urinary diseases, dyspepsia and diarrhoea. In India it is given in honey for asthma and rheumatism. The essential oil (EO) is employed for external usages in dermatosis (1). This study was undertaken to evaluate the antimicrobial, antioxidant and anti-inflammatory properties of the EO and the crude ethanolic 80% extract. The samples were tested by the hole plate diffusion method (PhEur). However, the pure EO showed a broad activity spectrum against gram-positive (*Staph. epidermidis*, *Staph. aureus*, *Bac. subtilis, Strept. mitis*) and gram-negative (*E. coli, Ps. aeruginosa, Prot. mirabilis, E. faecalis)* bacteria. The extract failed to inhibit the growth of the test microorganisms. No activity was observed against three *Candida* sp. The DPPH method was used to test for antioxidant activity. The EO and the extract showed low activity (EC_{50} = 125.68 and 2540.65µg/ml, resp.) The anti-inflammatory activity was tested by cyclooxygenase (COX-1 and COX-2) and 5-lipoxygenase (5-LOX) inhibitory assays *in vitro*. The ethanolic extract showed a stronger inhibition (COX-1 90.8%, COX-2 94.4%; 5-LOX 55.37%) in comparison to the EO (COX-1 70.4%, COX-2 88.9%; 5-LOX 7.57%). The EO was obtained by hydrodestillation (yield 2.30%) of the ripe fresh fruits harvested in 2002 in Phetchaburi Province, Thailand and analysed by GC-MS. More than 40 compounds were identified. The major representative constituents were sabinene (54.8%), terpinen-4-ol (12.5%), p-cymene (7.0%), myrcene (2.7%), α-pinene (1.9%), α-terpineol (1.7 %) and *n*-decanal (1.6%). The fruits were dried, the seeds separated from the pericarps which yielded 0.95% flavonoids consisting mainly of hyperosid and isoquercitrin. The tannin yield was 0.78% (mean N=3, PhEur).

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Antifungal compounds from *Piper amalago*

P 154

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Piper amalago L. (Piperaceae) is a shrub or small tree which grows in Central and South America. Several 2-acyl-3-hydroxycyclohex-2-en-1-ones were previously identified by our group (1), mainly by GC-MS and ^{13}C-NMR analysis of the total essential oils obtained from different parts of the plant.
The essential oils and the hexane and dichloromethane extracts from leaves and stems have been now assayed for their antifungal activity using the agar disk diffusion assay, showing to be active against *Candida albicans* and *Saccharomyces cerevisiae*.
Bioautography of the dichloromethane and hexane extracts showed a major inhibition zone at the same Rf of the main zones observed on the TLC of the essential oils. Isolation of the active compounds was carried out from the essential oil of the stems by semi-preparative HPLC using a C-18 column and eluting with a gradient of AcOH 10%, MeOH (2:8 to 0:1). Four 2-acyl-3-hydroxycyclohex-2-en-1-ones were isolated and their structure was established by EI-MS and NMR spectroscopy (^{1}H-NMR, ^{13}C-NMR, DEPT, COSY, HSQC, HMBC) as 2-hexanoyl- (**1**), 2-octanoyl- (**2**), 2-decanoyl- **(3)** and 2-dodecanoyl-3-hydroxycyclohex-2-en-1-one (**4**).
1 and **2**, the two main constituents of the essential oil (9.7% and 75.6%, respectively, determined by GC-FID), were tested against the two yeasts strains, showing a good antifungal activity.

Acknowledgements: Iberoamerican Program CYTED (Project X.7).
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P 155 Antibacterial activity of the essential oil of Catnip (*Nepeta cataria* L.)

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Catnip, *Nepeta cataria* L. (Lamiaceae) has been used in traditional herbal medicine for the treatment of coughs and disorders of the digestive system. By the composition of its essential oil, not by morphological properties, two types of catnip can be distinguished: the genuine catnip, whose essential oil consists mainly of stereoisomeric iridoid lactones (Nepetalactones) and the lemon scented type, *N. cataria var. citriodora*, with acyclic monoterpene alcohols as main constituents of the essential oil. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil of catnip from different commercial and natural sources were determined *in vitro* against grampositive and gramnegative bacteria with a modified broth microdilution method according to the German DIN regulation 58940-8 (1). The chosen bacteria represented the normal flora of human skin and/ or epithelia of respiratory and gastrointestinal tract and included pathogenic and facultatively pathogenic strains. All tested catnip oils showed a remarkable antibacterial activity against the tested grampositive strains with MIC values from 0.03 % to 0.5 % (v/v), while the tested gramnegative bacteria except of *Shigella flexneri, Moraxella catarrhalis and Haemophilus influenzae* were less susceptible, showing MIC values from 0.5 % to >2 % (v/v). Most susceptible to catnip oils were *Streptococcus pneumoniae and H. influenzae* with MIC values of 0.03 % and 0.06 % (v/v), respectively.

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P 156 Composition, antifungal and in vitro antioxidant activities of *Monarda didyma* L. essential oil

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Monarda didyma L. (Golden balm or Honey balm) is a wild plant in North America and is cultivated also in Europe (1). This plant was allowed to growth at 500 m above sea level near Urbino (Italy). The chemical composition of the essential oil obtained from *Monarda didyma* stem with leaves and flowers was analyzed by GC and GC/MS and the components identified were 22, mainly thymol (57.3% and 51.7% respectively), γ-terpinene (9.3% and 14.3%), p-cymene (10.5% and 9.7%), Δ^3-carene (4.5% and 6.2%) and myrcene (3.7% and 3.7%). The two oils were qualitatively similar but some quantitative differences were found. The antifungal activity of the oil was evaluated against 4 phytopathogenic fungi, post-harvest pathogens (*Rhizoctonia solani*, *Fusarium oxysporum*, *Botrytis cinerea*, *Alternaria solani*) by direct contact with the Agar diffusion method and with the fungistatic action of the vapors using the Micro-atmosphere method. The most sensitive fungus resulted *Rhizoctonia solani* in the first test (MIC µg/mL = 125 ± 2.5, MFC mg/mL = 300± 2.6) and *Botrytis cinerea* in the second (MFQ, Minimal Fungicidal Quantities, = 5.0 µL). The antioxidant activity of the oil was evaluated by DPPH test, were the oil showed an effect comparable to Trolox (IC_{50} = 6.6 ± 0.9 µg/mL and 7.7 ± 0.9 µg/mL, respectively), and by lipid peroxidation test, where the activity of the oil was similar to that of BHT (IC_{50} = 4.4 ± 0.9 µg/mL and 3.9± 0.8 µg/mL, respectively). The good antifungal effect of the oil showed in all tests is correlated with thymol content, as reported in literature (2, 3) and the presence of phenolic hydroxyl groups of thymol is also correlated with the high antioxidant activity (4).

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Composition and comparison of the essential oils of eight *Anthemis* species growing wild in Greece. Study of their antimicrobial activity

P 157

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The chemical composition of the essential oils obtained from the aerial parts of eight *Anthemis species* (Asteraceae), growing wild in Greece and used in folk medicine, was analyzed in quality and quantity by GC and GC-MS. Specifically, the essential oils of the following taxa were studied: *Anthemis chia* L., *A. auriculata* Boiss. and *A. tomentosa* L. belonging to the Anthemis section, *A. cotula* L. belonging to the Maruta section, as well as *A. altissima* L., *A. tinctoria* L, *A. werneri* L. and *A. melanolepsis* L. which belong to the Cota section (1). The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries (2) and those described by Adams (3), as well as on comparison of their retention indices (4) and with literature values (3). The main components of the essential oils were for *Anthemis chia* L. chrysanthenyl acetate, germacrene D and spathulenol, for *A. auriculata* Boiss spathulenol and γ-curcumene, for *A. tomentosa* L. linalool, linalyl acetate, spathulenol and T-cadinol, for *A. cotula* L. germacrene D, for *A. altissima* L. benzaldehyde and trans-caryophyllene, for *A. tinctoria* L. 1, 8-cineol and bicyclogermacrene, for *A. werneri* L. nopol and trans-caryophyllene and for *A. melanolepsis* L. benzaldehyde, p-cymene, trans-verbenol and benzene methanol. The essential oils were screened for their antimicrobial activity against *Bacillus cereus* (clinical isolates), *Candida albicans* (clinical isolates), *Escherichia coli* ATCC 35218, *Micrococcus luteus* ATCC 9341 and *Proteus mirabilis* (clinical isolates) following the microdilution method (5).

Aknowledgements: The authors thank Dr. Th. Constantinidis for the identification of the plants.

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Antimicrobial and antioxidative activities of *Detarium microcarpum* and *Hymenocardia acida*

P 158

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Detarium microcarpum Guill. (Perr) and *Hymenocardia acida* Tul. are two shrubby plants growing in the sub-saharan region of Africa (1). *Detarium microcarpum* is catalogued as the major African medicinal plant (2) for its uses in the healing of many diseases as infections (eg. meningitidis, panari, furonculosis, malaria, syphilis, gonorrhrea), rheumatism and asthma. *Hymenocardia acida* is also used against infection diseases (eg. malaria, syphilis, gonorrhea, abscess, tuberculosis, cholera, amibiasis, leprosy), against rheumatism and asthma. The antimicrobial and antioxidative activities of *Detarium microcarpum* (leaves, root bark, stem bark) and *Hymenocardia acida* (leaves, stem bark) were investigated *in vitro*. The antimicrobial activity was tested by disc diffusion (PhEur) and broth dilution methods on crude extracts (petroleum ether, dichloromethan, methanol, water) and on fractions obtained by chromatographic methods. The methanolic extract of *Hymenocardia acida* stem bark showed an interesting activity against *S. aureus* (MIC=125 µg/ml), *S. epidermidis* (MIC=250 µg/ml), *E. faecalis* (MIC=250 µg/ml), *M. luteus* (MIC=125 µg/ml) and *M phlei* (250 µg/ml). The aqueous extract was also active against the microbial strains mentioned above. The methanolic extract of *Detarium microcarpum* root bark inhibited *S. aureus* (250 µg/ml), *S. epidermidis* (MIC=250 µg/ml), *E. faecalis* (MIC=250 µg/ml), *M. luteus* (MIC=125 µg/ml) and *M. phlei* (MIC=250 µg/ml). The dichloromethan extract showed a weaker effect on the described bacterial strains. Bioguided fractionation of the crude plant extracts was monitored by means of chromatographic methods. Significant activities against *M. luteus* as leading strain were observed. The antioxidative activity was investigated by the DPPH and the lipidperoxidase assay on aqueous extracts of each plant part. All samples were active. The strongest activity was proved by the stem bark of both plants as well as the root bark of *Detarium microcarpum*. The activity decreased considerably after removing the tannins. It could be suggested that the tannins have a strong influence on the antioxidative effect. Further investigations for isolating compounds responsible for these activities are in progress.

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P 159 Antimicrobial activity of phenolic triterpenoids from Celastraceae. Structure-activity relationship

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The Celastraceae family have different therapeutic properties wich must be atributed to unsaturated and oxygenated D:A-friedo-*nor*-oleanane triterpenoids.

As part of our research on antimicrobial activity of this type of compounds we have investigated the structure-activity relationship of phenolic triterpenes with unconjugated doble bond in B ring, pristimerol **(1)** and their derivates, 2,3-diacetoxy-pristimerol **(2)** and 6-α-hidroxy-diacetoxy-pristimerol **(3)**, or without double bond[1] such us 8-*epi*-6-deoxoblepharodol **(4)**, 6-deoxoblepharodol **(5)** and their derivates 2,3-diacetoxy-8-*epi*-6-deoxoblepharodol **(6)** and 2,3-diacetoxy-6-deoxoblepharodol **(7)**. All the compounds assayed were inactive against the Gram negative bacteria and the yeast *Candida albicans* (MIC > 40 µg/ml). The results showed that the double bond increased the activity (**1** *versus* **4**, **5**). On the other hand, the blocked for acetylation of the hydroxyl groups in ring A give to lose of the activity.

Refernces: 1. Rodríguez, F. M. *et al*. (2005). Tetrahedron 61: 2513-2519

P 160 Activities of three 6-oxophenolic alone and in combination against spore forming bacteria

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Zeylasteral, demethylzeylasteral and zeylasterone are 6-oxophenolic triterpenoids presented in the *Celastraceae* family, which have the highest antimicrobial activity against spore forming bacteria, such as the genera *Bacillus* and the yeast *Candida albicans*. (**1**)

According this, *in vitro* activities of these phenolic triterpenoids alone and in combination against *B. subtilis* were evaluated. All compounds had bactericide effect ($\geq 3\log_{10}$ reduction in CFU/ml) at twice the MIC during the first nine hours of treatment, while at subinhibitory concentrations the greatest effect was observed in the case of zeylasteral at ½ and ¼ MIC values.

Combination time-kill curves were tested at one-quarter, one-half, one and two times the MIC to detect for synergistic, antagonistic, or additive effects. Sub-MIC of zeylasteral added to demethylzeylasteral resulted in synergy for the first six hours. It is of interest that when zeylasterone at bactericidal concentrations was combined with zeylasteral or demethylzeylasteral at bacteriostatic concentrations antagonistic effect was observed.

The post-antibiotic effect (PAE) of these compounds were also evaluated at twice the MIC. Zeylasteral showed a PAE clearly longer (3h) than demethyzeylasteral and zeylasterone (1h).

Refernces: 1. León, L. et al. (2005) Planta medica 71.

In vitro anti-*Propionibacterium acnes* activity of Thai *Ocimum* oils and their microemulsions

P 161

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Anti-*P.acnes* activity of essential oils extracted from Thai *Ocimum* was screened using agar disc diffusion method. Minimum inhibitory concentration (MIC) values of *O. Basilicum* and *O. sanctum* oil were 2.0% and 3.0% v/v, respectively, whereas *O. americanum* oil did not show activity against *P. acnes* at the highest concentration tested (5.0% v/v), resulting from agar dilution assay. GC-MS analysis revealed that methyl chavicol (93.0%) was the major compound in *O. basilicum* oil, and eugenol (41.5%), γ-caryophyllene (23.7%) and methyl eugenol (11.8%) were major compounds in *O. sanctum* oil. *O. americanum* oil contained high amount of geraniol (32.0%) and neral (27.2%) and small amount of methyl chavicol (0.8%). O/W microemulsions of individual *Ocimum* oil with concentration corresponding to its MIC value were formulated. The stable o/w microemulsion system for *Ocimum* oil consisted of 55.0% v/v water phase, 10.0% v/v oil phase (3.0% v/v *O. basilicum* or 3.0% v/v *O. Sanctum* oil plus 7.0% v/v isopropyl myristate), 29.2% v/v Tween 80 and 5.8% v/v 1,2-propylene glycol. Hydroxyethylcellulose at a concentration of 0.5% w/v was used as thickening agent. According to disc diffusion assay, the formulations containing *O. basilicum* oil exhibited higher activity against *P. acnes* than those containing *O. sanctum* oil, and the thickened formulations tended to give a lower activity against *P. acnes* than the non-thickened formulations. The prepared microemulsions were stable after being tested by a heat-cool cycling method for 5 cycles. These findings indicate the possibility to use Thai *Ocimum* oil in suitable formulations for local application in acne treatment.

Biological activity of compounds isolated from *Lithospermum canescens* roots of different origin

P 162

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Lithospermum canescens (Michx.) Lehm., *Boraginaceae* grows in open prairies in North America. The presence of pigments of the shikonin-type and pyrrolizidine alkaloids (PAs) is characteristic for this species. The chemical analysis of roots of plants of *L.canescens* collected in Canada and transformed roots of this species was done. Seven pyrrolizidine alkaloids (PAs) and four shikonin derivatives have been isolated and their structures were elucidated (1, 2). These compounds were the first time isolated and identified in *L. canescens*. Biological activity of both fractions: pigment and PAs, was studied (3, 4, 5, 6, 7). Acetylshikonin (ACS) and isobutyrylshikonin (IBS) showed immunomodulatory activity on female and male inbred Balb/c mice, and on F1 hybrids (Balb/c x C3H). ACS inhibited cutaneous angiogenesis induced by cells isolated from the mouse L-1 sarcoma tumour. The pigment fraction showed strong antibacterial and antifungal activities against *Staphylococcus aureus* FDA 209P, *S.aureus* Kg+, *Enterococcus faecalis* ATCC 8040, and *Candida albicans* PCM 1409 PZH, which was compared to chloramphenicol and amphotericin B activities. ACS and IBS showed also strong effect on the *S. aureus* FDA 209P, similar as activity of chloramphenicol. The PAs extracted from *L. canescens* caused a high mortality of juveniles, decrease in female fecundity and a shortened longevity of the *Tetranychus urticae* (Koch). PAs had also strong antifeedant properties against the *Leptinotarsa decemlineata* (Say) larvae.

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P 163 Anti-dermatophyte activity of saponins from *Medicago* species

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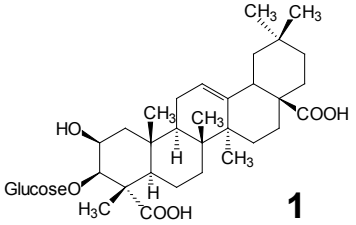
A series of 19 saponins and parent triterpenes were isolated from *Medicago* species and their minimum inhibitory concentration values against the dermatophytes *Microsporum gypseum* and *Trichophyton interdigitale* were determined using microtitre well plate serial dilution assays (1) with miconazole as a positive control. The saponins were more active than the aglycones. *Trichophyton interdigitale* was more sensitive to the saponins than *Microsporum gypseum*. Monodesmosidic glycosides of medicagenic acid were the most active compounds, especially the 3-O-β-D-glucopyranoside **1**, which displayed MIC < 0.0625µg/mL against both fungi), although those of hederagenin and zanhic acid showed weak activity. Bidesmosidic saponins had weaker activity than monodesmosidic ones.. This corresponds to activity of these compounds against other fungi (2).



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P 164 Antimicrobial Activity of Rhizome and Root of *Potentilla erecta* L. Raeuschel and *Potentilla alba* L. (Rosaceae)

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As the continuation of the previously performed research (1), this work represents a research of *Potentilla erecta* (L.) Raeuschel and *Potentilla alba* L. Tested plant material rhizome with root was collected in 2003. There was performed determination of the total phenolics content, non-tannin phenols, by applying the method of Folin-Ciocalteau reagent (2), and proanthocyanidin content by Porter (3). The method used was determination of antibiotic activity according to the European Pharmacopoeia (4) (procedure 2.7.2.) on medium A and bacteria *Staphylococcus aureus* ATCC6538 T= 80%, *Bacillus subtilis* ATCC 6633 T=30%, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231. The following extracts, which were prepared earlier, were tested: water, ethyl acetate, acetone and ethanol. Medium A was used for testing *Staphylococcus aureus* and *Escherichia coli* and Medium F for *Candida albicans*. The medium for *Bacillus subtilis* was as follows: peptone 5 g, meat extract 2,4 g, agar 15 g, purified water up to 1000 g. Values found during tests of phenolic compounds (% on dry plant material) was for *Potentilla erecta* : total phenolics 16,90%, Non-tannin phenolics 0,09%, Proanthocyanidins 2,70% and for *Potentilla alba* : total phenolics 11,74%, Non-tannin phenolics 0,71%, Proanthocyanidins 2,73%. Ethanol and acetone extracts have antimicrobial effect on *Escherichia coli*, ethyl acetate extract of rhizome *Potentilla erecta*, while water extracts of both tested species in dissolution 1:20 have antimicrobial effect on *Staphylococcus aureus*. There has not been noticed that tested species have any effect on fungus *Candida albicans*.

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Antibiotic activity of some wild *Allium* L. species

P 165

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Onions, garlic and some other species of the genus *Allium* L. (leek) have been used as phyto-pharmaceutics, seasonings and vegetables since ancient times. Carvings in pyramid walls of the Old Kingdom of Egypt and written sources of the ancient cultures of the Greeks and Romans mentioned the importance of common onion (*A. cepa*) and garlic (*A. sativum*) in the daily diet of mankind. Antibiotic activities are known since this time and used until our days in form of many traditionally prepared remedies. Antibiotic activity is related to sulphur containing compounds. Precursors of all of these sulphur compounds are cysteine sulphoxides. Antibiotic activity was visualised by a zone of inhibition surrounding a filter disc soaked with an ethyl acetate extract gained from different *Allium* species. In contrast to former investigations, no significant inhibition of gram (-) bacteria was observed. Of all tested samples *A. rosenorum* was the most active species (against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*). Surprisingly, the concentration of cysteine sulphoxides was found to be rather low (about 0.1 %). Another interesting result gave *A.* aff. *cristophii*. This extract was only active against *Streptococcus pyogenes*. This bacterium was not affected by extracts of most other tested *Allium* species. Also the results for *A. hymenorrhizum* are remarkable. This extract was rich in cysteine sulphoxides and showed an antibacterial activity against most of the tested bacteria strains. Unfortunately no information about the traditional use of this species could be gained until now. A number of extracts also displayed a good activity against the fungi *Erotium rubrum*, *Mycotypha microspora* and *Microbotryum violaceum* as well as against the alga *Chlorella fusca*.

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Antimycobacterial geranylated furocoumarins from *Tetradium daniellii*

P 166

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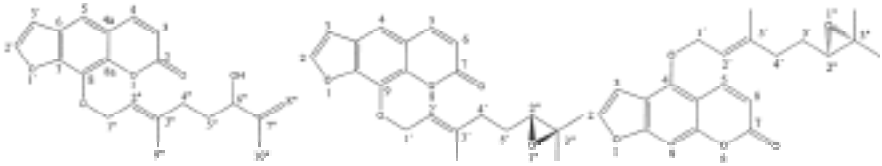
Eight geranylated furocoumarines were isolated from the fruits of *Tetradium daniellii* (Benn.) T.G. Hartley (Rutaceae). The substances, tested for their antimycobacterial activity against *Mycobacterium fortuitum, M. smegmatis* and *M. phlei,* were shown to be highly active, with minimal inhibitory concentrations (MICs) ranging from 2 to 16 uM. Xanthotoxin and xanthotoxol, representing furocoumarins lacking the lipophilic geranyl sidechain, were tested similarly, and were shown to be inactive. The antymycobacterial activity of the substances was dependant on the position and polarity of the geranyl moeity. Substitution at C-5 resulted in higher activity than C-8. Hydroxy and peroxy groups in the sidechain decreased the activity. The compound were purified using chromatographic methods. Structute elucidation was achieved with 1D and 2D NMR experiments.



P 167 Iridoids from *Scutellaria albida* ssp. *albida* and their cytotoxic and antimicrobial activities

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In continuation of our phytochemical investigations into *Scutellaria* species of the Greek flora (1), we report on the isolation and identification of twelve iridoids from the methanol extract from the aerial parts of *Scutellaria albida* ssp. *albida*. The main compounds of the plant are: catalpol and albidoside. The structures of the isolated compounds were established by means of NMR [^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HMQC, HMBC, NOESY]. The isolated compounds were tested for their antimicrobial activity against *Bacillus cereus* (clinical isolates), *Candida albicans* (clinical isolates), *Escherichia coli* ATCC 35218, *Micrococcus luteus* ATCC 9341 and *Proteus mirabilis* (clinical isolates) following the microdilution method (2), as well as for their immunomodulating potential (3). 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. Effect or 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. Preliminary data show that some of these compounds could be potentially used to enhance PBMC anticancer activity.

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P 168 Prenylated acetophenones from *Melicope* spp., chemotaxonomic significance and *in vitro* antiplasmodial activity

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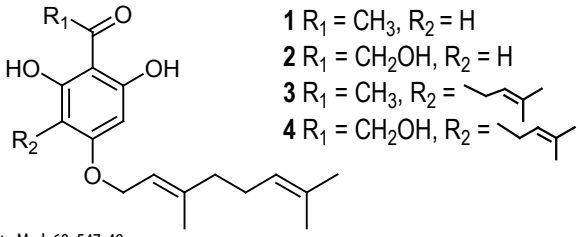
From *Melicope obtusifolia* ssp. *obtusifolia* var. *arborea* (Coode) T.G.Hartley and *M. obscura* (Cordem) T.G.Hartley (Rutaceae) four prenylated acetophenones, **1**-**4**, have been isolated. Both species are endemic to Réunion Island. The distribution of prenylated acetophenones in the Rutaceae appears restricted. Approximately 50 prenylated acetophenones have been recorded in the family and the distribution indicate that this type of compounds are valuable as chemotaxonomic markers in the subfamily Rutoideae and the tribe Xanthoxyleae, an assumption supported by the isolation of this type of compounds from the two *Melicope* species. All four compounds have shown *in vitro* antiplasmodial activity (*P. falciparum* 3D7 strain), and especially the two from *M. obtusifolia* **3** and **4** are highly potent with IC_{50} – values 0.8 ± 0.04 μM and 1.0 ± 0.07 μM respectively. The compounds had no effect on the erythrocyte membrane (1).



References: 1. Ziegler, H.L. et al. (2002) Planta Med. 68, 547-49.

Nematicidal bioactivity of natural glucosinolates

P 169

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Glucosinolates (GSL) represent a group of sulphur-containing phytochemicals, mainly found in the Brassicaceae plant family. These natural compounds and/or related enzymic breakdown products display different biological functions which allow their use as food, drugs or fine chemicals (1). Restrictions on soil treatments with synthetic fumigants make the application of glucosinolates as alternative biocidal products a further important use of these phytochemicals (2). The present study reports on the nematicidal in vitro assays with purified glucosinolates from Brassica species (sinigrin; iberin; gluconapin; sinalbin and a mix of progoitrin 63%, gluconapin 28% and glucobrassicanapin 5%) in the presence or absence of the hydrolytic enzyme myrosinase. Pure isothiocyanates, ITC (allyl-, butyl-, benzyl- and phenylethyl-isothiocyanate) have also been included in the tests. The nematicidal effect has been evaluated against Meloidogyne incognita eggs and Xiphinema index adult females. Four different concentrations (0.05, 0.3, 1.0 and 2 mg/ml) have been tested at three incubation times (2, 4 and 8 h). Mortality of X. index specimens was recorded at the end of each exposure time, whereas the egg masses of M. incognita were subjected to a 8 weeks hatching test after the above time x concentration combinations. In general, no bioactivity was observed with the intact glucosinolates, while both the GSL reacted with myrosinase and the ITC showed toxicity towards the test nematodes. That is, preliminary data indicate that myrosinase-treated sinigrin has an inhibitory trend against M. incognita mainly dependent on its dose, rather that on the incubation time: 73% inhibition was reached at the concentration of 1mg/ml. At the same concentration the mixture of GLS showed instead a 54% toxicity. X. index appears more susceptible than M. incognita, results obtained showed in fact a 100% mortality with myrosinase-treated GLS as early as 2 h after incubation at the concentration of 0.3 mg/ml. In comparison, pure isothyocyanates showed the same nematicidal effect on a longer time of incubation.

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Pharmacological validation of a new in vitro anthelminthic assay using 5- (6) carboxyfluorescein diacetate as an indicator of *Caenorhabditis elegans* viability

P 170

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A new *in vitro* assay of anthelminthic activity using *Caenorhabditis elegans* is described. The method is based on the ability of *5- (6) carboxyfluorescein diacetate* to indicate the worm viability. We show for the first time that the treatment of a suspension of worms with a solution of 5- (6) Carboxyfluorescein diacetate (4.2%) for 30 minutes induces fluorescence in dead worms only. The suitability of the assay has been evaluated by studying the activity of series of anthelminthic compounds with various modes of action (mebendazole, levamisole, niclosamide, pyrantel, piperazine, and thiabendazole) against *Caenorhabditis elegans*. To determine those activities, control worms and worms exposed to various concentrations of those compounds along seven days, were treated with *5- (6) carboxyfluorescein diacetate* for 30 minutes. The proportion of dead worms is, then determined by fluorescence microscopy. The worms were exposed to each drug at two concentrations, 50 and 100µg/ml for pyrantel and 5 and 10µg/ml for the others. We observed that, in the tested range of doses, piperazine and niclosamide were only moderately toxic (10 and 12% of dead worms). But, for the others, the results fully agree with those described in the literature and obtained by other more laborious techniques. In conclusion, the data exposed here clearly show that the proposed *in vitro* anthelminthic assay using 5- (6) carboxyfluorescein diacetate allows for sensible and sensitive measurement of worm viability.

P 171 Antitrypanosomal activity of *Mitragyna ciliata* and *Ritchea longipedicellata*

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Methanolic extracts of the roots of two plants, *Mitragyna ciliata Aubrex xpelleg (Rubiaceae)* and *Ritchea longipedicellata Gilg (Capparidaceae*) were evaluated for antitrypanosomal activity in mice. METHOD: Extracts were administered orally four days after the mice had been infected with *T. brucei brucei* and had expressed 0.2% parasitemia, at doses of (50& 100) mg/kg for *M. ciliata* and (400& 800) mg/kg for *R. longipe.* for the next four days. The combination of *M. ciliata* and *R. longipe* at a ratio 1:1 and at doses of (200 & 400) mg/kg were also administered. The levels of parasitemia were monitored for 28 days. Reference drug was1.05g Diminazene diaceturate &1.31g of Antipyrine. RESULTS: Drug / extract ratio for *M. cialata* is 1:25 and *R.longipe.* 1:17. *M. ciliata* consist mainly of indole bases and *R. longipe* consist of alkaloids and cardiac glycosides.

Table 1: dose, percent inhibition, survival rate and survival period

COMPOUND	-ve control	+ve control	*M.ciliata*		*R. longipe.*		COMBINATION	
DOSE (mg/kg)	-	3.5	50	100	400	800	200	400
INHIBTN. (%)	0	100	54.24	0	0	0	12.45	99.83
SURV.RATE(%)	0	100	60	0	0	0	0	100
SURV. PERD.	19	28	28	13	17	25	15	28

These results demonstrate that these plants extracts are potential trypanocides (1) and combination therapy is an effective strategy for treating parasites.(2)

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P 172 Molluscicidal saponins from the seeds of *Lagenaria breviflora* Robert family Cucurbitaceae

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Molluscicides are still considered the most important means of controlling schistosomiasis; but the high cost of synthetic molluscicides prohibit their use especially in developing countries like Nigeria where the prevalence is very high. Plant molluscicides are cheap, effective, and environmentally acceptable alternative.
Many plants have been screened for their intrinsic molluscicidal properties in Nigeria, with a few showing as promising plant molluscicides. However, only *Tetrapleura tetraptera* qualifies as a candidate plant molluscicide that can be used for control programme. **(1)** In the continued search for plant molluscicide, the molluscicidal bioassay of *Lagenaria breviflora* fruit extracts were made using intermediate host snail, *Biomphalaria pfeifferi*; the seed extract presented LD_{100} 30ppm and the pulp extract LD_{100} of 80ppm which can be considered a viable plant molluscicide for field test. (2) Bioassay guided fractionation of the methanolic seed extract resulted in the isolation of three molluscicidal bidesmosidic saponins; KJCF 9, 11 & 13 with LD_{50} 23, 13 and 8.5 ppm respectively. The saponins were characterized using IR, MS, 1D and 2D NMR spectroscopic methods.

Acknowledgement: University of Lagos is gratefully acknowledged for providing grant for part of this research.

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A *Rhodiola rosea* L. extract reduced stress- and CRF-induced anorexia in rats

P 173

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Rhodiola rosea L., is a popular plant in traditional medicine in Eastern Europe and Asia, with a reputation for improving depression, enhancing work performance, eliminating fatigue and treating symptoms of asthenia subsequent to intense physical and psycological stress (1,2,3). Many central effects of the drug have been attributed to its ability to influence levels and activity of biogenic monoamines, to interfere with opioid system and to reduce the secretion of corticotropin releasing factor (CRF). The aim of this work was to study whether *Rhodiola rosea* is able to modify food intake in freely feeding and deprived rats. In particular our interest was focused on investigating the ability of the drug to reverse hypophagia induced by **1)** physical stress due to 60 min immobilization; **2)** intracerebroventricular (i.c.v.) injection of CRF (0.2 μg/ml) which is known to be a major mediator of stress and to possess anorectic properties; **3**) intraperitoneal injection of *Escherichia coli* lipopolysaccharide (100μg/kg; LSP), wich induces a moderate infection associated with reduction of food intake (4). A rosavin 3% *Rhodiola rosea* extract (RHO, a generous gift of EPO S.r.l., Milano, Italy) was used, it was administered by gavage to male Wistar rats 1h before the experiments.
The results showed that RHO (10, 15, 20 mg/kg) did not modify food intake both in freely feeding ($p>0.05$) and in 24h food-deprived rats ($p>0.05$). Instead RHO abolished, at 15, 20 mg/kg, the anorectic effect induced either by immobilization [$F(4,48)=59.843$; $p<0.01$] or by i.c.v. CRF injection [$F(4,35)=3.94$; $p<0.01$]. Moreover RHO failed to reduce the anorectic effect induced by LSP ($p>0.05$). These findings provide original evidence that *Rhodiola rosea* could selectively attenuate stress-induced anorexia, probably by interacting with CRF system.

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Antipyretic effects of aqueous root extract of *Dalbergia saxatilis* (Hook, F.) in animals

P 174

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Dalbergia saxatilis, Hook, F. (DS) plant parts are employed for various medicinal uses including febrile convulsions (1). We have reported the anti-convulsant (2) as well as sedative and muscle-relaxant (3) effects of DS. In this study, we investigated the antipyretic effects of DS.
Groups of rats were pretreated, orally with DS (100 or 200 mg/kg), aspirin (100mg/kg) or normal saline (10ml/kg), immediately there was a minimum of 1^0C rise in rectal temperature after administation of *Klebsiella aeruginosa* (0.1ml/kg, i.v.) and turpentine (0.2ml/kg, i.v.) in animals; and 2,4–Dinitrophenol (2,4-DNP; 20mg/kg, i.p.) and *d*-amphetamine sulphate (10mg/kg, i.p) in rats. The rectal temperature of each rabbit was then measured, immediately, at 0 h, 0.5h. and at 1h. interval, until the temperature was constant or there was a drop in the temperature of the control animal. Thesame dosing schedule and procedure was used for measurements of antipyretic effects in each model.
The results showed that DS produced a dose-dependent and significant ($P<0.01$-0.001, Student *t*-test) reduction in hyperthermia induced by *Klebsiella aeruginosa,* 2,4-DNP, *d*-amphetamine and turpentine.
These results indicate that DS possesses antipyretic activities, supporting the usefulness of the aqueous root decoction of this plant in folk medicine, for the treatment of convulsions due to fever. Preliminary phytochemical investigations of DS revealed the presence of glycosides, sugars and saponins.

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P 175 Anti-arthritic activity of cucurbitacin R, a natural product isolated from *Cayaponia tayuya* roots

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Cucurbitacin R (CCR) is a natural triterpene obtained from *Cayaponia tayuya* roots and used as an anti-rheumatic crude drug (1). Its anti-inflammatory activity has previously been demonstrated in different acute and chronic experimental models (2). The aim of our latest research is to demonstrate the effect of CCR on the type of arthritis induced by adjuvant (AA), an experimental model of inflammation which mimics rheumatoid arthritis, and to evaluate the compound's potential as a new anti-rheumatic and anti-arthritic agent. To do so, we have evaluated different pathological parameters, including paw swelling, animal weight, and bone and tissue damage. In addition, we have determined the amount of NOS-2 and COX-2 in paw homogenates using Western blot techniques (3). CCR was found to reduce paw inflammation by 50 % at a dose of 1 mg/Kg (** $P < 0.01$, Dunnett's *t*-test), which is a result similar to that caused by ibuprofen (10 mg/Kg, ** $P < 0.01$, Dunnett's *t*-test). We also found that the body weight of the animals decreased in both the CCR and ibuprofen-treated rats. The X-ray analysis of the articular joints of the CCR-treated rats showed a clear decrease in damage, with a reduction in bone resorption and osteophyte formation in comparison with the non-treated group. The histology and the immunohistochemistry of paws treated with CCR showed a clear decrease in leukocyte infiltration as well as a reduction of the presence of the inducible form of cyclooxygenase (COX-2). This data was corroborated by the study of the homogenate of paws obtained from arthritic rats, in which it was demonstrated that CCR decreases both the levels of COX-2 and the inducible form of NOS (NOS-2). These results demonstrate the anti-inflammatory effect of CCR on chronic inflammatory diseases, and justify the use of *Cayaponia tayuya* as an anti-arthritic plant in folk medicine.

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P 176 The role of nitric oxide and intracellular calcium in vasorelaxant effect of *Artemisia annua* in diabetic rats

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Diabetes mellitus is one of the most common endocrine diseases. Vascular complications represent the main cause of mortality in diabetic patients. For treatment of diabetic vascular disorders, investigators have recently considered medicinal plants containing flavonoids. Since the extract of *Artemisia annua* has protective effect on aortic contractile response in diabetes rats, in this study, we evaluated mechanisms for vasodilating action of *Artemisia annua* extract. On the basis of the obtained results, we observed that in the treated and untreated diabetic rats, the difference in the contractile response of aortic rings, before and after adding of L-NAME was significant ($p<0.05$). The comparing of aortic cantractile response with or without endothelium to phenylephrine in the calcium-free Krebs solution in the presence of EGTA showed that the difference between treated- and untreated-rats is considrable ($p<0.05$). The above results showed that extract of *Artemisia annua* exerts its protective effects through several mechanisms such as: 1- The endothelium-dependetnt effect via increasing of production and release of NO 2- Direct effect through inhibition of Ca^{2+} release from intracellular stores.

Dose-dependent vasorelaxant effect of quercetin in subchronic streptozotocin-diabetic rats

P 177

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Vascular dysfunctions have been known as a well-established complication of diabetes mellitus. Flavonoids including quercetin have been been proposed as one of the main treatment strategies to prevent diabetic complications. In this study, the vasorelaxant effect of the flavonoid quercetin was investigated in isolated aortic rings from streptozotocin (STZ)-diabetic rats. After 4 weeks, addition of quercetin (0.1 μM-1 mM) caused a significant dose-dependent relaxation of noradrenaline (NA)- and KCl-preconstricted rings in both control and diabetic groups with a significant inter-group difference of $P<0.01$. Furthermore, both nitro-L-arginine-methyl ester (L-NAME, 100 μM) and indomethacin (10 μM) markedly and significantly attenuated the vasorelaxant responses following quercetin application. On the other hand, the responses to quercetin were not significantly changed by pretreatment of the aortic rings in a calcium-free medium. It is concluded that the quercetin can relax the preconstricted rings of aorta in STZ-diabetic rats through nitric oxide- and -prostaglandin-dependent pathway.

Effect of Mixture of 6 Plant Extracts on Blood Glucose level in Diabetic Mice

P 178

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In the present study the hypoglycemic effect of six plant extracts and their mixtures was evaluated in two animal models; control group and diabetic mice group. Plants included : *Rosmarinus officinalis, Arctium lappa, Vaccinium myrtillus, Urtica dioica, Rosa canina, Citrullus colocynthis*. Diabetes was induced by intra-peritoneal injection of streptozotocin (STZ) at a dosage of 80 mg/kg per day. After 24 hours of food deprivation, blood sampling was done before oral administration of the extracts, immediately and after 1, 2, 3 and 48 hour by the tail vein puncture. Blood glucose was determined by glucose oxidase method. A mouse was declared diabetic if it was found to have glucose level >12.0 mmol/l.

In control groups, plant extracts had no significant effect on blood glucose reduction, except extract of *C.colocynthis* which only showed significant effect after 3 hours. In diabetic groups *R. officinalis* and *V.myrtillus* decreased blood glucose level after 1 hour, and *C .colocynthis* induced hypoglycemic effect after 3 hour. Oral administration of *U.dioica* and *A.lappa* extracts decreased blood glucose level throughout the 3 hour sampling period ($p<0.05$). *A.lappa* effect on glucose reduction was more pronounced (until 48h). The most significant effect was observed with mixture of 6 extracts which induced hypoglycemic effect up to 3 hours, and reduced blood glucose level to that of control group ($p<0.001$).

P 179 Potential health promoting effects of flavonoids - A comparative study on hypolipidemic and hypoglycemic activities

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Administration of flavonoids from different plant sources namely *Punica granatum, Solanum melongena, Piper betal, Zingiber officinalis, Trichossanthes diocia, Beta vulgaris, Mimosa pudica,* Inflorescence of *Cocos nucifera* and *Losonia inermis* at a dose of 1 mg/100gBW/day showed potent lipid lowering activitiy. Significant reduction in concentration of cholesterol, triglycerides and free fatty acids were observed. Concentrations of hepatic & fecal bile acids and fecal neutral sterols were elevated significantly in the experimental groups indicating hypolipidemic activity of the above flavonoid sources. Highly significant hypolipidemic activity was observed in animals supplemented with flavonoids from *Punica granatum, Solanum melongena and Inflorescence of Cocos nucifera*. Concentration of blood glucose showed maximum significant retardation in animals administered with *Mimosa pudica* flavonoids.

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P 180 Can stevioside in combination with soy-based dietary supplement be a new useful treatment of type 2 diabetes and the metabolic syndrome?

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Background: The Stevia rebaudiana Bertoni (SrB) plant has been used by the Guarani Indians in Paraguay and Brazil in the treatment of diabetes and dietary supplement of soy protein (Abalon®) has shown to have beneficial effects on CV risk markers. Aims: To investigate if the combination of stevioside (Ste) and Abalon® possesses beneficial qualities in the treatment of type 2 diabetes (T2D) and the metabolic syndrome. Materials and Methods: Diabetic GK rats were fed for 4 weeks with 4 different diets. A) Standard diet (Chow), B) Chow + Ste (0.03 g/kg BW/day), C) 80 % Abalon® + 20 % Chow and D) 80 % Abalon® + 20 % Chow + Ste 0.03 g/kg BW/day. An i.a. catheter was inserted in the artery of rats and which underwent an i.a. GTT (2.0 g/kg BW). Statistic: two way-ANOVA. Results: Ste exerts beneficial effects in the T2D GK rat i.e.: 1) lowers blood glucose (A vs. B a 31 % and C vs. D a 86 %, $p<0.00005$); 2) Increase of the first phase insulin secretion (0-30 min): (A vs. B a 80 % and C vs. D a 163 %; $p<0.003$); 3) Suppresses glucagon : (A vs. B by 28% and C vs. D by 49 %, $p< 0.0004$); 4) After 2 weeks of treatment with Ste a 12 % suppression of the systolic blood pressure was observed ($p<0.0002$). Abalon® had a beneficial effects on CV risk markers i.e : 1) Lowers total-cholesterol (A vs. C by 15%, $p<0.0043$); 2) Reduces Triglycerides (A vs. C by 47%, $p< 0.0028$); 3) Reduces FFA : (A vs. C by 13 %, $p<0.02$). Conclusion: The combination of Ste and Abalon® appears to possess the potential as effective treatment of a number of the characteristic features of MS

Hypoglycemic activity of *Bauhina monandra* leaves in alloxan induced diabetic rats

P 181

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Renewed interest in alternative medicines has stimulated a new wave of interest in traditional practices. The WHO Expert Committee on diabetes has also recommended further investigation into traditional methods of treating this ailment because of problems associated with insulin therapy and side effects of oral hypoglycemic agents. Our interest in *Bauhinia monandra* arose because we have noticed in previous field surveys that other species of the genus are used traditionally for the treatment of diabetes. We report the hypoglycemic activity of this specie for the first time. Hyperglycemia was induced in albino rats by glucose-loading and by intraperitoneal injection of alloxan. Leaf extract (1000mg/kg) were administered orally to groups of normal, glucose-loaded (1000mg/kg) and diabetic rats and compared with untreated control animals and also glibenclamide-treated rats. Blood glucose was determined by a glucometer. Crude extract exhibited significant anti-diabetic activity ($P<0.05$). Peak effect was observed on 5th day (56.2%). Flavonoids, alkaloids and saponins were detected in the leaves.

Salvia officinalis: a medicinal plant for the treatment or prevention of type 2 diabetes *mellitus*?

P 182

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A *Salvia officinalis* (sage) extract has been shown to have hypoglycaemic effects in normal and alloxan-diabetic mice (1). We also verified that the drinking of sage tea (the common form of consumption of this plant – composition as described in 2) for 14 days lowered fasting plasma glucose levels in normal mice but had no effect on the response to an intraperitoneal glucose tolerance test. In this study we purposed to elucidate the mechanism(s) behind the observed effects. We hypothesize that the lower plasma glucose observed in fasted tea drinking animals resulted from effects on the liver namely on inhibition of gluconeogenesis. Normal and streptozocin (45mg/Kg bw) induced diabetic Wistar rats were fed with normal laboratory pellet diet and given water or sage tea for 14 days. On day 14th hepatocytes were isolated and primary cultures were used to test cell responses to insulin and glucagon in media of different compositions. Glucose production by hepatocytes of sage tea drinking fasted normal animals was lower than that of controls (water drinking) and stimulation of gluconeogenesis by glucagon was also lower. When incubated in the presence of high glucose, glucose consumption by hepatocytes of sage tea drinking normal rats was also significantly increased. In co-incubations with essential oil there was a potentiation of the effect of insulin on glucose consumption and inhibition of glucagon's effect on gluconeogenesis. In hepatocytes from diabetic rats, however, none of the above described effects were significant. Nevertheless, metformin (a reference inhibitor of gluconeogenesis) was effective in lowering glucose production. This response to metformin was not changed by sage tea drinking which indicates that there are no negative interaction between sage and this drug used in the treatment of type 2 Diabetes Mellitus (T2DM). These results seem to indicate that sage tea does not possess antidiabetic effects at this level. However, due to its glucose lowering effects in normal animals, sage tea may be of use in the prevention of T2DM.

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P 183 Effects of soy and the *Cimicifuga racemosa* extract on fat tissue and bone mineral density in orchidectomized (orx) rats

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Isoflavones present in soy preparations are believed to partially protect against osteoporosis, an effect which did not remain undisputed on the basis of experiments in ovariectomized (ovx) rats. Recently we demonstrated that extracts of Cimicifuga racemosa (CR BNO 1055) protected ovx rats better against osteoporosis than soy. Therefore, we compared in the present study the effects of soy (12.5 or 50.2 mg/day) and CR (33.3 or 133 mg/day) in orx rats. Determination of bone mineral density of the metaphysis of the tibia was determined prior to and 3 months after orx when the animals were sacrificed. Within this time sham-fed control animals lost 41.2 % of their mineral density and increased the size of various fat depots, an effect almost totally prevented by estradiol (bone loss 13 %) and testosterone (bone loss 14 %; $P<0.05$ for both substances). BNO 1055 at the higher concentration had a bone-sparing effect (bone loss 26%) and was thereby significantly ($P<0.05$) more effective than phytoestrogen-free food to prevent bone loss. No significant bone-sparing or lipolytic/antilipotropic effects were observed in the soy-treated animals at both doses. – These results indicate that yet unidentified compounds in CR BNO 1055 have antiosteoporotic effects in the metaphysis of the tibia where it partially prevented the development of osteoporosis in male orx rats. Castration-induced obesity is also prevented by E2 and CR BNO 1055.

P 184 Adipogenic effect of genistein in prepubertal and juvenile male mice

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The adipose tissue has been recently shown to be a major endocrine system that plays a role in energy homeostasis, glucose and lipid metabolism, immune response and also reproduction.
Estrogens promote, mantain and control the typical distribution of body fat and adipose tissue metabolism, through a still uncharacterized mechanism. These steroids are known to regulate fat mass by increasing lipolysis through the regulation of the expression of genes that regulate adipose deposition (lipogenesis), differentiation and adipocytes maintenance.
Males and females both express estrogen receptor (ERα and ERβ) in the white adipose, although, their role in male fat is less clear than in females. In both genders ERα seems to play a major role with regard to fat regulation comparing to ERß. Adipose deposition and distribution, which develope with aging, may be an independent predictor of cardiovascular diseases and insulin resistant diabetes in men and in women
Here we test the hyphotesis that genistein can have very different effects on the adipose tissue depending on the dose of intake. We studied the genistein's effects on adipose deposition in male mice at different nutritional doses. Our results indicate that genistein is adipogenic in young mice at levels as are present in diets containing different amounts of soy, soy milk or in food supplements containing soy. The adipogenic effect of genistein was in contrast with the lipolytic action of estradiol.

***Acmella ciliata*: Phytochemical screening and investigations on inhibition of the activity of the human neutrophil elastase**

P 185

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Acmella ciliata (H.B.K.) Cass., Asteraceae, is an annual herbaceous plant indigenous to the South American tropics. *Acmella c.* is traditionally used by the natives to relieve toothache and as a spice because of its anesthetic effect and its slightly hot taste. Antimicrobial, insecticide, and antithrombotic effects have been reported. The preparation SPOLERA® (company: OTW) which is registered and traded on the German market contains an isopropylic extract of the epigeal plant. This preparation is recommended and is successfully medically used to treat strain, contusion, sprain, haematoma etc.. Precise phytochemical as well as pharmacological investigations have in the most part not been carried out. By DC and HPLC investigations of various extracts of the epigeal parts of *Acmella c.* the following substance groups could be detected: flavonoids, saponins, phytosterols, phenolcarbonic acids and isobutylamides. Some of these isobutylamides were first described by Martin and Becker in the 1980s (1). Regarding the indications mentioned above, enzymatic tests (2) have been done using the enzyme neutrophil elastase. This enzyme belonging to the serine proteinases is involved in the process of degradation of connective tissue. A high titre of this enzyme is supposed to be one of the factors which are responsible for the progression of inflammations, arthritis and cancer metastasis. An enzymatic screening on various extracts and fractions extracted by solvents of different polarities as well as on CO_2-extracts to determine their inhibitory activities (IA) and inhibitory concentrations (IC) was carried out. Some samples showed a significant inhibition. IA-values are in the range of 64 to 85% (50 µg/ml extract concentration). The corresponding IC_{50}-values rank from 1 to 10 µg/ml.

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Anti-inflammatory and cytotoxic activities of a new flavanone isolated from *Lippia graveolens* H. B. K. var. *berlandieri* Schauer

P 186

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Due to its high quality essential oils, *Lippia graveolens* has been subject to several chemical analyses (1). Now we wish to report the isolation of the new 7-O-(glucosyl)-5,6,7,3´,4´-tetrahydroxy-flavanone (**1**) from the stems of *L. graveolens*. The identification of **1** was achieved by conventional spectrometric methods (RMN ^{1}H, ^{13}C, 2D, IR, DO and HRMS). The hydrolysis of **1** afforded the 5,6,7,3´,4´-tetrahydroxy-flavanone (**2**), also the peracetate derivative (**3**) from **1** was obtained. The results of the anti-inflammatory activity, in the TPA-edema induced test, showed 34.2%, 35.5% and 30.4% of edema inhibition for **1**, **2** and **3** respectively. On the other hand, although **1**-**3** were cytotoxic, at 50µg/ml doses, to MCF-7 (breast), HCT-15 (CNS), K562 (leukemia) and U-251 (colon) human cell lines, nevertheless all the cell lines tested were more susceptibility to the presence of the peracetate derivative (**3**). The evaluation was carried out using the SRB assay (2).

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P 187 Phytochemical investigation and biological activity of *Cyclamen repandum*

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As part of a series of phytochemical investigations for bioactive compounds from medicinal plants we investigated *Cyclamen repandum* S. et S. (Primulaceae). The tubers of this plant were used in Sardinian folk medicine as abortive, others species of the genus are used in Turkish against infertility. Early investigation on the different *Cyclamen* species resulted in the isolation of triterpenic saponins (1-3). At our knowledge no phytochemical and biological data are available about *C. repandum*. In this paper we report the chemical investigation, anti-inflammatory and anti-nociceptive activities of *C. repandum* tubers. The powdered tubers were extracted using solvents of increasing polariry (petroleum ether, chloroform and methanol). The residues were tested for the biological activities. The activities were evaluated *in vivo* with two experimental procedures: rat paw's edema and writhing in mice. The results obtained with methanol extract indicate a significant reduction of edema, after oral administration of 75 mg/Kg of dried residue. As concerns the writhing test, a very significant decrease of the number of writhes was observed, after oral administration of 150 mg/Kg. As frequently natural active principles, endowed with anti-inflammatory activity exhibit antioxidant power, it seemed interesting to evaluate this capacity. For this purpose the same extracts were tested with Briggs-Rauscher reaction (4). No antioxidant power was recorded. Nevertheless the saponin content justifies the pharmacological results. The active extract was fractionated using combined column chromathography (silica gel, Sephadex LH 20 and preparative HPLC). Five compounds were isolated. Compounds **1** and **2** were characterized by HR mass spectrometry, 1D and 2D NMR experiments as triterpenoid saponins, namely deglucocyclamin and deglucoanagalloside B. The structural elucidation of **3**- **5** is in progress.

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P 188 Antinociceptive and Anti-inflammatory Activities of *Ballota inaequidens*

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Ballota inaequidens Hub.-Mor & Patzak (BI), is a member of *Lamiaceae* and is found in South Anatolia (1). *Ballota* species have been used in Turkish folk medicine as antiulcer, antispasmodic, diuretic, choleretic, anti-haemorrhoidal and sedative agent (2,3,4,5).
Water extract of BI was investigated for antinociceptive and anti-inflammatory activities in mice and rats. The tail flick test, acetic acid-induced writhing test and the carrageenan-induced rat paw oedema test were used to determine these effects. Our findings show that BI cause dose releated inhibition in the acetic acid-induced abdominal stretching in mice. BI showed no significant changes in the nociceptive threshold of the tail flick test. BI also showed an inhibition of paw oedema induced by carrageenan. The present study reveals that the water extract of *Ballota inaequidens* possesses promising antinociceptive and anti-inflammatory activities.

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Analgesic And Hepatoprotective Effects Of *Chelidonium majus* L.

P 189

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Chelidonium majus L. is a member of the Papaveraceae known and is found in North Anatolia (1). *Chelidonium majus* (CM) has been used in folk medicine as diuretic, choleretic and hipnotic (2). It was used for skin conditions such as blister rashes, scabies and warts (3). The most effective alkaloid components of the plant (chelidonine, chelerythrine, coptisine, sanguinarine, berberine etc.) have spasmolytic, antiulcer, antiinflammatory, antimicrobial, antiviral, antifungal and antitumor activities and cytotoxic properties (4). The thiophosphoric acid chelidonine derivative from this plant called ukrain preparation, has been extracted by J.W: Nowicky (USA Patent no. 2, 670, 347) and has been tested with good malignotoxic activities in many cell lines (5). Öztürk et al. reported that CM is used for the treatment of liver diseases as an infusion (6). Water extract of CM was investigated for analgesic and hepatoprotective effects in mice. Analgesic activity of the extract was tested using tail-flick test. Mice were injected CM extract intraperitoneally in doses of 50 mg/kg, 100 mg/kg and 200 mg/kg, respectively. Pain tresholds were measured with tail-flick test before administration and at 30th, 90th and 150th minutes after treatment. Data were analysed with one-way variance analyses. The aqueous extract of CM had no analgesic activity at 50 and 100 mg/kg dose. However at 200 mg/kg dose, it produced higher analgesic activity than aspirin at 90th minute. At the 150th minute, its analgesic activity was equal to that of aspirin. Hepatoprotective activity of CM on carbon tetrachloride (CCl_4) induced acute liver toxicity was also studied. The extract showed no significant influence on the increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubine in CCl_4 treated animals (P>0.05). Histopathological examination did not reveal any significant difference between CM extract and CCl_4 groups. Thus, these results show that the extract of CM has no hepatoprotective effect on CCl_4-induced acute liver toxicity.

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Anti-inflammatory effects of total extract of *Curcuma amada* in a rheumatoid arthritis rat model

P 190

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Previous studies have suggested efficacy of parenteral administration of *Curcuma amad*a in animal models. In this study, topical effect of total extract of curcuma amada (CA) on chronic inflammatory response induced by Freund's Complete Adjuvant in rat was studied.
The adjuvant contained heat killed mycobacterium tuberculosis suspended in mineral oil (Arlacel A) and light paraffin. Injections were made in the right ankle foot and tibio-tarsal joint region of rats. On day 15 following adjuvant administration, animals were treated with 100 mg/kg dose of the total alcoholic (50%) extract on paw daily for 20 days. Body weight, arthritic index, joint diameter and blood level of TNF-alpha were recorded during the experiment and at the last day of treatment. It was concluded that the alcoholic extract could significantly ($p<0.05$) reduce joint diameter (29%) , arthritic index and blood level of TNF-alpha (61%) of treated animals compared with those of the control group. The data points out to the potential anti-rheumatoid efficacy of topical administration of CA extract in rats.

P 191 An anti-inflammatory and anti-nociceptive effects of hydroalcoholic extract of *Satureja khuzistanica* Jamzad extract

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Inflammation is a pathophysiological response of living tissues and a defense mechanism (1). Numerous studies have been carried out to develop more powerful anti-inflammatory drugs with lesser side effects. *Satureja khuzistanica* Jamzad (Lamiaceae) is an endemic plant that widely distributed in the southern parts of Iran. This plant has been used as analgesic and antiseptic among the inhabitants of southern parts of Iran (2). The *Satureja khuzistanica* hydroalcoholic extract was prepared and its anti-inflammatory and anti-nociceptive effects were investigated using the carrageenan-induced rat paw edema and formalin test. A similar anti-inflammatory activity was seen between *S. khuzistanica* hydroalcoholic extract (150 mg/kg; i.p.) and indomethacin (4 mg/kg; i.p.) in carrageenan test. The extract showed anti-nociceptive activity in a dose-dependent (10-150 mg/kh; i.p.) manner at the second phase of formalin test which was comparable with morphine (3 mg/kg; i.p.). This study confirms that anti-inflammatory and anti-nociceptive properties of *S. khuzistanica* are comparable to those of indomethacin and morphine. Presence of flavonoids, steroids, essential oil, mainly carvacrol and tannin might be responsible for anti-inflammatory and anti-nociceptive activities of this plant.

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P 192 Investigation of anti-inflammatory activity of complex herbal oily extract *in vivo*

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Arthritis is a common, chronic, progressive, skeletal, degenerative disorder. Bases on information gleaned from literature and own preliminary experiments we have prepared complex oily extract of *Boswellia serrata* resin, *Curcuma longa* roots and *Pinus sibirica* seeds. Complex extract called as Artroflex. Anti-inflammatory activity was studied on model of carrageenin and formalin induced edema of rat's paw and formalin induced edema of rabbit's knee joints. Forty Wister rats of either sex were used for each model and 51 rabbits of the Chinchilla race were used in formalin induced edema. Paw edema was induced by injection of 0.05 ml carrageenin in the planter aponeurosis of right hind paw of rats. In next experiments formalin 0.1 ml was injected into the subplanter area of right hind paw of albino rats and in the right knee joints of rabbits. Tested Artroflex, and reference drugs Phenylbutazone and Diclophenac were given 2 days one time and on 3rd day 4 hours prior to carrageenin or formalin injection. In experiment on formalin-induced edema test drugs were given subsequently for 7 consecutive days. Results were registered 3, 12 and 24 hours after carrageenin injection and 1, 4 and 8 days after formalin injection.In 24 hours after carrageenin injection paw volume was normalized in Artroflex group. Artoflex was in 19% effectively than phenylbutazone. In 8 day after formalin injection Artroflex was in 15% effectively than phenylbutazone. The response time of rats (on hot plate test) receiving Artroflex in 3 hours after carrageenin injection increase in 3 times in comparison with carrageenin group. While phenilbutazone had lover effect. Maximal effect of Artroflex was observed in 4 day in experiments of formalin-induced edema on rats. It was established, that application of Artroflex practically normalizes temperature of rat paw in 12 hours after carrageenin injection and in 4 day after formalin injection. Artroflex in dose 0.3 g/rat inhibited formalin-induced inflammation in rats in 4th day. Artroflex did not stimulate negative reactions in red and white cells of blood of rabbits. A pathomorphologic investigation has shown that destruction of knee cartilages stopped in 7 day of treatment and some thickening of cartilages was observed.

Evaluation of the anti-inflammatory activity of peniocerol, macdougallin and chichipegenin

P 193

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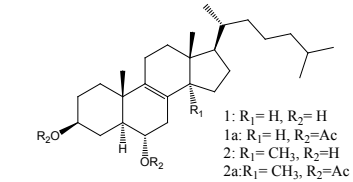

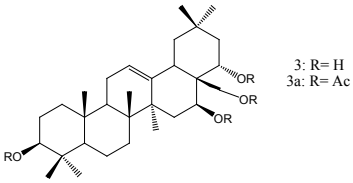

Continuing with our systematic study of natural anti-inflammatory compounds from the Mexican medicinal plants, now we wish to report the anti-inflammatory activity of peniocerol (**1**, 3β,6α-diol-Cholest-8-ene), macdougallin (**2,** 14α-methyl-3β,6α-diol-cholest-8-ene) and the triterpene chichipegenin (**3**). All the compounds were isolated from the roots and aerial parts methanolic extracts of *Myrtillocactus geometrizans* (Martius). Every compounds showed potent anti-inflammatory activity in the TPA-induced mouse ear edema model (**1**, IC_{50} =0.091, **2**, IC_{50} = 0.27 and **3** IC_{50} = 0.172 μmol/ear). However, only **1** was active in the carrageenan-induced rat paw edema (50.0 % of inhibition of edema). All compounds were inactive at dose of 1 mg/ear, in the EPP-induced mouse ear edema model. The two hydroxyl groups appears to be important for the anti-inflammatory activity in the TPA model, since the di- and tetra-acetate derivatives **1a**, **2a** and **3a**, showed a decrease activity in comparison with the parent compound.

Scrovalentinoside, an iridoid from *Scrophularia auriculata*, as an anti-inflammatory agent in experimental delayed-type hypersensitivity

P 194

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Scrovalentinoside (SV), an iridoid isolated from *Scrophularia auriculata* (1), has previously been found to exhibit anti-inflammatory activity (2). In the present work, we now report on the effect of SV on the delayed-type hypersensibility (DTH) in mice induced by oxazolone (OXZ), dinitrofluorobencene (DNFB), and sheep red blood cells (SRBC) (3). Because lymphocytes are the main type of cell implicated in DTH, the *in vitro* studies focused on the effect of SV on their proliferation and cell cycle, as well as on the production of different mediators by human lymphocytes (4). We found that at a dose of 0.5 mg/ear, SV significantly reduced the DTH reaction induced by OXZ by 57% at 96 h (* $P < 0.05$, Dunnett's *t*-test); however, it exhibited no activity in the early stages of the process (24 and 48). In contrast, SV had no significant effect on the DTH reaction induced by DNFB. For its part, the DTH reaction induced by SRBC in mouse paws was clearly and significantly reduced by SV (10 mg/kg) by 46% at 48 h (* $P < 0.05$, Dunnett's *t*-test), but had no significant effect at 18 h and 24 h. In addition, SV was found to be an inhibitor of lymphocyte proliferation with an IC_{50}= 68.8 μM, arresting the cell cycle in the G_1 phase. The production of mediators such as interleukin-1β (IL-1β), interferon-γ (INF-γ), and tumour necrosis factor-α (TNF-α) by lymphocytes was significantly inhibited by SV: IC_{50} values of 27 μM for IL-1β, 16.6 μM for INF-γ and 68.3 μM for TNF-α. In conclusion, all the reported data justify the effect of SV as an anti-inflammatory agent for the skin in cases of DTH reactions.

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P 195 Topical anti-inflammatory activity of *Salvia hierosolymitana* Boiss. leaves

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Salvia hierosolymitana Boiss. (Lamiaceae) is a perennial herbal plant, which aerial parts are used in Jordan folk medicine for the treatment of inflammatory-based diseases (**1**). To verify the therapeutic effectiveness of the plant, *S. hierosolymitana* leaves were investigated for their *in vivo* topical anti-inflammatory properties following a bioassay-guided fractionation procedure. By extraction with solvents of increasing polarity, hexane, chloroform, chloroform-methanol and methanol extracts were obtained and evaluated for their ability to inhibit the Croton oil-induced ear dermatitis in mice (**2**). Each extract, except the methanol one, exerted a significant anti-inflammatory activity. The chloroform extract was the most active, inducing a dose-dependent oedema inhibition, comparable to that of the reference non steroidal anti-inflammatory drug indomethacin (ID_{50} = 120 and 93 μg/cm^2, respectively). Phytochemical investigation of this extract led to the isolation of eight polyhydroxylated triterpenes of ursane and oleanane series, besides rosmarinic acid. Four triterpenes were new compounds, whose structures were elucidated by NMR and MS spectroscopy as 3β,6α,23-trihydroxy-urs-12,19(29)-dien-28-oic-acid; 23-(*trans-p*-coumaroyloxy)-3β,6α,23-trihydroxy-urs-12-en-28-oic-acid, 2α,3β-dihydroxy-olean-28-oic acid and 2α,3β-dihydroxy-olean-24-nor-4(23),12-ene. Each compound (0.3 μmol/cm^2) induced significant oedema inhibitions, ranging from 11 % to 60 %. Equimolar dose of indomethacin reduced the oedematous response by 57 %. The anti-inflammatory activity of the polyhydroxylated triterpenes play a significant role in the overall antiphlogistic effect of *S. hierosolymitana* leaves, whereas rosmarinic acid seems do not contribute significantly to the activity of the plant. These findings support the use of this species in the traditional Jordan medicine as an anti-inflammatory remedy.

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P 196 Evaluation of the topical anti-inflammatory activity of ginger dry extracts

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The rhizome of *Zingiber officinale* Rosc. (ginger; Zingiberaceae) is widely used for motion sickness, nausea and vomiting in pregnancy and in antineoplastic therapy. Since it inhibits *in vitro* production of inflammatory mediators, such as prostaglandins and leukotrienes, we focused on its topical anti-inflammatory properties. Cutaneous application of a ginger extract appears interesting as its probable active principles, gingerols and shogaols, are low molecular weight molecules with moderate solubility in water and in oil. Therefore, a commercial and a gingerol-enriched ginger extract were evaluated for their topical anti-inflammatory activity as inhibition of Croton oil-induced ear oedema in mice (**1**) as well as for skin permeation using modified Franz diffusion cell and human stratum corneum and epidermis (SCE) as membrane (**2**). The quantitative analysis of the extracts, performed according to the USP-NF ginger monograph, showed that the 6-gingerol content was 1.6 % w/w and 5.5 % (w/w) in the commercial and enriched extract, respectively. The amounts of all the other main components increased in the enriched extract of about four fold. Ginger commercial extract exerted a significant anti-inflammatory effect, comparable to that of the reference drug indomethacin: 100 and 300 μg/cm^2 of the extract induced 42 % and 67 % oedema inhibition, respectively, similarly to the non steroidal anti-inflammatory drug indomethacin which induced 55 % reduction at 100 μg/cm^2. The same doses of gingerol-enriched extract (100 and 300 μg/cm^2) showed slightly lower effect, reducing the oedematous response by 35 % and 62 %, respectively. The tested commercial extract showed a dose-depending anti-inflammatory activity not far away from the potent reference drug. The 6-gingerol and the other considered constituents seem to be not involved in the anti-inflammatory activity of the tested ginger extracts.

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Evaluation of the anti-inflammatory and antinociceptive activity of *Pistacia vera* L.

P 197

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The ethanolic and aqueous extracts prepared from different parts of *Pistacia vera* L. (Anacardiaceae) were evaluated for *in vivo* anti-inflammatory and antinociceptive activities. Among the extracts screened, only the resin possessed remarkable anti-inflammatory activity against carrageenan-induced hind paw edema model in mice without inducing any gastric damage at both 250 and 500 mg/kg doses whereas the rest of the extracts were inactive. While the resin was found to display significant antinociceptive activity at 500 mg/kg dose, the ethanolic and aqueous extracts belonging to fruit, leaf, branch and pedincul of *Pistacia vera* did not show any effect on antinociceptive activity in varying degrees against p-benzoquinone induced abdominal contractions in mice.

Anti-inflamatory activity of extracts obtained from different *Hypericum* species

P 198

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St. John`s wort has been used as a medicinal plant for more than 2000 years. It is one of the most popular remedies for depression, anxiety and unrest. Its external use for relieving inflammation and promoting wound healing is well known. The aim of this study was to investigate anti-inflammatory activity of extracts obtained from some *Hypericum* species, present in Flora of Serbia. Ethanolic extracts of six *Hypericum* species: *H. barbatum* (H1), *H. androseamum* (H2), *H. richerii* (H3), *H. hirsutum* (H4), *H. perforatum* (H5) and *H. perforatum* cultivated on mountain Tara (H6) were tested in comparison with indomethacin (IND) by using carrageenan-induced rat paw edema test as a model for general inflammation. Air-dried aerial parts were extracted by 96% EtOH in Soxhlet and solvent was evaporated. Dry extracts were used in doses of 25-200 mg/kg (p.o.). Indomethacin was used as reference drug in doses of 1-8 mg/kg (p.o.). Our results (Table 1.) indicate that all examined extracts posses anti-inflammatory activity especially extracts H4, H5 and H6. There was no statistically significant difference among tested doses. Dose-dependent effect was noticed in other three extracts (H1, H2 and H3) which showed significant, but, lower activity than extracts H4, H5 and H6. A qualitative reversed-phase HPLC method has been developed for the analysis and characterisation of the extracts. The method enables separation of the main constituents: flavonoids, hypericins, phloroglucinols and phenolic acids.

Table 1. Mean effective doses (ED-50) of extracts and indomethacin

Extracts	ED-50 (mg/kg p.o.)	95% Conf. limits
H1	90,15	35,42 – 229,46
H2	148,01	62,73 – 349,20
H3	120,52	55,47 – 261,86
H4	46,75	11,11 – 196,81
H5	47,55	8,35 – 270,71
H6	50,86	10,96 – 236,17
Indomethacin	2,48	0,67 – 9,13

P 199 Antioxidant and anti-inflammatory activity of *Hypericum rumeliacum* Boiss. methanol extract

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Aerial parts of *Hypericum rumeliacum* Boiss. (Guttiferae), a perennial herb growing wild in Greece, were subjected to biological and phytochemical studies *H. rumeliacum* methanol extract showed, on the DPPH test (1), antioxidant activity ($IC_{50} = 23.61$ μg/ml). The anti-inflammatory activity of the extract (70 mg/kg, i.p.) in the carrageenan-induced paw oedema in rat was studied (2). In this experimental model an inhibition of paw oedema, significant from first to third hour, was observed. The results were statistically analyzed by Student's "t" test. Moreover, histological examination was carried out. Biopsies of paws, taken 3 hours following the intraplantar injection of carrageenan, were observed and photographed with BH_2 Olympus microscopy. The histological examination confirmed the resolution of inflammatory process observed *in vivo*.
The HPLC analysis of *H. rumeliacum* methanol extract led to the identification of the naphtodianthrones hypericin, pseudohypericin and some polyphenol compounds as chlorogenic acid, rutin, and isoquercitrin.
The antioxidant activity of *H. rumeliacum* could be related to polyphenol compounds that are, as it is well known, the main contributors to the free radical-scavenging effects. The anti-inflammatory effect of *H. rumeliacum* methanol extract could be due to the synergic action both of hypericin and polyphenol compounds (3, 4, 5).

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P 200 Antiinflammatory Effect of Rhizome and Root of *Potentilla erecta* L. Raeuschel and *Potentilla alba* L. (Rosaceae)

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Potentilla erecta is greatly found in European and Asian countries and in Northwest Africa. *Potentilla alba* is found in Central and South Europe. *Potentilla erecta* is used for treatment of diarrhea, ulcerous colitis and mouth rinsing due to high percentage of tannin it contains. (1). As the continuation of the previously performed research of Potentilla (2) species, there has been performed a research of local anti-inflammatory extract of rhizome and root of plants *Potentilla erecta* and *Potentilla alba*. Plant material was collected in autumn 2002. Local anti-inflammatory effect of the extract of the tested plants was tested on a modified model of a mouse ear (3). For the purpose of provoking inflammation, both mouse ears were applied 3% *Oleum crotonis* acetone solution, in quantity of 10 μL. Application of extract was a one-off application, two hours after provoking the inflammation. Ear was observed for three days, and appearance changes were expressed in scores 0 to 14. Before application to a mouse ear, the acetone & ethanol extract was prepared, which had total 5% of phenolic compounds. Hydrocortisone 1% ointment was used as a comparative substance. The strongest pharmacological reaction was found with acetone extract of rhizome *Potentilla alba*, whose pharmacological reaction was similar to Hydrocortisone ointment, and then ethanol extract *Potentilla erecta* and *Potentilla alba*, while the weakest reaction was found with acetone extract *Potentilla erecta*. Acetone and ethanol extracts of the tested plant species Potentilla have significantly reduced inflammation in time for 30-50% in relation to control ear. According to the results, the tested extracts' effect on the inflammation process is weaker than the effect of Hydrocortisone ointment.

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Antianiogenic saponins from *Maesa lanceolata* leaves

P 201

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Cancer chemoprevention, a term coined by Sporn in 1976, can be defined as the prevention, inhibition, or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet. Because carcinogenesis is a multistage process there is considerable opportunity for intervention and a number of potential targets for chemoprevention have recently been identified. Compounds that interact during the tumor promotion/progresson phase can exhibit their activity by the inhibition of angiogenesis. Amongst the four most frequently used *in vivo* models belong the rabbit corneal micropocket assay, the hamster cheek pouch assay, the rodent subcutaneous sponge model and the chicken chorioallantoic membrane assay. Ten pentacyclic triterpenoid saponins with a different substitution pattern in positions 16 (R_1), 21 (R_2) and 22 (R_3), isolated from the leaves of *Maesa lanceolata*, were tested for there antiangiogenic activity in the CAM-assay. From the results a structure activity relationship could be established and it was apparent that for maesasaponins 16,21-disubstitution is associated with a 'non-membrane irritating antiangiogenic activity' (1). Although experimental animal *in vivo* models bring a lot of light to the understanding of partial steps of angiogenesis, the transcription of obtained results to the human environment is not always successful. Therefore an *ex vivo* human tissue assay to evaluate pro-/anti-angiogenic capacity of test compounds was optimised and validated (2). Results of the maesasaponins in both assays are compared.

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Experimental study of plant polyphenols and carotenoids for treatment of macular degeneration

P 202

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A problem of treatment of the central forms of macular degeneration of retina gets the increasing importance for practical ophthalmology. Lutein and zeaxanthin are famous antioxidant complexes at treatment of macular degenerations. Their effects may be amplifies by polyphenolic, in particular anthocyanins of bilberriy and flavonoids. The aim of work was experimental study of pharmacological efficiency of complex, containing plant polyphenols and carotenoids, at treatment of rabbits with model of macular degeneration. The experiment was carried out on rabbits of the Chinchilla race. Macular degeneration modeled by direct laser coagulation in the right eye of rabbits. The left eye was the control. Polyphenol-carotenoids complexes were prepared from a powder of freeze dried bilberry, taxifolin from wood of larch and lutein from marigold extract in different proportions. Mirtilene® forte was a reference preparation. Rabbits were treated with preparations (0.46 g/kg, per os) during 21 days. The results of the test were assessed for parameters: changes of retina, electrophysiological parameters, ophthalmoscopic changes of the central area of retina (the area of the center of macular degenerations, atrophied changes, neovascularization retinas, choroidal vascularization). After of therapy it was observed complete resorption of exudates and bloods from vitreous and the central area of retina. The dystrophic centers tended to merge and make more plane. Choroidal circulation restored. The area of the center of macular degenerations under influence of preparations decreased on the average on 40-60 %. Atrophic changes of retina statistically significantly decreased, thus the maximal expressiveness of medical action was observed (decrease of inflammation by 60-65%). All complexes had positive effects in vascularization retinas, keeping choroidal vascularization and interfering with development of neovascularization. Choroidal vascularization and neovascularization retinas of polyphenol-carotenoids complex was 60% and 20%, while at Mirtilene® forte was 40% and 45%. The results have shown high efficiency of plant complex containing 81.2% bilberry, 7.1% taxifolin and lutein in therapy of macular degeneration.

P 203 Hepatoprotective activity of aqueous root extract of *Lecaniodiscus cupanioides* in rats

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The hepatoprotective effects of aqueous root extract of *Lecaniodiscus cupanioides* (LC) was investigated against bromobenzene (BB) and acetaminophen (ACN) induced acute liver damage in rats. For BB set, adult rats were divided into groups treated thus: Group I: olive oil (1mg/kg, p.o.); group II: BB (9mmol/kg in 1:1 olive-oil, i.p.) alone; group III: LC (100mg/kg, p.o.) 1h before BB; Group IV: LC (500mg/kg, p.o.) 1h before BB; and group V: methionine (36mg/kg, p.o.) 1h before BB. For ACN set, group I received methyl cellulose (13mg/kg, p.o.); group II: ACN (640mg/kg in 1% methyl cellulose, p.o.); Group III: LC (100mg/kg, p.o.) 1h before ACN; Group IV: LC (500mg/kg, p.o.) 1h before ACN; and group V: N-Acetyl-cysteine (N-AC; 150mg/kg, p.o.) 1h before ACN. 24h later, blood samples were collected through cardiac puncture of ether- anaesthetized rats and sera analysed for hepatic enzymes - aspartate amino transaminase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and the total protein (TP). In the BB groups, pretreatment with LC (100mg/kg), like methionine produced a significant ($p<0.05$) lowering of ALP. Lowering of AST and ALT was at 500mg/kg of LC. In the ACN groups, pretreatment with LC (500mg/kg), like N-AC produced a significant ($p<0.05$) lowering of AST, ALT, ALP and TP. Histological examination revealed a distinct prevention of BB –induced necrosis by LC. However, prevention was not noticeable in ACN-treated rats. The extract has been shown to contain saponins and tannins as phytochemical constituents. The results obtained in this study showed that LC possesses some Hepatoprotective effect, justifying the traditional use of the plant in the treatment of liver abscess.

P 204 Hepatoprotective effects of *Micromeria cristata* extracts against carbon tetrachoride induced hepatic injury in rats

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The hepatoprotective effects of ethyl acetate extract of the aerial part of *Micromeria cristata* have been investigated using carbon tetrachloride (CCl_4) induced liver damage in albino Wistar rats as the experimental model. Dose of 25 mg/kg of the examined extract was administered intraperitoneally to the test group. The positive control group received CCl_4 (3 mL kg^{-1} 50 % in olive oil) and the negative control group received normal saline (10 mL kg^{-1}). Sylimarin, a natural antihepatotoxic agent, has been used as a standard (25 mg/kg). Twenty four hours after intoxication with CCl_4, animals were sacrified. Blood levels of the antioxidant enzymes: superoxid dismuase (SOD), glutatione peroxidase (GPX) as well as reduced glutathione (GSH), total antioxidative status (TAS) and intensity of lipid peroxidation (LP) were used as biochemical markers of hepatotoxicity. Histopatological studies were also done to confirme the biochemical changes (1, 2). Administration of CCl_4 induced significant impairment in hepatic antioxidant status, decreasing the SOD activity, the GSH content, modified the GPX activity and stimulating liver LP. Pretreatment with *M. cristata* extract significantly improved hepatic antioxidant status in rats, which most pronounced in the reduction of CCl_4-mediated LP. In addition, in pretreated animals, an increase GSH level and SOD activity, as well as a decrease of GPX activity, was found. Histopatological studies showed that CCl_4 caused ballooning degeneration, fatty change, cell necrosis, lymphocyte infiltration and increase in Kupffer cells. *M. cristata* extract exhibit liver protection, expresed by reduction of histopatological changes. The effects of *M. cristata* extract were comparable to those of sylimarin. The results of this study indicate that *M. cristata* ethyl acetate extract exhibit hepatoprotective action in acute CCl_4 hepatotoxic model.

References: 1. Slater, T.F., Sawyer, B.C. (1971a) J.Biochem. 123: 805-814. **2.** Halliwell, B., Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, 2nd edn, Oxford Unuversity Press, Oxford.

Comparison of hepatoprotective activity of *Phyllanthus amarus* with other *Phyllanthus* species growing in south Vietnam

P 205

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Phyllanthus amarus Schum.& Thonn. (Euphorbiaceae) is a medicinal plant that has been used in traditional medicine of Vietnam and many other countries for treatment of jaundice (**1,2)**. Pharmacological studies have also confirmed its anti-hepatitis activity in vivo (**2**]) Aerial parts of *Phyllanthus* species were collected in Ho Chi Minh City (Aug. 2001). Extracts were made of *P. amarus* with water, 50% ethanol, 70% ethanol, butanone and chloroform to find out a suitable one for further formulation studies Their hepatoprotective activity was compared with that of *P.urinaria (P.u)* in a mouse model challeneged with carbon tetrachloride (**2**). The 50% ethanol extract was found to be the most active with *P.a***.** having a greater effect than *P.u* . The extracts of both species were shown to decrease the serum AST and ALT levels in comparison with a control group receiving no extract in post-treatment liver injury models (hepatoxic recovery and hepatoprotective activity).
The hepatoprotective activity of the *P.a*. 50% ethanol extract was comparable to that given by the same dose of Chophytol®, a commercial extract of *Cynara scolymus*. Isolated Gallic acid and nirurine were isolated from the active fractions of *P.amarus* (**3)**].
These results show that *Phyllanythus* species growing in Vietnam have a hepatoprotective effect, although it is not as great as that given by *P. amarus*

Acknowledgements: Prof. Peter.J.Houghton,Prof.Hyland,Group Pharmacognosy, King's College Univ.of LonDon
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Effects of Benzyl Glucoside and Chlorogenic Acid from *Prunus mume* on ACTH and Catecholamine Levels in Plasma of Experimental Menopausal Model Rats

P 206

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To investigate the effectiveness of benzyl β-D-glucopyranoside (**BG**) and chlorogenic acid (**CA**), the constituents of the fruit of *Prunus mume*, for relieving tension in experimental menopausal model rats (M-rats) caused by ether stress, the effects of **BG** and **CA** on ACTH and catecholamine (adrenaline, noradrenaline, and dopamine) levels were examined in the plasma of M-rats. Caffeic acid, quinic acid, and rosmarinic acid, which are compounds structurally related to **CA**, were also examined. **BG** obviously recovered catecholamine levels decreased by ether stress and increased dopamine to high levels. On the other hand, **CA** significantly decreased the ACTH level increased by ether stress and showed the greatest effect of all compounds. These results suggest that **BG** and **CA** may contribute to relieving the tension in M-rats caused by ether stress (1).

Reference: 1. Ina, H. et al. (2004) Biol. Pharm. Bull., 27, 136.

P 207 Effects of lavender oil and linalool on plasma ACTH, catecholamine and gonadotropin levels in experimental menopausal female rats

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The effects of inhaling the vapor of lavender oil(LO:*Lavandula burnatii super*) and linalool(LI) on plasma ACTH, catecholamine(CA) and gonadotropin(GO)levels in model rats under ether-inhalation(EI) were studied. The increased plasma ACTH levels induced by EI tended to decrease by pre-inhalation of LO and LI vapor was induced the decrease of ACTH level. The decrease in CA levels induced by EI tended to recover, especially, the dopamine level significantly recovered to the normal level by the inhalation of LO and LI vapor. The increased plasma GO levels in model rats was significantly decreased by the inhalation of LI. These results suggest that LO or LI may contribute to relieving tension and may be applicable to the treatment of menopausal disorders in human beings(1).

Reference:1. K.Yamada et al.,(2005),Biol. Pharm. Bull. 28: 378-379.

P 208 Effects of isoflavones from Red clover (*Trifolium pratense* L.) on skin changes induced by ovariectomy in rats

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Several factors contribute to the skin changes that are associated with menopause, including chronologic aging, photoaging and the withdrawal of ovarian hormones. Ovariectomy involves an absolute decrease in the production of estrogens and progesterone and tends to accelerate skin aging, just as it accelerates bone and vascular aging. The absence of estrogens slows the mitotic activity of the basal layer ot the epidermis, reduces the synthesis of collagen and that of elastic fibers. It contributes to the thickening of the general dermoepidermal junction. There is evidence that diets which high levels of phytoestrogenic isoflavones are associated with a low incidence of menopausal symptoms and osteoporosis. Plant extracts as Red clover, which contain high levels of isoflavones, have been used to reduce menopausal symptoms and have been shown to reduce bone loss in healthy women (1). In this study we have evaluated the effects of Red clover isoflavones on histoarchitecture of the skin of ovariectomized rats in comparison with the skin of no treated ovariectomized and intact rats. Bilaterally ovariectomy (2) was performed on female wistar rats. Starting 1 week the operation the rats were treated with an oral dose of 40 mg of total isoflavones daily for 14 weeks. The histological examination showed that the skin of the ovariectomized rats presented typical atrophied features. The thickness and keratinization of the epidermis were reduced; glands were less in number and vascularity was poor; the distribution and morphology of the collagen bundles and elastic fibers were altered. Whereas the skin of the ovariectomized rats treated with isoflavones appeared well organized with a normal epidermis with uniform thickness and regular keratinization; vascularity, collagen and elastic fibers were well developed. These skin changes were significantly different ($P < 0.05$) in the treated group in comparison to the control group. These findings suggest that Red clover isoflavones are effective to reduce skin aging induced by estrogen deprivation.

References: 1. Clifton-Bligh, P.B., Baber, R.J. (2001) Menopause 8: 259-265. **2.** Edgren , R. A., and Calhoun, D. W., (1957) Contraception, the chemical control of fertility. In Edgren, R.A. (ed.), Marcel Dekker, New York, p. 537.

Chemical composition and immunological investigation of *Achillea millefolium* I. Population golestan, Iran

P 209

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The genus *Achillea* (Astraceae)[1] is well known for medicinal properties such as anti-inflammatory and inhibition of bacterial growth. *A.millefolium* grows widely in the north, northeast and central parts of Iran. In this research we have studied MeOH-Water extract of *A. millefolium* for flavonoids. The plant was collected in June 2001 from Golestan province. The dried and powdered oftop flowered plant was extracted by percolation with MeOH–Water (80-20) and solvent was evaporated under reduced pressure. This extract was washed with petrol ether and $CHCl_3$ and the residue was chromatographed on Watman No.3 paper using 15% AcOH and BAW (4:1:5) as solvent respectively. The spots were detected under UV fluorescent at 365 nm before and after exposure to ammonia fumes, and after isolation and purification on Sephadex LH20 were identified by UV, MS and NMR spectroscopic methods as:luteoline-7-O-glucoside(24mg), apigenine-7-O-glucoside (24mg) and trans-caffeic acid-4-glucoside (54mg). For immunological investigation[2] BALB/c albino female mice (17-22 g) were used as experimental animals. Eight groups, each consisting of seven mice were used. These test groups received different fractions (MeOH, MeOH-Water, Water, CHCl3) of *A. millefolium* extracts intraperitoneally in doses of 31.5, 62.5, 125 and 250 mg/kg body weight daily for six days and the effects of extracts on anti-SRBC (sheep red blood cells) haemagglutinating antibody titer (HA) were studied. Among different Extracts only water extract with dose of 62.5 mg/kg indicated a significant decrease in the antibody titer ($p<0.01$ by Kruscal–Wallis test) and other fractions showed mortality in doses of more than 31.5 mg. since different extract of *A.millefolium* showed poisonous effect on mice and it seems there are some poisonous substances in fractions, it is candidate for further investigation in our laboratory.

References: 1. D. Podlech, A. Huber-Morath (1986). Achillea In: Flora Iranica, Compositae. No.158. Edits., K. H. Rechinger and I. C. Hedge, pp 49- 72, Akademische Druck Verlagsantalt, Graz, Austria. 2. R. Rezaeipoor, S. Saeidnia (1999), J. Ethnopharm., 65, 273-276.

Antiviral activity of taxol derivatives (*in vitro* and *in vivo*)

P 210

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Derivatives of taxol were considered to be biologically inactive. Now it is known that baccatin III induces apoptosis, assembly of microtubules and shows cytotoxic activity against various cell lines. The aim of our study was to estimate *in vitro* and *in vivo* antiviral activity of taxol and its three derivatives: *N*-benzoyl-(2'R,3'S)-3'-phenylisoserine, methyl (*N*-benzoyl-(2'R,3'S)-3'-phenylisoserinate), and 10-deacetylo-baccatin III (the different „parts" of taxol moiety). Cytotoxicity of the compounds was evaluated on Vero cells using MTT assay. Antiviral activity *in vitro* was assessed against *Herpes simplex virus* type 1 (HSV-1). The compounds were added in different concentrations after HSV-1 inoculation (0,01 $TCID_{50}$/cell). After 24 h viral-induced CPE was estimated and titer of the virus was calculated. In *in vivo* experiments two groups of NMRI male mice were infected with M-MSV i.m. The compounds were administered to the mice i.p. on 1st, 2nd and 4th day after virus inoculation, 100µg each day. In M-MSV-infected mice dynamics of tumor progression and regression was estimated. In *in vitro* experiments all of tested taxol derivatives significantly inhibited, in low concentrations, the replication of HSV-1 virus. Taxol did not affect virus replication. In *in vivo* experiments 10-deacetylo-baccatin III significantly lowered the diameter of the M-MSV-induced tumor. It seems that 10-deacetylo-baccatin III appear to be promising chemical compound, which show antiviral activity *in vitro* and *in vivo.* This compound and other taxol derivatives may constitute a potential source of antiviral chemotherapeutics.

P 211 An extract and phenolic compounds from *Apocynum venetum* L. exert antidepressant activity in the tail suspension test in mice

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Apocynum venetum L. (AV, Apocynaceae) is a perennial wild shrub growing in mid and north-western China. It has been shown previously that an extract prepared from the leaves of AV significantly decreased immobility time in the forced swimming test in rats, an animal model which is commonly used to detect antidepressant activity **1**. In the present study the Tail Suspension Test (TST) in mice was used as screening model for antidepressant activity. The lowest active dose of AV in the TST was 3 mg/kg (i.p.) (control: 140 ± 18 s; AV 3 mg/kg: 87 ± 12 s). Fractionation of AV extract yielded mainly a fraction S3 enriched in flavonoids. After further fractionation glycosides of kaempferol, quercetin and myricetin were isolated and characterized by NMR, MS and [α]. These compounds were tested in the TST as well but they did not show an activity. In addition, three compounds consisting of a flavonoid moiety (either quercetin or myricetin) and a caffeic acid residue (phenylpropane substituted flavan-3-ols) were isolated and characterized by NMR, MS, and [α]. Since the amount of these so called "cinchonains" or "apocynins" is relatively low in AV extract, these compounds were synthesized as described in **2** and tested for antidepressant activity in the TST.

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P 212 Evaluation of neuro pharmacological effects of *Nelumbo nucifera* leaves

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In recent years, the management and treatment of CNS disorders is a vexing problem due to its inherent complexities and the limitations of allopathic medications. There are scanty reports on the putative neuro pharmacological effects of the leaves of *Nelumbo nucifera (Family: Nymphaceae)*[NN]; hence the present work investigated gamut of its neuro-pharmacological effects. The aqueous extract of the dried powdered leaves of NN revealed the presence of alkaloids and phenolic compounds. The extract was evaluated for anti-depressant activity in Swiss albino mice using tail suspension test, despair swim test, reserpine antagonism, amphetamine-induced excitation and anorexia, and potentiation of nor-epinephrine toxicity. The quantitative estimation of catecholamine levels in mice brain was performed by fluorimetric methods. Anxiolytic activity was assessed using plus maze model, social interaction test and light dark model. Marble burying behavior model assessed anti obsessive-compulsive disorder (OCD) potential. Further, the nootropic potential of extract was evaluated using Morris water maze test. Anti-epileptic activity was evaluated using strychnine induced convulsion model in mice. One-Way ANOVA followed by Dunnett's test was applied for statistical significance. Pretreatment with NN extract resulted in decreased immobility time in tail suspension and despair swim test. Reduction in degree of ptosis and catalepsy revealed reserpine antagonism while enhancement of amphetamine induced excitation and anorexia as well as potentiation of nor-epinephrine toxicity revealed anti-depressant activity, which was confirmed by increased catecholamine levels in mice brain. Administration of NN extract resulted in preference to open arm in plus maze test, increased social interactions and increased number of crossings in light dark model. Decreased marble burying behavior contemplated its anti OCD potential. Further, the NN extract improved cognitive function with respect to spatial and working memory processes. NN extract also offered protection in strychnine induced convulsion model. In conclusion, NN extract exhibited anti-depressant, anxiolytic, anti OCD, nootropic and plausible anti-epileptic activity.

Central nervous effects of the *Salix* extract BNO 1455

P 213

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The antiinflammatory effects and the clinical efficacy of *Salix spp.* extracts cannot be explained solely by their content of salicyl alcohol derivatives. A significant reduced incidence of side effects in patients treated with willow bark extract points towards a central effect the drug. Thus, the standardized willow bark extract BNO 1455 was investigated for central effects in animal experiments. Anxiolytic activity was studied using the modified open field test and the elevated plus maze test in mice. Antidepressant activity was tested using the tail suspension test in mice and the forced swimming test in rats. The extract BNO 1455 proved to be anxiolytic as it caused a clear increase in curiosity in the open field test and increased the time on the open arms as well as the number of entries into open arms. Evidence for an antidepressant activity was obtained in the tail suspension test as well as in the forced swimming test. False negative results were excluded since no stimulation of motility was observed at active doses. The antidepressant activity was confirmed after a two weeks pretreatment period excluding false positive results. The anxiolytic and the pronounced antidepressant activity of the extract BNO 1455 – in particular- are likely to contribute to the clinical efficacy, since neuroleptics and antidepressant are used as adjuvants in patients suffering from chronic pains to increase the activity e.g. of the NASD.

Phytochemical investigation and anticonvulsant activity of *Paeonia parnassica* radix

P 214

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Plants of the genus *Paeonia* have been used since the antiquity in the traditional medicine against epilepsy. The present study concerns the Greek endemic plant *Paeonia parnassica* Tzanoud. (Paeoniaceae). The plants' roots were collected in Parnassos, air-dried, pulverized and extracted with CH_2Cl_2, MeOH and H_2O. All the extracts were studied using chromatographic and spectroscopic methods and totally 17 natural products were isolated. Seven products (e.g. paeoniflorin, albiflorin) contained the characteristic cage-like terpenic skeleton which is found only in Paeonia plants. Two of the above products (4-O-methylpaeoniflorin (1) and paeonidanin(2)) are isolated for the first time as natural products. The plants' extracts were tested for their in vivo anticonvulsant activity. The water extract of the roots was found to be the most active one in the 6 Hz test, while the methanol extract was less active. It is noteworthy that both extracts (mainly the water extract) were found to contain albiflorin, a compound that according to literature data posses anticonvulsant activity(1).

References: 1. Sugaya, A. et al. (1991) J. Ethnopharmacol. 33: 159-167

P 215 Testing for anxiolytic activity in experimental animals - Influence of the test conditions on the test result

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Since extracts from *Piper methysticum* were taken from the market there is an increasing interest in new anxiolytic phytomedicines. One of the most used and relevant test systems to detect such an activity is the elevated plus maze paradigm in mice and rats. As plant extracts may exert weaker effects as e.g. benzodiazepines the test system has to be sufficient sensitive. The impact of various test conditions on sensitivity as well as on accuracy were studied using the standard anxiolytic drug diazepam as reference.
Test parameters indicative for anxiolytic activity in the elevated plus maze are the percentage of time spent on the open arms as well as the percentage of open arm entries. Changes of the experimental conditions as the background colour of the arms, the presence or absence of small ledges on the open arms, the intensity of light and the diet as well as the gender and the strain were studied to assess the influence on the test result. Test results gained from mice were compared to those from rats. A good sensitivity of the test was obtained with naive female NMRI mice, in a maze without ledges and dark coloured arms.The outcome of the test results obtained using plant extracts proved the suitability of these test conditions.

P 216 Antioxidant and anxiolytic effects of *Apocynum venetum L.*

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The antioxidant and anxiolytic effects of *Apocynum venetum* (AV) L. were investigated in two well established assays. The Oxygen Radical Absorbance Capacity (ORAC) assay is a common and comparable method to measure the total antioxidant activity of samples *in vitro*.(1) In the present experiment, an AV extract containing tannins (TCAV), a tannin-free AV extract (TFAV) and several pure phenolic compounds of AV were investigated in the ORAC assay. The TCAV extract showed significant antioxidant activity with 1636 ± 42 µmol TE/g whereas the TFAV extract had a lower activity of 642 ± 24 µmol TE/g. The reference compound ascorbic acid showed an antioxidant activity of 289 ± 6 µmol TE/g. The pure phenolic compounds quercetin (1123 ± 34 µmol TE/g), hyperoside (1167 ± 24 µmol TE/g), and chlorogenic acid (1314 ± 29 µmol TE/g) had the highest antioxidant activity. The anxiolytic effect of AV was investigated using the Elevated Plus Maze (EPM) test in male BL6/C57J mice.(2) Different concentrations of an AV extract were compared to diazepam as reference compound. The test was performed for 6 min. 1 hour after oral administration. Diazepam (1 mg/kg) as well as higher concentrations of the extract (60 mg/kg and 125 mg/kg) increased the time spent ($p<0.05$) (Diazepam Δt 156 sec., AV 60 mg/kg Δt 123 sec., AV 125 mg/kg Δt 194 sec.) and entries on the open arms ($p<0.05$) (Diazepam Δ 25, AV 60 mg/kg Δ 21, AV 125 mg/kg Δ 31), indicating the anxiolytic effect.

References: 1. Ou B. Et al. (2001) J. Agric. Food Chem. 49: 4619-4626 **2.** Pellow S. et al. (1985) J. Neurosc. Methods 14: 149-167

Medicinal plants and epilepsy in rabbit

P 217

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St. John`s wort, celery and parsley are widely used in folk medicine. St. John`s wort (*Hypericum perforatum* L.) has external (as antiseptic and in wounds treatment), and internal application (against stomach aches, diarrhea in treatment of depression). Celery (*Apium graveolens* L., *Apiaceae*) is used chiefly as diuretic in bladder and kidney complaints as well as, in arthritic and rheumatic conditions. Parsley (*Petroselinum crispum* mill. A. w. Hill., *Apiaceae*) is used as an emmenagogue, galactogogue and stomahic. In the present study the effects of different extracts of St. John`s wort, celery and parsley on epileptic discharges were analysed. The bioelectric activity in kindking rabbits was registrated before and after intramuscular application some extract of plant. Dried roots of every plants were hashed in small pieces and extracted were successive extracted aparature with 89% ethanol. In investigation were used: ether, ethyl-acetate and $CHCl_3$ extracts. Extracts were given to the experimental animal parenteraly on a single dose of 1 ml/kg. The obtained results showed: the effects of plant extracts on epileptic discharges are dependent of the type of extract. Also, our results shows that the extracts of *Hypericum perforatum* L. have biologically active substances which influenced on epileptic observation. Effects of extracts from *Hypericum perforatum* L on epileptic discharges in rabbits depends on the type of the extract: ether extracts has increasing effect on epileptogenic discharges, while ethyl-acetate extract suppresses specific activity.

Gastroprotective effect of Astragaloside IV: Role of Prostaglandins, Sulfhydryls and Nitric Oxide

P 218

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This investigation evaluated the gastroprotective activity of Astragaloside IV, a cycloartane-type triterpene glycoside. Gastric mucosal damage was induced in rats by intragastric ethanol (1ml/rat). Rats treated orally with Astragaloside IV suspended in Tween 80 at 3, 10 and 30 mg/kg, showed 15, 37 and 52 % gastroprotection respectively. The gastroprotection observed at 30 mg/kg for this compound was attenuated in rats pretreated with L-NAME (70 mg/kg, i.p), a nitric oxide (NO)-synthase inhibitor, suggesting that the gastroprotective mechanism of this glycoside involves, at least in part, the participation of NO. The gastroprotective effect of Astragaloside IV was not affected by the inhibition of prostaglandins synthesis with indomethacin (10 mg/kg, s.c.) nor for the block of endogenous sulfhydryls with N-ethylmaleimide (NEM, 10 mg/kg, s.c.). Carbenoxolone (3-30 mg/kg) was used as gastroprotective model drug and showed dose dependent gastroprotective effect. The partial participation of prostaglandins, sulfhydryls and nitric oxide was observed in the gastroprotective mechanism of carbenoxolone.

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P 219 Pharmacological and biochemical properties of *Mentha x piperita* L. (Lamiaceae) essential oil

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The appearance of common and on one's own initiative usage of various herbal preparations in everyday practice and life imposes the question of possible interactions with drugs. In this study, acute and chronic effects of *Mentha piperita* L. essential oil (PO) (prepared as emulsion for oral use) on gut motility, pentobarbitone induced sleeping time, analgesic effect of codeine in mice, and its influence on antioxidative enzymes activity in mice liver were examined. Acute pretreatment by PO was performed 60-90 min before the examination, and chronic pretreatment during 5 consecutive days. Applied doses of PO for mice, calculated from daily human doses, were 0.1 and 0.2 mL/kg. The activity of liver enzymes (peroxidase – Px, catalase – CAT, xanthinoxidase – XOD), lipid peroxidation (LP) and reduced glutathione content (GSH) was determined in the liver homogenate. Examined essential oil increased gut motility only by acute intake in higher dose. Chronic intake of PO (in both applied doses) led to significant decrease of analgesic effect of codeine, especially 50 min. after the drug application. Acute intake of PO didn't changed this effect. Acute PO pretreatment in higher dose caused significant prolongation of pentobarbitone induced sleeping time, while it was significantly shortened by chronic PO pretreatment in the same dose. Multiple application of PO did not caused changes in the activity of examined antioxidative enzymes, LP and GSH in liver. Obtained results show possible influence of PO on drug effects, either by changing drugs' gut passage (and absorption), or by affecting liver metabolic enzymes, which should be additionally examined. Also, essential oil didn't expressed harmful effect on antioxidative systems, but its protective effect should be furthermore examined.

P 220 Protective efficacy of *Moringa oleifera* (Lam) seed extract on alcohol treated male rat's testes

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The aim of the present investigation was to assess the seed extract of *Moringa oleifera* on the testis of alcohol treated rats. Adult male rats were treated with 1) 0.5 ml of 1 % (percentage) alcohol (group II) 2) a combination of 0.5 ml of alcohol and 0.5 ml (400mg) extract of *M. oleifera* (group III) and 3) finally 0.5 ml of extract only administered (group IV), simultaneously control was maintained as group I for 50 days . The biochemical parameters such as total protein, serum cholesterol , and marker enzymes ; alkaline and acid phosphatase, lactose dehydrogenase and sperm concentration were also measured and it was significantly decreased in alcohol treated rats but these parameters were remarkably maintained in the extract plus alcohol treated rats. Significantly increased all the parameters in extract only administered rats than control. The histopathological studies of testes also shown significant changes in both the alcohol and extract treated animals.

A pilot phytopharmacological study of a standardized extract of *Solanum indicum,* ss. *distichum*, on experimental hypertension in rats

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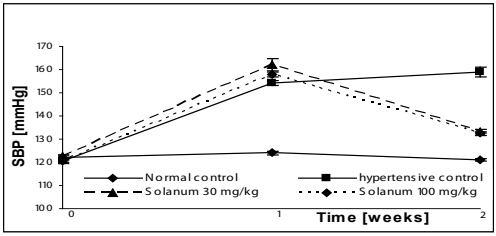

S. distichum fruits have been used in African folk medicine as an antihypertensive, but so far no studies have been carried out on the pharmacological effects of this plant. In the present work, an ethanolic extract of the fruits has been standardized by a spectrophotometrical method to contain 0.2 % total glycoalkaloids. The standardized extract was then subjected to a pilot study to investigate its potential antihypertensive action in an L-NAME model of hypertension in rats. Hypertension was induced in rats by the i.p. injection of L-NAME daily for 1 week. Systolic Blood pressure (SBP) was measured non-invasively in conscious rats. Simultaneous treatment of animals with L-NAME (i.p.) and the extract (orally) prevented the development of hypertension. Treatment of hypertensive rats with the extract in doses of 30 and 100 mg/Kg orally tended to normalize the SBP of the animals compared to control (fig.). Treatment with the extract was started 1 week after induction of hyper-tension and continued for a further week, whilst still continuing L-NAME administration. However, oral administration of the extract to normal rats for four weeks in doses up to 300 mg/kg did not show any significant hypotensive effects. The present results show a definite blood pressure lowering effect of the extract in hypertensive, but not in normal rats.

Multi-target-effect of a phytomedicinal combination containing *Iberis amara* and eight other herbal components: STW 5 (Iberogast®) in functional dyspepsia

P 222

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The phytomedicine STW 5 (Iberogast®) is a fixed combination of the fresh plant fluid extract of *Iberis amara* totalis and the fluid extracts of eight herbal drugs, Angelica root, camomile flower, caraway fruit, milk thistle fruit, melissa leave, peppermint leave, celandine herb and liquorice roots. Its clinical efficacy in functional gastrointestinal diseases is shown by several randomized controlled clinical studies and more than 40 years of clinical experience (1). STW 5 is effective in a very broad spectrum of symptoms of these multicausal diseases. Therefore the question for its mechanisms of action arises. To answer this question studies on STW 5 and its herbal components were evaluated. Pharmacological studies with regard to all pathomechanisms discussed as important causes of these diseases exist, showing the effects of STW 5 on gastric and intestinal motility, a main reason of functional disturbances, where it had a dual mechanism of action, spasmolytic in hypermotile and tonicising in hypomotile gastric muscle. As a mechanism of action a change of frequency and amplitude of the electrophysiological slow waves of the intestinal muscle was identified. In in vivo studies of gastrointestinal hypersensitivity, STW 5 decreased significantly the afferent neuronal reaction on mechanical and inflammatory stimuli. It significantly inhibited gastric acid hypersecretion and stimulated mucosa protecting mechanisms like mucus secretion in vivo. It showed anti-inflammatory and anti-oxidative effects in vivo and in vitro, so having positive effects on an important trigger of functional gastrointestinal diseases. *Iberis amara* and the other herbal components of STW 5 contribute each in a specific way in this multi-target-effect, acting in parallel or synergistically in the different pharmacological targets discussed as relevant in the therapy of functional gastrointestinal diseases (multi-drug concept, 2). Therefore the example of STW 5 (Iberogast®) verifies, that phytomedicine allows a modern, evidence-based multi-target therapy.

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P 223 "Chemical" versus "Phyto": Is efficacy equivalent? Results of a clinical study

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In the treatment of minor blunt injuries several topical drugs are known to have anti-inflammatory and analgesic properties. They represent, however, two fundamentally different major pharmacological therapy approaches: the "chemical-synthetical" and the "phytotherapeutical" approach.
The main objective of this trial (CODEC_2004) was to compare the efficacy and tolerability of an ointment of comfrey extract (Extr. Rad. Symphyti) with that of a diclofenac gel in the treatment of acute unilateral ankle sprain (distortion).
In a single-blind, controlled, randomized, parallel-groups, multicenter and confirmatory clinical trial ambulant patients with acute unilateral ankle sprains received four times a day either a 6 cm long ointment layer of Kytta-Salbe® f (Comfrey extract) or of Diclofenac gel containing 1.16 g of diclofenac diethylamine salt for 7 ± 1 days.
Primary variable was the area-under-the-curve (AUC) of the pain reaction to pressure on the injured area measured by a calibrated caliper (tonometer). It was confirmatorily shown that Comfrey extract is non-inferior to Diclofenac. The 95 % confidence interval for the AUC (Comfrey extract minus Diclofenac gel) was 19.08 to 103.09 h*N/cm^2 was completely above the margin of non-inferiority.

P 224 Effect of *Echinacea* on human immune responses in vivo

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The immunomodulatory effects of oral dosing with an Echinacea preparation were investigated in a small scale clinical trial ($n = 11$). Expression of leucocyte heat shock protein 70 (hsp70), serum chemistry, haematological values and plasma alkylamide concentrations were evaluated in eleven healthy individuals (26 to 61 years of age) at baseline (day 1) and on day 15 after consuming two commercially blended Echinacea tablets (containing both Echinacea angustifolia and Echinacea purpurea root) per day for fourteen days.
Plasma total alkylamide levels were determined one hour after ingestion of one Echinacea tablet and concentrations were found to be 12 ± 2 ng equiv/mL plasma. Echinacea supplementation significantly enhanced the fold increase in leucocyte hsp70 expression after a mild heat shock from 2.2 ± 0.4 to 3.3 ± 0.7 ($p = 0.03$). Serum chemistry and haematological values for subjects after Echinacea supplementation did not vary significantly from baseline levels with the exception of white cell counts which increased from 6.6 ± 0.4 to $7.2 \pm 0.3 \times 10^9$/L ($p = 0.04$). Differential white cell counts displayed modest increases after Echinacea supplementation although only the lymphocyte subpopulation approached significance ($p = 0.06$).
The enhanced hsp70 stress response found is indicative of an improved immune response given that increases in hsp expression following cellular stress may play a critical role in antigen presentation, lymphocyte effector function and cytokine induction. This pilot study therefore suggests that supplementation with Echinacea may invoke an immune response through altered expression of hsp70 and increased white cell counts.

Open, Multicentre Study to Evaluate the Tolerability and Efficacy of Echinaforce® Forte Tablets in Athletes

P 225

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In western civilisation the common cold belongs to the most frequent diseases and accounts for 40% of all absences from work. Adults in average are affected 2-4 times annually, such that in a lifetime we spend up to two years suffering from colds (1). Mainly in the winter when the cold and dry air impairs the barrier-functions of mucosal membranes we become susceptible for viral infection. In addition after exhaustive physical training we are exposed to increased risk due to a transient fall in activity of our immune system (open window)(2).
Based on an increasing demand for phytomedical remedies and 50 years of successful application of Echinaforce®, a new formulation made from Echinacea purpurea fresh plant tincture (Echinaforce® forte tablets) was developed. In order to investigate the tolerability and efficacy an open, multicentre study in athletes was performed.
A two-months treatment in the winter showed good efficacy in the prevention and treatment of common cold. During the time of observation 71% of participants were devoid of any cold. The majority of physicians and patients rated the prophylactic efficacy to be "very good".
By assessing adverse events (AE), change in blood parameters and the patients and physicians personal impression, a very good tolerability of Echinaforce® Forte tablets was deduced. Two events (2.5%) clearly could be reduced to the study medication and were of moderate and mild degree.

Echinaforce® Forte tablets are efficacious in the treatment and the prevention of common cold and show good tolerability.
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Thujae occidentalis herba - A safe and effective herbal remedy in the treatment of common cold

P 226

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Nowadays *Thuja occidentalis*, a native European species, is used in homeopathy and traditional phytotherapy. The current investigation reviews an evidence-based update on quality, pharmacology, safety and efficacy of this medicinally used plant. For this reason literature was performed through a search with MEDLINE and pharmaceutical manufacturers were asked for published and unpublished information pertaining to scientific data on Thuja occidentalis. The results show various in vitro and in vivo test models which clearly demonstrate the immunostimulating and antiviral activities of *Thuja* ingredients. Notwithstanding this positive research data, no information on clinical trials are available for *Thuja occidentalis* as a single product. However, substantial literature on clinical studies conducted with a herbal product containing an aqueous ethanolic mixture extract of *Echinacea purpurea/pallida*, *Baptisia tinctoria* and *Thuja occidentalis* (Esberitox®) is published. At least 3 GCP-compliant double-blind and placebo-controlled studies have been identified demonstrating a very good efficacy and tolerability/safety of this herbal remedy in the treatment of common cold as well as its effectiveness as an adjuvant to standard antibiotic treatment of severe bacterial infections. Only in rare cases mild to moderate adverse events were documented. However, none of them was assessed as serious. In addition, it has been shown that a special pharmaceutical production procedure can reduce the thujone content by approximately one third. Taking into account the recommended daily dose (up to 36 mg *Thuja*) and the production procedure (with ethanol 30 %) the ingested amount of thujones per day are well below the threshold which is considered as harmless for humans. Further, this is even below the maximum permitted level of thujones in alcoholic beverages. From these data it can be concluded, that *Thuja occidentalis* is an effective and safe herbal remedy in the treatment of common cold.

P 227 Experimental approach to determine intracellular genistein concentrations in human buccal mucosa cells by using a specific buccal mucosa brush technique

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Genistein, an isoflavone most abundant in soy, possesses estrogen receptors binding properties and shows estrogenic effects during supplementation in human and animals. Although, the general safety of genistein seems to be established, certain questions about its long-term safety during high-dose supplementation (treatment) remained unclear. With the intention to develop a non-invasive monitoring tool for genistein exposure we explored the feasibility to analyze genistein in buccal mucosa cells (BMC). BMC represent a high turn over tissue and could be used for monitoring tissue concentrations (1, 2). 12 elderly women were supplemented with 30 mg synthetic genistein (Bonistein™) for one week. Blood samples as well as BMC were taken at pre-dose, on study day 8 and after one week wash-out (post-dose). Genistein were analysed by using a standardised LC/MC method (LoQ plasma 2 ng/ml, BMC 15 pg/cell pellet) corrected with the DNA content (µg) per cell pellet (BMC only). For harvest of BMC from inside the cheeks the method established by BioTeSys was applied (3). Prior to genistein supplementation only in one subject genistein was measurable in plasma (34.7 ng/ml) and in none of the BMC samples. The same was through for the post-study assessment: plasma genistein was detected in 4 subjects (mean 4.0 (SD ± 2.6) ng/ml), but not in BMC. On study day 8 average fasting genistein plasma level (trough value) was 112.8 (SD ± 95.8; min, max 25.2, 305.0) ng/ml, but only three subjects had accumulated genistein in BMC (DNA corrected data): 1.04 pg genistein/µg DNA (plasma 33.8 ng/ml), 2.36 pg genistein/µg DNA (plasma 187.0 ng/ml) and 1.76 pg genistein/µg DNA (plasma 56.6 ng/ml). In conclusion, to use BMC to monitor genistein status and exposure seems to be generally feasible. The method is sensitive, *ie* subjects avoiding dietary genistein have no detectable genistein in BMC (r=0.999). Obviously, the duration of supplementation was too short to allow intracellular accumulation of orally administered genistein in BMC.

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P 228 Detection and identification of acetylcholinesterase inhibitors in plant extracts

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The purpose of this study was to investigate crude methanol extracts of plant origin for their ability to inhibit acetylcholinesterase and butyrylcholinesterase. Clinically used cholinesterase inhibitors, galanthamine and physostigmine, have been isolated originally from plants, galanthamine from *Narcissus* species e.g. *Galanthus nivalis* and physostigmine from *Physostigma venosum*.
The primary screening of the extracts for the inhibitory effect as well as enzyme selectivity was carried out on TLC (1). Compounds in the extracts were further separated and fractionated directly into 96 well plate by HPLC coupled with fraction collector. Another acetylcholinesterase inhibitory assay in 96 well plate was performed to find out which compound in the extract inhibited the enzyme. The quality of the enzyme assay in 96-well plate was assessed using statistical parameters Z', S/N and S/B to ensure sufficient dynamic range and acceptable signal variability. The active compound was identified utilizing LC-MS-MS.
A compound in the flower extract of *Mentha arvensis* inhibited acetylcholinesterase in a dose dependent manner. The effect was selective for acetylcholinesterase because butyrylcholinesterase inhibition was not detected even at 100-fold dosage. The compound was identified to be acacetin-7-O-rhamnoglucoside (2, 3).
In conclusion, the present results indicate that the used strategy to find and identify acetylcholinesterase inhibitors from plant extracts is workable. Acacetin-7-O-rhamnoglucoside isolated from the flower extract of *Mentha arvensis* could be a promising lead compound for future studies as it inhibits acetylcholinesterase in dose dependent manner.

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Single-dose and steady state pharmacokinetics of 30 mg Bonistein™ in 12 postmenopausal women

P 229

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For the isoflavone genistein loads of research activities are currently ongoing, continuously elucidating its various, dose-dependent pharmacological properties. Among them, the selective estrogen receptor modulating (SERM) properties are of specific interest, because they might provide a phytomedicinal alternative to prevent postmenopausal osteoporosis and to treat climacteric syndrome (1). First clinical studies with genistein have shown surprisingly good efficacy (2, 3). Bonistein™, a new product containing 99.4% synthetic genistein, is in development for these clinical conditions (4). In a phase I study pharmacokinetics (PK) in response to oral dosing of 30 mg Bonistein™ were assessed, once after single-bolus and, twice after 7 days repeated dosing oid at PK-equilibrium (steady state (SS)) in 12 postmenopausal women (mean age 60 yrs.). Plasma genistein (fraction unbound, free genistein) and its conjugates (total genistein) were determined by a standardised LC/MS analytical method using D^4-genistein as internal standard. Orally ingested Bonistein™ was rapidly absorbed from gut (< 0.h h, T_{lag} zero). The plasma concentration-time profiles for total genistein showed a fast, monophasic one-peak course until T_{max} (5.5 h (bolus), 5.3 h (SS); C_{max} (445.3 ng/ml (bolus), 498.5 ng/ml (SS)) followed by a multiphasic decrease consisting of a distribution phase and an elimination phase. Elimination half-lives ($t_{1/2}$) were calculated to be 9.2 h (bolus) and 9.7 h (SS), respectively. Determination of $AUC_{(0\text{-inf.})}$ (bolus) was good with a low percentage of extrapolation (3099.8 h*ng/ml). $AUC_{(0\text{-24h})}$ at SS was 3239.2 h*ng/ml. No statistically significant effects on extent or rate of absorption were found by ANOVA across time. Albeit some residual genistein pre-dose concentrations (trough values) no relevant accumulation occurred (R = 1.04). In conclusion, 30 mg Bonistein™ reaches pharmacokinetic steady state in elderly women after 4 to 5 days and no systemic accumulation occurs. In comparison to the PK profiles assessed in young subjects (4) genistein is slightly better bioavailable in the elderly women (assessed by $AUC_{(0\text{-inf.})}$), reaches higher C_{max} values and has a prolonged terminal elimination half-life ($t_{1/2}$).

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P 230 Efficacy of *Satureja khuzistanica* extracts and carvacrol preparation in the management of recurrent aphthous stomatitis

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Recurrent aphthous stomatitis (RAS) is among the most prevalent and complicated disorders of oral cavity. The causes of this disease remained poorly understood and treatment is directed largely toward symptomatic (1).The purpose of this study was to evaluate the efficacy of Satureja khuzistanica extract (SKE) (2) and carvacrol preparations in the treatment of RAS. 60 patients with minor aphthae were selected and randomly divided into three groups. Groups A and B received topical preparations of SKE and carvacrol, respectively. Group C (control group) received hydroalcoholic solution as a placebo. The date of pain elimination and the duration of thorough healing were recorded. Mean time of pain elimination showed significant differences ($p<0.01$) between groups A (3.4±0.5 days), B (3.2±0.41 days) with group C (5.7±1.12 days). The mean duration of healing showed again significant difference ($p<0.03$) between A (5.9±1.24 days), B (6.85±1.3 days) again with C (10.4±1.66 days). No significant difference was seen among group A and B ($p=0.1$). The results obtained for SKE (group A) was similar to group B (received carvacrol). The findings of this study revealed that SKE and carvacrol preparation showed better effects in the treatment of RAS than placebo. It was concluded the SKE is effective prepration for the management of minor aphthae.

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P 231 Healing capacity of patented MIX 557 in severe, devastating or pathologic wounds in several animal species

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Wound healing is a complex sequence of cellular and biochemical events. It is a regenerative system for restoring the anatomy and the function of injured tissues (1). It is not a complete regenerative system because the final scar does not present the same anatomy and function of the original (2). Therapeutic tools for wound repairs space from traditional, natural or highly technological methods (3). However, scientists agree that no single exogenous available agent can effectively mediate all the numerous aspects of the wound-healing process and no special omnicomprehensive formulation for regulating wound-healing processes exists (4). MIX 557 is a mixture of natural extracts from plants in olive oil which seems to possess the capacity to properly regulate all, or most of all, the complex events of the healing process. Furthermore it shows to have repellent properties towards flies (myiasigenic and non). It is patented at national level but the international patent is still pending;. However it can be stated that all the components of M557 are already used in traditional medicine for different purposes. The study was conducted on accidentally injured animals under compassionate regimen. The patented MIX 557 was used as therapeutic dresses on severe and dramatic complicated wounds in horses, donkey, dogs and sheep. The course of the healing process was registered. Data show a physiologic and complete repair of the lesions in all treated animals; specie-specific timing of the wound healing is perfectly respected, the common complications (bacterial infection or pathologic processes as cheloid formation) are solved within a few days after initiation of treatment. MIX 557 favours the successful repair of severe and complicated wounds in different species and it could represent a all in one treatment both in human as in veterinary medicine.

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Acute oral toxicity of *Jungia Paniculata* and *Chuquiraga Spinosa (Asteraceae)* in wistar rats

P 232

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Jungia paniculata and *Chuquiraga spinosa* (*Asteraceae*) are widespread in the Peruvian Andes between 2500 - 3500 m of altitude. *J. paniculata* (matico) is an herbaceous plant employed in folk medicines as an anti-inflammatory and genitourinary antiseptic. *C. spinosa* (huamanpinta) is a bush used against prostate pathologies as well as diuretic and vermifuge. No toxicological investigations have been carried out on these plants. We have previously observed that single oral doses (500 mg/Kg) of 50% EtOH extracts in rat did not resulted any adverse effects. We have now investigated the oral toxicity of 50% ethanolic extract of both plants in rats though up and down procedure. LD50 values have been determinate by the AOT425StatPgm: Acute Oral Toxicity Statistical Program (175, 550 and 2000 mg/Kg). Oral doses of 2000 mg/Kg of 50% EtOH extracts have not produced mortality or significant changes in the behavior and gross appearance of internal organs of rats (heart, liver, kidneys, ovaries and pancreas). Nevertheless, an increase of the size of the spleens has been observed but no alterations have detected in further histological analysis. No toxicity was observed in wistar rats after oral administration of *Jungia paniculata* and *Chuquiraga spinosa*, evidenced by high LD_{50} value organ integrity, suggest a wide margin of safety for therapeutic doses of these plants.

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Drinking of Salvia officinalis tea increases CCl_4-induced hepatotoxicity in mice

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In a previous study we observed a beneficial effect of the drinking of sage tea (a traditional water extract of *Salvia officinalis* L) on some antioxidant parameters in mouse and rat livers, namely an enhancement of glutathione-s-transferase (GST) and glutathione reductase activities (1). When rat hepatocytes were cultured *in vitro*, sage tea drinking protected the cells against GSH depletion induced by *tert*-butyl hydroperoxide (1). Taking this into account, in the present study we decided to evaluate the potential protective effect of sage tea drinking on CCl_4-induced hepatotoxicity, in male and female mice. Forty mice (20 males and 20 females) were divided in two groups each. In half the groups drinking water was replaced by sage tea for 14 days. Twenty four hours before the end of the experiment, half the animals of each drinking group (of both genders) received an i.p. injection of 20 µl/kg of CCl_4 in olive oil, and controls vehicle only. Several plasma and liver parameters were evaluated. Confirming our previous results, sage tea drinking caused a 12-26% increase in GST activity – a phase II enzyme. The antioxidant enzyme glutathione peroxidase and some phase I enzymes activities measured (EROD and PNP hydroxylase) were also increased by sage drinking around 12%. However, sage tea drinking did not protect against CCl_4-induced liver toxicity. On the contrary, CCl_4 hepatotoxicity was strongly potentiated resulting in a 10-30 fold increase in plasma AST and ALT (female>male) when compared with CCl_4 water drinking animals. Once CCl_4 becomes toxic upon activation through P450 2E1 and an over-expression of this cytochrome correlates with higher toxicity of CCl_4 (2), we decided also to evaluate the level of this protein in the liver microsomes. A higher level of the cytochrome 2E1 was indeed observed (female - 24.5%; male - 8.5%) and may provide an explanation for the observed results. A possible herb-drug interaction between this extract and pharmaceutical drugs metabolized by the liver warrants further studies.

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P 234 Cytotoxic and antioxidant activities of *Petiveria alliacea* L.

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Petiveria alliacea, is salso known as skunk weed due to its characteristic odor resulting from the prresence of sulfurate compounds **(1)**, it belongs to the Phytolaccaceae family, which is widely distributed in Mexico. Various uses are attributed to this plant, such as: antirheumatic, analgesic, antigrippal, antitussive, antiinflamatory and anticarcinogenic **(2,3)**. Its antioxidant activity is unknown, thought flavonoids have been identified **(4)**. The objective of the present study was to evaluate cytotoxic activity, as well as the antioxidant activity in five cellular lines. The plant material was gathered in the southern part of the states of Veracruz, México. The cytotoxic evaluation of varous extracts was made with five cellular lines of Human cancer: U251 (CNS), PC-3 (prostate), HCT-15 (colon), MCF-7 (breast) y k562 (leukemia cronics mieloblastic) according to the method proposed by Monks **(5).** The assay was made using the protein-binding dye sulforrodamine B (SRB), all of which are microculture assays to measure cell variability and cell growth. The antioxidant activity of different leaf and root extracts were carried out according to the method described by Blois **(6).** The antioxidant activity of total extract was determined by free radical scavenging (2,2-diphenyl-1-picrylhydrazyl; DPPH*) method. Of the five extracts tested, the aqueous leaf extracts and the dichloromethane root extract showed the greatest cytotoxic effect on the cellular line of leukemia, with an inhibitory percentage of 70.1% and 81.1% at a concentration of 100 µM, respectively. These results are similar to reported by Jovicevic **(7).** The butanolic leaf extract presented an antioxidant effect, with the medium inhibitory concentration of IC_{50}=264.54 µl mg^{-1}.

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P 235 Antioxidant and antimicrobial activity of some plant species belonging to Fabaceae family

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The aim of this study was preliminary investigation of antioxidant and antimicrobial activity of nine Fabaceae species collected on the mountains of Serbia and Montenegro. Five examined species are endemic: *Lathyrus binatus* Pančić. (**1**), *Anthyllis aurea* Welden (**2**), *Onobrychis scardica* (Griseb) Halácsy (**3**), *Oxytropis halleri* Bunge ex W.D.J. Koch. (**4**) and *Oxytropis campestris* (L.) DC. Subsp. *dinarica* Murb. (**5**). Other four species are *Astragalus glycyphyllos* L. (**6**), *Anthyllis vulneraria* L. (**7**), *Coronilla emerus* L. (**8**) and *Trifolium pannonicum* L. (**9**).
Air-dried aerial parts or flowers of plants were extracted with methanol in Soxhlet and solvent was evaporated. Dry extracts (in different concentrations) were used for experiments. Antiradical activities were tested in reaction with stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by conventional colorimetry, as well as by on line HPLC-DPPH method. Trolox® and ascorbic acid were used as positive controls. The total phenolic content (chlorogenic acid equivalent) of each extract was estimated using the Folin-Denis reagent. Antimicrobial activity was determined by agar dilution method on Mueller-Hinton (for bacteria) and Sabouraud dextrose agar (for funghi). The extracts were screened against: *Micrococcus lysodeiktus* ATCC 4698, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli ATCC 25922*, *Candida albicans* ATCC 24433, *Fusarium sporotrichoides* and *Aspergilus niger* and the inhibition zone was measured. The antioxidant activity of plants extract was as follows **1** > **9** > **2** > **3** > **7** > **5** > **4**, while **6** and **8** were not active.
The most active were *L. binatus* with IC_{50} = 15.69 µg/m. Extracts of *O. scardica* and *O. campestris* subsp. *dinarica* contained, as major compound, flavonoid glucoside which was isolated using column chromatography on Silica gel. Using UV-VIS and NMR techniques, its structure was confirm as quercitrin. Quercitrin showed strong antioxidant activity with IC_{50} = 5.13 µg/ml. There was a significant correlation between the total phenolic content of the extracts (7.06% for *L. binatus* to 1.27% for *A. glycyphyllos*) and the scavenging capacity of free radicals. Antibacterial activity was noticed in *O. campestris* subsp. *dinarica* against *M. lysodeictis* and *O. halleri* against *S. aureus*.

Antioxidant and antimicrobial activity of *Alnus viridis* (Chaix) DC. subsp. *viridis*

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Bark, cones and leaves of *Alnus glutinosa, A. incana and A. viridis* are used in folk medicine of Serbia and Montenegro for the treatment of gastrointestinal and skin diseases as well as, for gargle of mouth and throat. Due to the little literature information about biological activity of endemic plant species *A. viridis* (Chaix) DC. subsp. *viridis*, the aim of this study was preliminary investigation of its antioxidant and antimicrobial activity. Plant material (green cones and seeds) was collected in September and October 2004, in Montenegro, on mountain Čakor (1600 m).

Air-dried powdered material were extracted with methanol in Soxhlet and solvent was evaporated. Dry extracts (in different concentrations) were used for experiments. Antiradical activities were tested in reaction with stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by conventional colorimetry, as well as by on line HPLC-DPPH method. Trolox® and ascorbic acid were used as positive controls. The total phenolic content (chlorogenic acid equivalent) of extracts was estimated using the Folin-Denis reagent. Antimicrobial activity was determined by agar dilution method on Mueller-Hinton (for bacteria) and Sabouraud dextrose agar (for funghi). The extracts were screened against: *Micrococcus lysodeiktus* ATCC 4698, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli ATCC 25922*, *Candida albicans* ATCC 24433, *Fusarium sporotrichoides* and *Aspergilus niger* and the inhibition zone was measured.

Both extracts showed strong antioxidant activity with IC_{50} = 4.34 µg/ml (green cones) and IC_{50} = 21.81 µg/ml (seeds). There was a correlation between the total phenolic content of the extracts (10.2% for cones and 3.34% for seeds) and the scavenging capacity of free radicals. HPLC method has been developed for the analysis and characterisation of the extracts. The method enables separation of flavonoids and phenolic acids. Antibacterial activity was noticed against *M. lysodeiktus, S. aureus* and *E. coli* while antifungal activity was noticed against *F. sporotrichoides*. Further assays to assess the ability to inhibit peroxidation of membrane lipids are in progress.

Investigation on Antioxidant Properties and Inhibition of MAO Activity of *Stachys alpina* L. subsp. *dinarica* Murb

P 237

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Stachys alpina L. subsp. *dinarica* Murb. (Labiatae) is endemic for Balkan Peninsula (Croatia, Bosnia and Herzegovina, Montenegro, Serbia and southwestern Bulgaria). Aerial blooming parts of this plant were collected in Bosnia and Herzegovina (Mt. Jahorina), in July 2004. Air-dried and powdered plant material was extracted with $CHCl_3$, and then with MeOH. Dry MeOH extract, obtained by solvent evaporation under reduced pressure, was investigated in *vitro* on antioxidant properties and inhibition of MAO activity. Total antioxidant ability (TAA), evaluated using FRAP assay (1) was 1.36 µmol/mg. This value was in correlation with total polyphenols content (0.14%, calculated as gallic acid). TBA test was used in order to measure the inhibitory effect on Fe^{2+}/ascorbate induced lipid peroxidation (LP) in liposomes (2). Inhibition of LP was concentration dependent: 4.64, 18.72, 60.49, 69.24, and 43.35% at concentrations 12.5, 25, 62.5, 125, and 250 µg/ml, respectively. Extract strongly scavenged 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (3). At concentrations of 5, 10, 20, 50 and 100 µg/ml inhibition was 6.28, 18.18, 35.57, 91.6, and 94.47%, respectively (IC_{50}=26.14 µg/ml). $OH^{\cdot}$ radical generated in Fe^{3+}-EDTA-H_2O_2-deoxyribose system (4), was also scavenged in dose depended manner, reaching maxima of 50.94% at concentration of 12.5 µg/ml. *In vitro* radioassays of MAO A and MAO B inhibition were performed by ^{14}C-5-HT and ^{14}C-β-phenyelthylamine, respectively (5). The extract exhibited a modest MAO A inhibition (IC_{50}=390 µg/ml) and insignificant influence on MAO B (IC_{50}=2.6 mg/ml).

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P 238 Radical scavengers flavonols from *Chuquiraga spinosa* (Asteraceae)

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In the search for bioactive principles from Peruvian medicinal plants, we examined *Chuquiraga spinosa* (Asteraceae), a plant used in folk medicine as a anti-inflamatory herbal remedy. The $CHCl_3$, MeOH, EtOH 50% and H_2O extracts of *C. spinosa* aerial parts were tested for antioxidant potency using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The highest activity was exerted by the EtOH 50% extract (EC_{50}=23,20µg/mL). Phytochemical studies by HPLC-UV and spectrophotometrics methods showed the presence of flavonoids and phenolic acids, in the extract (11,47% of total polyphenols). A bioassay-oriented fractionation was carried out using Sephadex G-25, to yield 6 fractions (I-VI). The most active fraction was IV (EC_{50}=6,53µg/mL), and further fractioned by Sephadex G-10 and HPLC-prep to give three flavonols: quercetin 3-O-glucuronide, kaempferol 3-O-glucuronide and isorhamnetin 3-O-glucuronide. These compounds were isolated for the first time from this plant. These flavonols also interaced with superoxide anion generated by enzyme system or by human neutrophils. Quercetin 3-O-glucuronide was the highest scavenger of anion superoxide generated by humans neutrophils stimulated with TPA (EC_{50}=619,44 µg/mL) and superoxide generated by hipoxanthine/xanthine oxidase (EC_{50}=94,84 µg/mL). Scavenging of superoxide and DPPH radicals determine the antioxidant properties of these active flavonols.

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P 239 Antioxidant activity of medicinal plants from Navarra (Spain) (I)

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Navarra (Spain) has very rich vascular flora comprising 2650 plant species. In our search for new classes of biologically active plant metabolites, we have collected 20 medicinal plants of different families, mainly Asteraceae. All of these plants are used in traditional medicine. Different organs of collected plants were extracted with dichlorometan, ethyl acetate and methanol. We investigated the potential radical scavenging capacity of all 60 extracts using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (yellow spot on purple background) on the plates (200 µg/spot). The highest scavenging activity was obtained with the methanol extracts of *Equisetum arvense*, *Equisetum telmateia*, *Jasonia glutinosa*, *Lythrum salicaria, Silybum marianum, Tanacetum parthenium* and *Verbena officinalis*; and ethyl acetate extracts of *Achillea millefolium subsp. millefolium, Cichorium intybus, Equisetum telmateia, Santolina chamaecyparissus subsp. squarrosa, Silybum marianum* and *Verbena officinalis*. Further studies that are on going in our laboratory could allow the isolation of new antioxidant compounds.

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Antioxidant activity of medicinal plants from Navarra (Spain) (II)

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Navarra (Spain) has very rich vascular flora comprising 2650 plant species. In our search for new classes of biologically active plant metabolites, we have collected 20 medicinal plants of different families, mainly Lamiaceae. All of these plants are used in traditional medicine. Different organs of collected plants were extracted with dichlorometan, ethyl acetate and methanol. We investigated the potential radical scavenging capacity of all 60 extracts using 2,2-diphenyl-1- picrylhydrazyl radical (DPPH) (yellow spot on purple background) on the plates (200 μg/spot). The highest scavenging activity was obtained with the dichlorometan extracts of *Calamintha sylvatica, Mentha suaveolens, Origanum vulgare* subsp. *vulgare*, and *Thymus vulgaris* subsp. *vulgaris*; ethyl acetate extracts of *Lycopus europaeus, Melissa officinalis*, *Mentha aquatica, Mentha longifolia, Mentha* x *piperita, Mentha pulegium, Mentha suaveolens, Origanum vulgare* subsp. *vulgare, Origanum vulgare* subsp. *virens, Teucrium chamaedrys* and *Thymus vulgaris* subsp. *vulgaris*; and methanol extracts of *Lycopus europaeus, Melissa officinalis*, *Mentha aquatica, Mentha longifolia, Mentha pulegium, Mentha suaveolens, Origanum vulgare* subsp. *vulgare, Origanum vulgare* subsp. *virens, Phlomis lychnitis*, *Teucrium chamaedrys, Thymus praecox* and *Thymus vulgaris* subsp. *vulgris.* Further studies that are on going in our laboratory could allow the isolation of new antioxidant compounds.

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Radical scavenging activity of two endemic Compositae species: *Cicerbita pancicii* and *Leucanthemum illyricum*

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Reactive oxigen species (ROS) can cause extensive damages to important biomolecules in cells (DNA, proteins and lipids) and are related to different diseases. Due to the toxic effects of some synthetic antioxidants, there is an increased tendency for their replacement with natural ones. The aim of this work was investigation of radical scavenging activity (RSC) of two endemic *Compositae* species: *Cicerbita pancicii* (Vis.) Beauverd, that inhabits Balkans mountains (1) and *Leucanthemum illyricum* Vogt & Greuter, which is Dinaric endemic species (2). As a material for this investigation herb of *C. pancicii* , collected on Mt. Maglić (Bosnia), and flowers of *L. illyricum* from Mt. Durmitor (Montenegro) were used. Air-dried and powdered plant material was extracted with $CHCl_3$ and then with MeOH. Dry MeOH extracts were used for investigation of RSC against 2,2-diphenil-1-picrylhydrazyl radical (DPPH) (3) and OH radical (4). In the reaction with free radical quencher, DPPH is reduced into DPPH-H, with the color change from violet to yellow, and RSC of investigated extracts is measured spectrophotometricly. Both extracts exhibited dose-dependet and significant activity: IC_{50}=55,5 μg/ml for *C. pancicii* and IC_{50}=44,2 μg/ml for *L. illyricum* extract. At TLC chromatograms, using DPPH as a sprey reagent (3), in both investigated extracts several polyphenols (flavonoids and phenolic acids) were revealed as antioxidant components. OH radical scavenging activity of the extracts was measured in Fe^{3+}-EDTA-H_2O_2-deoxyribose system, following degradation of 2-deoxyribose into TBA-reactive substances. Both extracts reached maximum of inhibition at concentration of 250 μg/ml. For *C. pancicii* extract maximum of inhibition was 38.74 %, and for *L. illyricum* 40.62 %.

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P 242 Antimicrobial and Antioxidant Activities of *Carlina acaulis* subsp. *caulescens* and *Carlina acanthifolia* subsp. *utzka* Root Extracts

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Roots of *Carlina acaulis* L. subsp. *caulescens* (Lam.) Schübler & Martens, collected at Mt. Durmitor (Montenegro), and of *C. acanthifolia* All. subsp. *utzka* (Hacq.) Meusel & Kästner, collected at Mt. Suva planina (SE Serbia), were extracted with 70% MeOH. Dry extracts (samples C1 and C2, respectively) were used for all assays. Antimicrobial activity of the samples was assayed using the agar diffusion method (1) against five bacterial strains (*Staphylococcus aureus* ATCC25923, *Enterococcus faecalis* ATTCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCIMB 9111 and *Pseudomonas aeruginosa* ATCC 27853) and two strains of *Candida albicans* (ATCC 24433 and ATCC 10259). Both samples exhibited similar antimicrobial activity in the following order: *P. aeruginosa* > *E. faecalis* > *S. aureus* > *E. coli* > *K. pneumoniae* > *C. albicans* ATCC 10259 > *C. albicans* ATCC 24433. Antioxidant activity was evaluated using FRAP assay (Total antioxidant ability, TAA) (2), TBA test (inhibition of Fe^{2+}/ascorbate induced lipid peroxidation in liposomes) (3) and "scavenging" of DPPH (4) and OH radicals (5). TAA values were 0.21 and 0.25 μmol/mg (samples C1 and C2, respectively), with polyphenol content of *ca* 0.03%. Inhibition of LP was dose dependent: both samples have shown moderate activity with maxima at 250 μg/ml (25.73 and 19.34%, respectively). Scavenging DPPH radical was in correlation with applied concentration: IC_{50} values were 208 and 155 μg/ml, respectively. OH radical, generated in Fe^{3+}-EDTA-H_2O_2-deoxyribose system, was also scavenged in dose depended manner, with maxima of 40.75 and 44.84% at concentration of 100 μg/ml, respectively.

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P 243 *In vitro* estrogenic and antioxidant activities of *Asplenium trichomanes* and *A. ruta-muraria*

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In the proceeding of our investigations on botanical species traditionally used to induce or regulate menstruation and to prevent or alleviate menopause problems we considered *Asplenium trichomanes* L. and *A. ruta-muraria* L. Few phytochemical data are available about these plants. Kaempferol and triterpenes derivatives have been isolated from *A. trichomanes* (1), while no reports, at our knowledge are present about *A. ruta-muraria*. Dried leaves were extracted with methanol and residues were used to perform both the antioxidant assay (DPPH radical scavenging activity) and the *in vitro* estrogenic test (MCF-7). The calculated IC_{50} in the DPPH assay were of 144.5 and 91.7 μg/mL for *A. ruta-muraria* and *A. trichomanes* respectively. The MCF-7 test showed moderate estrogenic effects for both the extracts at a range of concentration of 10^{-1}-10^{-3} mg/mL. The methanol extracts were subjected to several chromatographic steps (CC, TLC, preparative and analytical HPLC). A new phenol glycoside, 4-vinyl-phenol-1-0-[α-L-rhamno(1→6)-β-D-glucopyranoside], astriloside (**1**) together with five known compounds, three phenols derivatives and two kaempferol glycosides were isolated from *A. trichomanes*. From *A. ruta-muraria* a novel natural compound, kaempferol-3-0-[β-D-(6"-0-caffeoyl)-gluco(1→2)-β-D-galactopyranoside]-7-0-β-D-glucopyranoside, asplenioside (**2**) was isolated. The structures of all compounds were established by means of spectroscopic techniques 1D and 2D-NMR including HMQC, HMBC, NOSY, TOCSY, and COSY experiments as well as HR mass spectrometry. The above compounds are under investigation for their biological activities

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Antioxidant activity of *Laurus nobilis*

P 244

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Laurus nobilis L. (Lauraceae) is an evergreen tree widely distributed in the Mediterranean area and the leaves are extensively used as a spice for culinary and flavouring purposes. In the Sardinian traditional medicine the leaves decoction or infusion are widely used in the treatment of different diseases (1). Previous phytochemical investigations lead to the isolation of several classes of secondary metabolites and in particular sesquiterpene lactones, alkaloids, catechin and procyanidine derivatives, flavonoids and megastigmane glucosides (2). In this work the antioxidant properties of *L. nobilis* extracts were investigated. Sequential extracts were prepared using solvents in increasing polarity (petroleum ether, chloroform and methanol). In addition the leaves infusion was prepared and analysed. The residues were evaluated for their antioxidant activity with different *in vitro* assays (DPPH, TEAC and BR) (3). The antioxidant activity was established in the methanol extract. Phytochemical investigations lead to the isolation of five kaempferol-O-glycosides, five quercetin-O-glycosides derivatives and one catechin. In addition a flavone C-glycoside, 5,7,4'-trihydroxy-6-C-β-D-glucopyrano-2"-O-α-L-rhamnopyranoside was isolated and characterized. The structure elucidation of the isolated compounds was performed by 1D and 2D NMR experiments and by HR-MS analysis The our results showed that the total antioxidant capacities from DPPH, TEAC and BR assays were highly correlated with phenolic content ($r^2 = 0{,}915$, 0,971 and 0,952, respectively).

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Antioxidant Activity of Phenolic Fatty Acid Esters from cv. Annurca Apple Fruits

P 245

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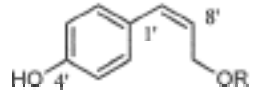
Excessive concentrations of radical species in the human body are able to determinate numerous pathologies(1). The aim of this study was to valuate antioxidant and radical scavenging activities of metabolites isolated from Annurca apple fruits. Apple (*Malus domestica*) is a rich source of medicinal substances(2) and cv. Annurca, an apple variety cultivated in the south of Italy, is a harmonic complex of nutritional compounds. In the framework of the Regional Research Center for the Agro-alimentary productions of the Campania Region (Italy), chemical study of organic extract of the fruits of Annurca apple has led to isolate and characterize some fatty acid esters. Structure of these esters was elucidated by GC-MS and NMR 1D and 2D after purification of individual compounds by HPLC. The metabolites isolated from cv. Annurca apple fruits have been characterized as the Z and E isomer of the *p*-coumaryl esters of C18-C24 saturated and unsaturated fatty acids. The presence of a hydroxyl group in the isolated molecules suggested their possibility to act as antioxidant agents. All the compounds have been tested for their antioxidant activity to inhibit the production of peroxides in the methyl linoleate(3) and for their radical scavenging activity by measuring the reduction of DPPH radical. In the both tests, α-tocopherol was used as positive standard. The antioxidant activity of cv. Annurca fatty acid esters showed that these compounds have upright antioxidative properties. Compounds **2c** and **2f** showed an antioxidant activity higher that the standard, inhibiting the autoxidation of methyl linoleate for 60%. When scavenging of DPPH radical was tested, the strongest activity was observed for compound **2b**.

1



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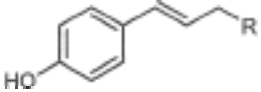

a. R = linolenyl; **b.** R = linolyl; **c.** R = oleyl;
d. R = steryl; **e.** R = arachidyl; **f.** R = behenyl; **g.** R = lignoceryl.

Acknowledgment: This work has been supported by CRdC (Centro Regionale di Competenza) "Produzioni Agroalimentari" in the framework of the project line A: "Mela annurca per l'industria" (P. O. R. 2000-2006, misura 3.16).

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P 246 Antioxidant activity of methanolic extract of cucumber (*Cucumis sativus*) fruits (Greek cultivar)

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Bioactive components from cucumber fruit (Greek cultivar) were isolated and their antioxidant activity was characterized by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (1). Fruit peel and pulp were homogenized, lyophilized and extracted with dichloromethane and methanol successively. Total polyphenols and antioxidant activity of the methanolic extract were determined. The components of the methanolic extract were separated by column and thin-layer chromatographic methods. Major chemical constituents were further characterized by spectroscopic methods. We found that fruit pulp contained about 2.5-fold higher polyphenol concentration than the peel. Both pulp and peel methanolic extracts showed important antioxidant activity (EC_{50} 1310 μg/ml and 1840 μg/ml respectively; EC_{50} indicates the amount of antioxidant which reduces the initial concentration of DPPH free radicals by 50%), while EC_{50} of the standard phenolic antioxidant BHT was 250 μg/ml (1.1 mM). The most significant finding was the presence of lactic acid in fruit pulp.

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P 247 Antioxidant activity of nigerian dietary spices

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Antioxidants are compounds that help to inhibit the many oxidation reactions caused by free radicals. The antioxidant activity of 20 extracts from 12 Nigerian spices *Aframomum danielli K. Schum (Zingiberaceae), Allium cepa* L. (Amarylliadaceae), *Allium sativa* L. (Amaryllidaceae), *Capsicum frutescens* L. (Solanaceae), *Citrus sinensis* (*L.*) Osbeck (Rutaceae), *Curcuma longa* L. (Zingiberaceae), *Justicia flava* (Forssk) Vahl. (Acanthaceae), *Ocimum gratissimum* L. (Lamiaceae), *Piper guineense* Schum. et Thonn.(Piperceae), *Syzygium aromaticum* (L.) Merr. et Perry (Myrtaceae), *Xylopia aethiopica* (Dun.) A. Rich.(Annonaceae) and *Zingiber officinale* Rosc. (Zingiberaceae) was evaluated by using the ferric thiocyanate method and reducing power. The total phenolics of the extracts was determined spectrophotometrically as Tannic Acid Equivalent (TAE) method of relative astringency of the plant extracts as a direct measurement of total soluble tannin. The anti-oxidant activity (expressed as per percent inhibition of oxidation) ranged from as high as 82.5% in turmeric extracts to as low as 8.6% in sweet orange peel. Anti-oxidant activity correlated significantly and positively with total phenolics (($R^2 = 0.83$) while there was no linear correlation between total antioxidant activity and reducing power ($R^2 = - 0.53$) neither between reducing power and total phenolic content ($R^2 = - 0.20$). The results indicate that reducing power does not fully characterize the antioxidant activity, spices containing high phenolics provide a source of dietary anti-oxidants in addition to imparting flavor to the food, they possess potential health benefits by inhibiting lipid peroxidation and justifies their traditional use as *pepper soup* and a cure for all medicine for the sick.

Free radical scavenging activities of five *Salvia* species from Iran

P 248

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It is generally accepted that free radicals especially oxygen radicals play an important role in the development of molecular damage, cell injury and pathological events. The radicals are implicated in the etiology of cancer, multiple sclerosis, Parkinson's disease, senile dementia, diabetes mellitus, Alzheimer's disease etc. A large amount of research has been carried out to find antioxidant drugs, which participate as radical scavenger in living organisms. Currently, there is an increasing interest in the antioxidative activity of natural compounds. *Salvia* species are rich in constituents that are known to be effective radical scavengers. The genus *Salvia* is represented by approximately 700 species in the world and 58 taxa in Iranian flora. The *Salvia* species are used in Iranian traditional medicine for their anti-inflammatory properties. The medicinal properties of this genus are mainly attributed to the presence of terpenoides and flavonoids. This study was designed to examine the in-vitro free radical scavenging (FRS) activities of the ethanol extracts obtained from aerial parts of five *Salvia* species found in Iran(*S. hypoleuca*, *S. officinalis*, *S. reuterana*, *S. verticillata* and *S. virgata*). The in-vitro FRS activities were spectrophotometrically evaluated by quantitative DPPH assay. The positive control used was rutin. The FRS activity of each extract was expressed as an IC50% value and calculated from Log concentration–response curve. The results were statistically compared by one-way ANOVA to see the significance. The results showed that all examined extracts have FRS activities and *S. verticillata* exhibited the highest FRS activity. The extract showed an IC50% of approximately 16.51 mg/ml ($P<0.001$). The results indicate that the extract of *S. verticillata* has a very potent FRS activity compared with *S. officinalis* extract (IC50%=21.91 mg/ml).

Antioxidative properties of *Filifolium sibiricum* kitam

P 249

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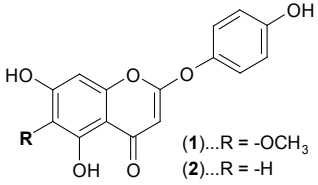

(**1**)...R = -OCH_3
(**2**)...R = -H

The species *Filifolium sibiricum* (L.) Kitam. [syn.: *Tanacetum sibiricum* L., *Artemisa sibirica* Maxim.] (*Asteraceae*) is widely distributed in Mongolia, Eastern Siberia, China, Korea and Japan. Aerial parts of *Filifolium sibiricum* (drug name: Сибирийн зүр өвс/ sibiriin zur ovs) are used in the traditional Mongolian medicine for the treatment of respiratory diseases, typhoid fever, diphtheria, and gastrointestinal complains. Phytochemical investigations of the aerial part of *F. sibiricum* resulted in the isolation and structure elucidation of eleven compounds, 4-hydroxy-acetophenon, filifolin, eriodictyol, 6-methoxy-naringenin, 6-hydroxy-naringenin, naringenin, homoeriodictyol, quercetin-4′-methylether, scopoletin and the rare phenoxychromone derivatives capillarisin (**1**), and 6-demethoxy-capillarisin (**2**). Isolation was performed by means of silica-CC and Sephadex® LH-20-CC. Structures were established by LC- MS and 1D- and 2D-NMR spectroscopy. The anti-oxidative properties of the isolated constituents of the DCM extract of the aerial plant parts were evaluated by DPPH decolourizing assay. The most active compound was identified as quercetin-4′-methylether (IC_{50}: 3.4 µM; CI_{95}:1.9 - 5.8). Other constituents showed IC_{50} values between 13.4 µM (CI_{95}: 11.0 - 17.1; homoeriodictyol) and 17.7 µM (CI_{95}: 11.7 - 21.8; 6-hydroxynaringenin). The lowest activity was found for naringenin (IC_{50}: 25.3 µM; CI_{95}:21.6 - 30.5), its 6-methoxy-derivative (IC_{50}: 35.2 µM; CI_{95}:29.7 - 43.6) and 4-hydroxy–acetophenon (IC_{50}: 37.8 µM; CI_{95}:31.4 - 38.4).

P 250 Antioxidant and anti-inflammatory constituents of *Sideritis perfoliata* subsp. *perfoliata* (Lamiaceae)

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Sideritis species (common Greek name "mountain tea") are occurring mainly in the Mediterranean area and are often used as herbal teas in Greece as well as in Turkey, because of their anti-inflammatory, antirheumatic, digestive, anti-ulcer and antimicrobial activities. In continuation of our phytochemical and biological studies of the genus *Sideritis* (Lamiaceae) we have investigated the aerial parts of *S. perfoliata* subsp. *perfoliata*. Three flavonoid derivatives and three phenylethanoid glycosides were isolated so far, by repeated chromatographic isolation of butanol soluble fraction. Their structures were elucidated as isoscutellarein - 7 - O - [6′′′- O - acetyl- β-D-allopyranosyl - (1→2)] - 6′′-O-acetyl-β - D - glucopyranoside **(1)**, isoscutellarein - 7 - O - [6′′′- O – acetyl - β - D – allopyranosyl - (1→2)] - β - D - glucopyranoside **(2)**, 4′-O-methylisoscutellarein - 7 - O -[6′′′-O-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside **(3)**, acteoside **(4)**, lavandulifolioside **(5)** and leucoseptoside **(6)**. The structures were determined by UV and NMR (^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, HMBC) analysis. The extracts, as well as the isolated compounds were tested for their scavenging activity (87-96%) using the free stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and for their inhibitory activity toward soybean lipoxygenase, using linoleic acid as substrate

P 251 A preliminary study on the antioxidant and anti-inflammatory activity of *Anthemis tinctoria* L. subsp. *tinctoria* var. *pallida* DC. (Asteraceae)

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The genus *Anthemis* (Asteraceae) is represented by 62 species in Europe. The species of the *Anthemis* genus are widely used in the pharmaceutics, cosmetics and food industry. The flowers of the genus have well-documented use as antiseptic and healing herbs, the main components being natural flavonoids and essential oils. Continuing our chemotaxonomic examinations of the Greek flora belonging to Asteraceae and our search for new compounds of pharmacological interest, we now report the investigation of the aerial parts of *Anthemis tinctoria* L. subsp. *tinctoria* var. *pallida* DC. Four flavonoid derivatives were isolated so far, by repeated chromatographic separation of methanol soluble fraction, on silica gel 60 (Merck) and Sephadex LH-20. Their structures were elucidated as kaempferol-3-O-rutinonoside **(1)**, quercetin-3-O-glucopyranoside **(2)**, quercetin-3-O-rutinonoside **(3)** and patuletin-7-O-rutinonoside **(4)** by the analysis of spectroscopic evidences (UV and NMR: ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, HMQC, HMBC). Extracts and pure isolated compounds were examined on their free radical scavenging activity by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable radical and for their inhibitory activity toward soybean lipoxygenase, using linoleic acid as substrate. From the flavonoids, patuletin-7-O-rutinonoside showed strong scavenging effect in the DPPH radical assay.

The Flavonoidal Constituents of *Salicornia fruticosa* (L.) and Their Antioxidant Activity

P 252

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The genus *Salicornia* is occasionally utilized as a vegetable in Europe, especially the tetraploid species. Several species possess antibacterial and anti-hypertensive properties, also mentioned in folk medicine for a relief of toothache and chronic rheumatic (1). The genus *Salicornia* is represent in Egypt by only three species and nothing was reported about the phytoconstituents of *Salicornia fruticosa*. In the series of searching for natural antioxidants, we tried to isolate a new antioxidant from plants, in particular from the plants which were not investigated before. Plant materials (aerial parts) were dried, powdered and defatted with pet. ether and extracted with 80% methanol. The metabolic extract after partitioned with chloroform followed by ethyl acetate and finally with n-butanol yielded a crude extract containing flavonoid. The previous three extracts were subjected separately to preparative PC (3MM, 15% acetic acid) and the main flavonoidal bands were cut and eluted with 90% methanol. The eluted fractions were subjected separately to further purification using Sephadex LH-20 column (2). The flavonoidal constituents isolated from the chloroform, ethyl acetate and n- butanol fractions of the aqueous alcoholic extract of *S. fruticosa* were identified as apigenin, isorhamnetin, isorhamnetin-3-O-galactoside, acacetin and apigenin-7-O-galactoside. The flavonoidal compounds were identified by using PC, TLC, UV and MS analysis in addition to acid hydrolysis of the flavonoidal glycosides (3). This is the first record of the flavonoids in *Salicornia fruticosa* (L.). The radical scavenging effects of the extracts and isolated compounds on DPPH free radical were studied, n-butanol and ethyl acetate extracts showed strong antioxidant activity, also the isolated flavonoidal compounds showed high antioxidant activity compared to Trolox (standard antioxidant compound).

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Antioxidant activity of some species from Ericaceae family

P 253

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The herb extract of five species from Ericaceae family (*Erica arborea*, *Erica carnea*, *Calluna vulgaris*, *Bruckentalia spiculifolia*, *Arbutus unedo*) were investigated for their antioxidant activity. Plant material was collected during the summer of 2004., dried, reduced to a fine powder, extracted with ethanol (70%, *v/v*) by percolation and evaporated to the dryness. The content of total flavonoids was determined according to DAB 10 monograph *Crataegi folium et flores* (1) and calculated as hyperoside. The total polyphenols in the extracts were determined by Folin-Ciocalteu procedure (2), as well as the tannin content. Antioxidant activity of extracts was investigated by inhibition of lipid peroxidation in lipozomes induced by Fe^{2+}/ascorbate system (3) and by DPPH free radical scavenging assay (4). The highest scavenging activity was obtained with extract of *Arbutus unedo* (IC_{50} = 7.14 µg/m), while the best inhibition of LP has been shown by *Bruckentalia spiculifolia* herb extract (Table 1). The herb of *Bruckentalia* contained the highest content of flavonoids (3.42 %). The largest quantities of total polyphenols were determined in the extract of *Erica arborea* and *Erica carnea* (28,0 and 25,96 %, respectively).

Table 1. Antioxidant activity of investigated species from Ericaceae family

	Inhibition (%) of lipid peroxidation					Radical scavenging activity IC_{50} (µg/ml)
	6.25 µg/ml	12.5 µg/ml	25.0 µg/ml	62.5 µg/ml	125.0 µg/ml	
Erica arborea	- 5.63	14.79	22.63	78.97	67.44	46.97
Erica carnea	- 0.95	3.61	15.50	65.97	29.54	13.24
Calluna vulgaris	22.04	34.06	85.97	90.26	88.25	12.05
Bruckentalia spiculifolia	50.32	70.70	83.44	95.33	96.32	10.29
Arbutus unedo	12.85	23.71	36.73	66.16	74.31	7.14

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P 254 Similarities and differences between leaves of *Arctostaphylos alpinus* and *Actostaphylus uva-ursi*

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Arctostaphylos alpinus (L.) Spreng., black bearberry is deciduous, creeping and mat-forming dwarf shrub, with steams up to 60 cm long. It is distributed in the sub-alpine and alpine zone, in the dwarf shrubs and mountain pine wood region, together with *Arctostaphylos uva-ursi*, an evergreen shrub. The leaves of *A. alpinus* are 1-4 cm long, spatulate or narrowly obovate with short stalk and with finely and closely serrate, fringed with spreading, white hairs and emergences. Those leaves hardly could be mistaken for thick and heavy *Uvae-ursi folium*. The margines of *A. uva-ursi* leaves are entire, slightly rolled back, with short hairs on the young leaves. After morphological research the chemical analyse of the *A. alpinus* leaves was performed. They contained of 2.14±0,005 % of total phenolic glycosides calculated as arbutoside (1), 1.40±0,004 % of total flavonoids calculate as hyperoside (2), 9.11±0.17 % of total phenolics and 6.02±0.44 % of hydrolysable tannins (3). The quantity of poliphenolic constituents was less than it is request for official drug, Uvae-ursi folium (1). Further, antioxidant activity of the acetone and methanol extracts of the *A. alpinus* leaves was measured trough the test of inhibition of the lipid peroxidation induced in the lipozomes by Fe^{2+}/ascorbate system (4). From the obtained results value of IC_{50} were calculated; for acetone extract IC_{50} was 1.05×10^{-4} mg/ml and for methanol extract IC_{50} was 1.36×10^{-4} mg/ml. For this activity, probably, the polyphenolic constituents of the *A. alpinus* were responsible. The acetone extract contained 23.43±0.40 % of total phenolics and 15.73±1.15 % of tannins, while methanol extract contained 23.48±0.39 % of total phenolics and 18.27±0.52 % of tannins.

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P 255 Antioxidant activity of *Epimedium alpinum* L.

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South-European species *Epimedium alpinum* has modest use in folk's medicine and hasn't been studied chemically and pharmacologically as Asian *Epimedium* species, which have long traditional use.
Plant material (herba and rhizome with roots) was collected in June 2001. at mountain Maljen in western Serbia; dried powedered material was extracted by maceration, first with chloroform, then with methanol. From rhizome/roots' methanolic extract aporfine alkaloid magnoflorine was isolated (1). Total flavonoid content (calculated as hyperoside) in methanolic extracts was determinated by spectroscopic method (2). Antioxidant activity of extracts was tested by the method of Fe^{2+}/ascorbate induced lipid peroxidation in liposomes (3), the FRAP method (4) and the DPPH free radical scavengging assay (5). Methanolic extract of herba (HM) showed stronger inhibition of lipid peroxidation (IC_{50} 0.47 mg/ml) and DPPH scavenging activity (IC_{50} 70 µg/ml), than rhizome/roots' methanolic extract (RM) (lipid peroxidation IC_{50} 0.82 mg/ml; DPPH scavengging activity IC_{50} 101 µg/ml); however, RM showed higher reductive activity in FRAP test (1339 AAE/g) than HM (628 AAE/g). Magnoflorine showed very strong reductive activity in FRAP test (7794 AAE/g). Methanolic extracts didn't differ much in total flavonoid content, as in magnoflorine content (HM: 8.15 % flavonoids, 0.46 % magnoflorine; RM: 9.02 % flavonoids, 11.82 % magnoflorine).

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Antioxidant properties of some fraction of bilberry fruits and their combinations with carotenoids in vitro

P 256

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Bilberry fruits and its extracts have positive effect on eyes. This effect connected first of all with carotenoids and polyphenoles. The aim of this study was to characterize the antioxidant activities of different fraction of fruits of Vaccinum myrtillus L and their combination with caratenoids. Freeze dried bilberry fruits were extracted according to the scheme (1). Chemical composition of fractions was analyzed by RP HPLC and HPTLC. Cyanidin, malvidin, delfinidin were identificated in crude extract. Rutin, quercetin, gallic acid, chlorophylles were identificated in polyphenoles fraction. β-sitosterol and lipids were found in lipophylic fraction. In vitro antioxidant [neutralization of HO• radical generated by the Fenton reaction (2) and inhibition of ROO• radical generated in system with AMVN (3)] properties of crude extract, ethylacetat, hexan fractions, lutein (L), zeaxantin (Z) and their combination were examined.

Fraction	Main compounds	IC_{50} ROO•			IC_{50} HO•		
		Fraction	Fraction+L	Fraction+Z	Fraction	Fraction+L	Fraction+Z
Crude extract	Anthocyanes	160±4*	107±3*	100±1*	360±3*	170±10*	180±9*
Ethylacetat	Polyphenoles	340±4*	167±10*	328±42	160±10*	130±1*	90±6*
Hexan	Lipophylic	250±30*	253±26	197±1*	270±1*	150±4*	110±9*

Values (mg/ml)x 10^{-3} are expressed as means ± standard error. * Significant different from fraction, $p<0.05$

Crude fraction is more active to ROO•, but less effective to HO•. Hexan fraction has shown practically identical activity to ROO• and HO•. Ethylacetat fraction was in 2 times more active to HO•, than to ROO•. It was shown that combination of fractions with lutein and zeaxanthin had synergism or additive effects.

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Bioguided screening focusing the antioxidant activity of coumarins and furanocoumarins in natural extracts

P 257

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Several natural products with a coumarinic moiety have been reported to have multiple biological activities. It is to be expected that, in a similar way to isomeric flavonoids, coumarins might affect the formation and scavenging of reactive oxygen species (ROS) and influence processes involving free radical-mediated injury. In this context, a class of compounds that is receiving increasing attention is furanocoumarin. This interest has been sparked by several large epidemiological studies in which the intake of dietary furanocoumarin is inversely related to the risk of cancer. So, we screened alpine medicinal plants for antioxidant activity, and we found *Peucedanum ostruthium* (L.) Koch. extracts very interesting. The roots and rhizomes of *P. ostruthium,* a rather handsome robust perennial herb of the Apiaceae family, are used in alpine ethnobotany mainly for digestive problems. The experimental work started with a sequential extraction procedure steps of the underground parts, from *n*-hexane to methanol. The extracts were screened by measuring the DPPH radical-scavenging potential. The methanolic extract showed strong antioxidant activity. This extract was subjected to HPLC-UV and HPLC-FD separation. The HPLC fractions corresponding to each compound were then subjected to MS and DPPH analysis. Bergapten, xanthotoxin, khellin, isopimpinellin, imperatorin, isoimperatorin, as linear furanocoumarins, and ostruthin were identified as main compounds. Ostruthin, 6-geranyl-7-hydroxycoumarin, showed a reduced antioxidant activity if compared with linear furanocoumarins.

P 258 Effects of *Micromeria cristata* extracts on stable DPPH radical and lipid peroxidation in rat liver microsomes

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The diethyl ether, ethyl acetate and n-butanol extracts that were obtained from aerial parts of *Micromeria cristata* (Lamiaceae) were tested for their possible antioxidant activity. The antioxidant activity was evaluated *in vitro* by the ability of the extracts 1) to interact with the stable free radical DPPH and 2) to inhibit the spontaneous and induced lipid peroxidation (LP) in rat liver microsomal fraction. DPPH scavenging effect was recorded spectrophotometrically, monitoring the transformation of DPPH stable radical into the reduced form (DPPH-H) (1). The LP in microsomes was proceed through an enzymatic pathway (NADPH; Fe(III)-ADP) and through processes that are non-enzymically catalyzed (ascorbic acid), measured by the amount of thiobarbituric acid-reactive substances (2). The *M. cristata* extracts act as moderate scavengers of DPPH radicals. The highest scavenging effect was obtained with n-butanol extract (IC_{50}=20 mg/mL), and the lowest one with diethyl ether extract (IC_{50}=50 mg/mL). The n-butanol extract exhibit the highest value of inhibition of spontaneous LP in rat liver microsomes (more than 30%) as well as in microsomal LP induced by NADPH, Fe (III)-ADP and ascorbic acid systems (more than 60%). The inhibition of the induced microsomal LP depends on the extract's concentration. The highest activity was obtained from the 0.01 g/mL extracts. The results were compared to those obtained for luteolin, quercetin and caffeic acid, that were used as standard substances. The results suggest that *Micromeria cristata* extracts act as non-specific donators for hydrogen atoms or electrons in the DPPH-assay and exhibit a potent antioxidant effect on spontaneous and induced microsomal LP in both enzymatic (NADPH; Fe (III)-ADP) and non-enzymatic (ascorbic acid) systems.

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P 259 Screening of Radical Scavenging Activities in Phytoextracts by means of HPLC Analysis with on-line $ABTS^{\bullet+}$-Bleaching

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Natural antioxidants have become a topic of increasing interest in medical and nutritional research driven by the belief that they are effective nutrients in the prevention of stress-related diseases. Due to the complexity of biological samples (phytoextracts, food) it is required to separate each antioxidant and to study individually their antioxidant capacity after time-consuming fractionation steps. Aim of this paper is to describe a rapid on-line method for screening of complex plant extracts for radical scavenging components. A paper published by Koleva e.al. showed a very appealing approch as it combines on-line HPLC separation with an assay for radical scavenging capacities (1). In our laboratory we have used reversed-phase HPLC with post-column derivatization with the stable radical 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), $ABTS^{\bullet+}$. HPLC separated compounds with radical scavenging abilities induce bleaching of the $ABTS^{\bullet+}$ detectable by a UV-VIS detector at 650 nm as negative peaks. The experimental set-up has been optimized. Quantification has been achieved based on Trolox equivalents. The method shows excellent repeatability (standard deviation <3%) and sensitivity and selectivity towards polyphenols. For instance, the detection limit of catechin is below 2 µmol /L. The potential of the method for optimizing extraction processes of antioxidants from herbs is demonstrated for rosmary and oregano. Furthermore we have screened alpine plants and wood decay fungi (*Ganoderma lucium*) for potential antioxidant ingredients.

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Comparison of antioxidant activity of acetone and aqueous extracts of raspberry (*Rubus idaeus L.*) leaves

P 260

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During last two decades numerous studies investigated various plant origin sources attempting to find new natural bioactive components for food and other applications. Some of these studies resulted in a development of natural antioxidant formulations. However, taking into account vast biodiversity in the plant kingdom scientific information on antioxidant and other bioactive properties which might be present in numerous plants is still scarce. The aim of this work was to assess radical scavenging capacity (RSC) and variations in the composition of phenolic compounds in the leaves from 35 raspberry (*Rubus idaeus* L.) accessions. The plants were collected in different natural habitats of Lithuania and replanted in the experimental field. The leaves were extracted with acetone and water and the extracts were tested for their antioxidant activity (AA) by using $ABTS^{\bullet+}$ decolourisation and $DPPH^{\bullet}$ radical scavenging methods. Antioxidant activity of raspberry extracts varied in a wide range; RSC of acetone extracts in $DPPH^{\bullet}$ reaction was from 39.4 to 88.7 %, that of aqueous extracts from 2.9 to 93.5 %; RSC of acetone extracts in $ABTS^{\bullet+}$ reaction system was from 19.0 to 97.8 %, that of aqueous extracts from 41.9 to 97.5 %. However, 94 percent of all tested acetone and aqueous extracts exceeded 50 % of $DPPH^{\bullet}$ RSC, while 69 % of acetone and 97 % of aqueous extracts exceeded 50 % of $ABTS^{\bullet+}$ RSC. The total amount of phenolic compounds in acetone extracts varied from 22.1 to 52.2 mg of gallic acid equivalents (GAE) in 1 g of plant extract, while in aqueous extracts phenolics constituted from 19.2 to 38.7 mg. The extracts were also analysed by HPLC/UV/MS. Quercetin glucuronide, quercetin-3-glucoside and quercetin glucosyl rhamnoside (rutin) were identified in the extracts. Remarkable differences in the composition of the extracts were observed indicating that herbal tea preparations containing *Rubus idaeus* leaves need more detailed examination in order to standardise their possible functional properties and pharmacological effects.

Free radical scavenging activity of the flavonoids isolated from *Tecoma radicans*

P 261

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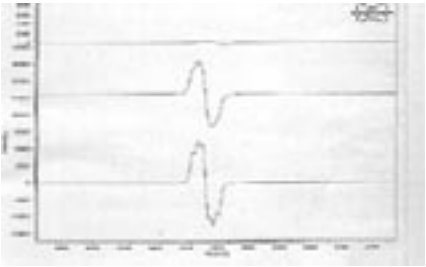
Successive extracts of aerial parts of *Tecoma radicans* F. (Bignoniaceae) showed free radical scavenging activity(1), the most inhibition produced with the stable DPPH radical(2), recorded by electron spin resonance (ESR) was from ethyl acetate extract, Fig 1. Vitamin C is used as standard scavenger. The double integration area is 62 for DPPH alone, it decreased to 55, 62,61 and 14 after addition of vitamin C, pet. ether, chloroform and ethyl acetate extract, respectively, Table1. Activity guided fractionation led to the isolation of ten flavonoids(3).The most potent of them as free radical scavenger is quercetin 3-methyl ether giving 69% inhibition of DPPH radical then luteolin giving 40% and sciadopitysin 19%.

Table1.Inhibition of DPPH radical by Tecoma radicans acetate ext. of Tecoma radicans.

Compound	Double integration area	Percentage inhibition
DPPH	62	
Vitamin C	55	11.3
Pet. ether ext.	62	0
Chloroform ext.	61	1.0
Ethyl acetate ext.	14	77.4
Quercetin 3-methyl ether	19	69
Luteolin	37	40
Sciadopitysin	50	19

Fig 1. ESR of control, standard and ethyl



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P 262 Flavonoids from *Rosa rubiginosa* L. and their antioxidant activity

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Twenty five species (fourteen native) of the genus *Rosa* L. (Rosaceae), mostly belonging to caninae Section, are known in the flora of Poland area (1). *Rosa rubiginosa* L. is one of the species became a main source of Polish Pharmacopeias material Fructus Rosae (FP IV). Rose hips have been used extensively for pharmacological purposes (e.g. purgative, anti-inflammatory, antiulcers, cytotoxic and antioxidant) and as food, mainly for preparing jam, tea and alcoholic beverages after fermentation. Flavonoids are a very valuable group of natural compounds, which posses a wide range of pharmacological activities, such as antioxidant, anti-inflammatory, cardiovacsular and anticancerogenic (2). Several flavonoids were reported in *Rosa* L species in the past (3, 4, 5), but there are no intensive chemical and biological works on the constituents of *R. rubiginos*a. Subsequent separation of the aqueous methanol extract from the dried leaves of analyzed species on polyamide, silica gel and Sephadex LH 20 columns (CC) led to isolation of 16 known flavonoids (derivatives of kaempferol or quercetin). Structures of the compounds were elucidated by chemical methods (co-chromatography, hydrolytic degradation, melting point) and spectroscopic techniques (UV, MS, 1D and 2D NMR). The extracts from *R. rubiginosa* and isolates were investigated for their radical scavenging abilities through spectrophotometric assay on the reduction of 2,2-diphenyl-pycryl hydrazyl (DPPH). These results were correlated to structural features and contents of the flavonoids.

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P 263 Total radical-trapping antioxidant capacity of kaiware-daikon (*Raphanus sativus* L.) extract and glucoraphasatin before and after treatment with myrosinase

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A great deal of scientific evidence suggests that the intake of Brassica vegetables (e.g. broccoli, cabbage, etc.) may be very effective in reducing cancer risk. Their effectiveness is mainly attributed to their content of phytochemicals known as glucosinolates (GL), which upon intake are hydrolysed into isothiocyanates (ITC) by the endogenous enzyme myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1). Of the many GL identified, several possess an alkenyl side chain bearing a sulphur atom at different oxidation states. Kaiware-daikon (*Raphanus sativus* L. sprouts) contains large amounts of one of them, the 4-methylthio-3-butenyl GL (glucoraphasatin, GRH). Sprouts have been known for centuries to have therapeutical and nutrient properties, but recently a mixed Brassica sprout extract was found to protect human colon cells against H_2O_2 damage. Studies on the antioxidant capacity of sprouts and their components (namely GL) are of great interest due to fact that oxidative stress is a feature of many diseases. We investigated the total radical-trapping antioxidant parameter (TRAP) of both the kaiware-daikon extract (KDE) and purified GRH before and after myrosinase-catalysed hydrolysis. Our results show that KDE has relevant antioxidant activity by means of inhibiting free radical generation, this activity flagrantly increasing after myrosinase-catalysed hydrolysis.

Bioassay-guided isolation of the antioxidant constituent from *Punica granatum* fruit peel

P 264

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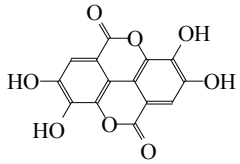
Using DPPH radical scavenging assay (1) to investigate the antioxidant activity of crude ethyl acetate extract from the fruit peel of *Punica granatum* found that the fruit peel extract possessed a significant antioxidant activity (ED_{50} 5.8 ± 0.30 μg/ml). On the basis of DPPH radical scavenging assay-guided isolation, the ethyl acetate extract of *P. granatum* fruit peel was separated by silica gel vacuum chromatography and Sephadex LH-20 gel filtration chromatography afford a cream colored needles (PG1), which was identified as ellagic acid. This compound exhibited antioxidant activity (ED_{50} 2.1 ± 0.02 μM) that was 4 times stronger than quercetin (ED_{50} 8.3 ± 0.23 μM). In addition, PG1 was also subjected to evaluation of HIV-1 protease and HIV-1 integrase inhibitory activities (2,3). It was found that GP1 possessed only anti HIV-1 integrase activity with IC_{50} of 14.2 μM.



Ellagic acid (PG1)

Acknowledgements: Faculty of Pharmaceutical Sciences, Prince of Songkla University

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Investigation of Phenolic compounds and Antioxidant Activities of *Zeravschania aucheri* (Boiss.) Pimenov an Endemic Plant of Iran

P 265

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Z.aucheri was selected for analysis among the endemic species of zeravschania from *Peucedanum* s.l complex of parsley family (Apiaceae), collected from Gilan province and powdered aerial parts of plant (300g) were extracted with MeOH-Water (80-20) respectively, extract was concentrated under reduced pressure and then washed with $CHCl_3$. 12 g of methanol extract was chromatographed with different methods such as CC and TLC. Different fractions were collected according their UV quenching at 254, 365 nm and each fraction was purified by Sephadex LH20 and PC chromatography on Watman No.1 and 3. Chrysoeriol-7-O-glucoside (27 mg), 6-hydroxy kaempferol-7, 3-O-diglucoside (21 mg), 6-methyl kaempferol (27 mg) and caffeic acid (30mg) were isolated and identified by NMR, MS and UV spectroscopic methods. These compounds were isolated for the first time from *Z. aucheri*. The antioxidant activity (lipid peroxidation and radical scavenging) of the plant MeOH extract was determined with FTC (ferric ammonium thiocyanat) method and DPPH (1, 1-diphenyl-2-picrylhydrazyl) (1). The antioxidant activity of MeOH extr. With FTC method was (IC_{50}=294μg/ml) after 96 hr and with DPPH was (IC_{50}=437μg) after 30 min. which their activities were near the BHT and ViteE. The flavonoid containing plants have antioxidants, anticancer, antispasmodic, anti-inflammatory, antibacterial and antifungal activity and S.J.Cutler 2000 was reported Kaempferol has antileukemic effects (2). The antioxidant activity of extract may be due to caffeic acid (3) and two kaempferol derivatives (4).

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P 266 Antioxidant and neuroprotective properties of *Scutellaria lateriflora*

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American skullcap (*Scutellaria lateriflora* L., Lamiaceae) extracts have been used to treat several nervous disorders. There is still some uncertainty about the effective compounds, nevertheless, it is accepted that the flavonoids are important active constituents in skullcap. In the present study, we evaluated the antioxidant and neuroprotective potential of an ethanolic extract (SLE) prepared from leaves of *S. lateriflora*. The SLE main compounds were identified and quantified by HPLC-DAD. The flavones baicalein and baicalein-7-*O*-glucuronide were the major compounds found. The extract was very effective in scavenging the DPPH free radical (EC50, 83µg dwb/ml) and in reducing the lipid peroxidation of rabbit synaptossomes (EC50, 10µg dwb/ml), induced with ascorbate/Fe^{2+}. We also assessed the potential antioxidant role of SLE in PC12 cells submitted to lipid peroxidation with ascorbate/Fe^{2+}, in conditions that cause a significant increase of TBARS products (5x more). An SLE equivalent amount of 10µg dwb/ml significantly reduced (10%) the ascorbate/Fe^{2+} induced lipidic peroxidation, namely when PC12 cells were pre-incubated for 2 hours with the extract (35% reduction). The SLE extract by itself, even in a non-diluted concentration (100 mg dwb/ml), did not affect cell viability of PC12 cells under the experimental conditions used for lipid peroxidation. In a condition where ascorbate/Fe^{2+} induced lipidic peroxidation cause a significant loss of PC12 cell viability (up to 30%), this effect was attenuated by the use of SLE (10µg dwb/ml). When the PC12 cells where pre-incubated for 2 hours with the same concentration of SLE, cell death was significantly reduced (only 10% of the cells died). In conclusion, SLE could act as a strong antioxidant agent and have neuroprotective properties, which can account for the traditional use of this plant.

P 267 Redox activity in vitro and in vivo of minor compounds of extra virgin olive oil

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In order to evaluate the beneficial effects of extra virgin olive oil in the prevention of cardiovascular pathologies onset, oils produced in different locations (Tuscan and Liguria, Italy) were considered. Minor polar compounds (MPC) and other new minor constituents, i.e. nitro-derivates of oleic acid (0-NO2) and linoleic acid (L-NO2), recently identified in virgin oil, were analyzed. L-NO2 a platelet anti-aggregant, inhibits neutrophile activation, and is a signalling molecule related to stimulation of peroxisomal proliferator-activated receptor gamma. The extracts of the different oils were characterized by different MPC content (prevalently lignans and secoiridoids) totals (600-150 mg/L) and nitro-derivates content (10-40 µM). Furthermore, they presented notable qualitative differences with potential differences in biological response. The relative *in vitro* antiradical activity in the DPPH test, the antioxidant capacity on LDL and antiaggregant properties in animals and humans were evaluated. The degree of oxidation of human LDL induced by copper has shown a different antioxidant effect in two extracts, higher in a Tuscan sample (ED_{50} = 0.46 ±0.07 µM; n=8) compared to that obtained from the area of Liguria (ED_{50} = 2.39 ±0.09 µM; n=8). The antiaggregant activity was evaluated in a platelet aggregation test in platelet enriched human and rat plasma, utilizing the following agonists: ADP and collagen. The ED_{50} levels of the agonists before and after the administration of oil per os (36g of oil for human, 0.4g oil per 400 g rat) were evaluated. ED_{50} levels in humans support the antioxidant role of olive oil in preventing cardiovascular pathologies. Dose-dependent effect in function of the growing MPC content has been determined.

Development of analytical methods and oxidative stress animal models for the evaluation of antioxidants

P 268

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There is great interest in the role of oxidative stress in aging and diverse disorders such as atherosclerosis, diabetes and cancer. Many polyphenolics have shown interesting antioxidant activity *in vitro*[1], but more research is needed to elucidate whether these dietary antioxidants exhibit relevant activity *in vivo*. This requires development of an "oxidative stress" animal model and a battery of validated assays, covering the different aspects of *in vivo* oxidative damage and antioxidant defence. For the latter, different methods for monitoring oxidative lipid damage (malondialdehyde, MDA), fat-soluble antioxidant status and glutathione peroxidase activity in plasma were developed and validated[2]. Using these optimised assays, three animal models were evaluated, including a vitamin E-deficient rat model, a diabetic rat model and an atherosclerotic rabbit model, in order to select a representative "oxidative stress" model. With respect to plasma lipid peroxidation status, a significant augmentation was observed in the diabetic (dia) [0.80 $\pm$ 0.02 µM MDA (dia), 0.54 $\pm$ 0.03 µM MDA (control), $p < 0.0001$] and atherosclerotic (ath) [2.28 $\pm$ 0.26 µM MDA (ath), 0.99 $\pm$ 0.09 µM MDA (control), $p < 0.001$] animal models, confirming increased oxidative damage, while no significant change was observed in the vitamin E deficient (Edef) animals [0.43 $\pm$ 0.02 µM MDA (Edef), 0.41 $\pm$ 0.04 µM MDA (control), $p > 0.05$]. In addition, the endogenous antioxidant coenzyme Q9 was significantly increased in diabetic rats [337 $\pm$ 45 ng/ml (dia), 186 $\pm$ 28 ng/ml (control), $p < 0.05$]. In conclusion, this optimised *in vivo* oxidative stress system can be directly applied for the *in vivo* evaluation of promising antioxidants.

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Effect of Bastard Balm (*M. melissophyllum* L.) Ether Oil on Lpx of Liposomes

P 269

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Bastard balm leaves (*Melittis melissophyllum* L.) contain no more than 0.1% of essential oil which is of complex and variable composition. The composition is similar to that of lemon grass, but balm oil can be identified by its typical pattern in chiral compounds. The leaves contain flavonoids, triterpenes, monoterpene and phenolpropanoid glycosides, phenolic acids, sterols and salts. In this study we investigated the effect of bastard balm ether oil on lipid peroxidation in liposomes (LPx). Beside that, we exhamined synergistic effects of this ether oil and ciprofloxacin. The crude n-hexane extract was obtained by the Soxhlet extraction of powdered plants material with n-hexane. 10% (v/v)solutions of extracts in 50% ethanol were prepared. The effects of these extracts on lipid peroxidation of liposomes was investigated according to Fukuzawa. As a model-system the commercial preparation of proliposomes: "PRO-LIPO S" (Lucas Meyer) with 30% phosphatidylcholine of soybean pH=5-7, was used. Lipid peroxidation was performed according to Afanas'ev and Buege-Aust. Pure preparations of ciprofloxacin and fullerene was used in different amounts. Addition of 30 µl of bastard balm ether oil caused decrease in the LPx activity. All added volumes of ether oil (10, 20 and 30 µl) in combination with ciprofloxacin reduced the LPx intensity, and the most efficient was the combination of 30 µl of ether oil with all added volumes of ciprofloxacin (10, 20 and 30µl). In combination with fullerene derivative, ether oil of bastard balm, containing 20 µl of ether oil and 30 µl fullerene, and 30µl ether oil and 30 µl fullerene induced decrease in LPx intensity. In conclusion, it is pointed out that ether oils of the above-mentioned plants contain various compounds with antioxidative properties. Those substances show different mechanisms of action, including synergism, to reduce LPx activity and are included in the antioxidative defence systems.

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P 270 Treatment with European mistletoe (*Viscum album* L.) grown on pears aqueous extracts may reduce the *in vivo* oxidative stress induced by CCl_4

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One of the paradoxes of life on the Earth is that oxygen is necessary for living of aerobic organisms. On the other hand, increased concentration of oxygen and it's metabolites (reactive oxygen species-ROS) may cause a number of diseases. Enviromental contamination, modern lifestyle, food rich in hormones and aditives, etc., are just some of the causes of increased production of reactive oxygen species and development of oxidative stress. In healthy cells, oxidative stress and antioxidative protection are in constant equilibrium. In the last decade, significant attention is focused on active principles from plants as potential antioxidants. European mistletoe (*Viscum album* L.) is used as a complementary therapy of certain number of carcinomas. Previously we have proved that one of the mechanisms of mistletoe cytotoxicity towards tumour cells is its prooxidative action and induction of oxidative stress. In this paper, we have tried to verify whether there is an pro- or antioxidative shift in healthy cells and tissues upon treatment of CCl_4-induced oxidative stress with an aqueous extract of European misltetoe grown on pears. We have followed the potential antioxidative properties of examined extract by antioxidative enzymes activities (xanthine oxidase, catalase, peroxidase, glutathione reductase, glutathione peroxidase), as well as amount of reduced glutathione, concentration of hydroxyproline in mice liver homogenate, and intensity of lipid peroxidation. Obtained results showed the high degree of oxidative tissue damage upon CCl_4 administration. Almost all examined parameters were significantly reduced after mistletoe extract application in comparison to CCl_4 control. Having these results in mind, we suggest that mistletoe grown on pears extract might exhibit certain cytotoxic effects towards tumour cells, but protective action towards healthy tissues.

P 271 Prooxidant-antioxidant shift induced by European mistletoe (*Viscum album* L.) grown on pears treatment of Ehrlich tumour cells *in vivo*

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Extracts from European mistletoe or *Viscum album* L. have been known as secondary medicaments, widely used in therapy of hypertension. Also, they have been reported to exert cytotoxic and immunomodulatory effects *in vitro* and *in vivo*. The mechanism of anti-tumoral activity is, however, largely unknown. In this study we tested the hypothesis that aqueous extract from the European mistletoe grown on pears, exhibit mentioned properties due to induction of oxidative stress in Ehrlich tumour cells *in vivo*. Aqueous *Viscum album* L. extract was given to experimental animals (NMRI mice) in three different ways – before implantation of Ehrlich carcinoma cells (EAC), after implantation and at the same time with implantation. We have observed significant reduction of cancer incidence in all groups that received mistletoe extract in comparison to Ehrlich control. Number of tumour cells was decreased up to almost 95% in male animals that received mistletoe extract before implantation, and up to 80% in female animals. Significantly reduced number of EAC cells was also obtained in animals with developed carcinoma. Levels of antioxidative enzymes were low in EAC, as well as intensity of lipid peroxidation. In contrast, oxidative stress of EAC was increased in a rather high degree after administration of *Viscum* extract. This was in accordance with with the increase of damaged cells percentage. These results indicate that oxidative stress, defined by the levels of antioxidative enzymes, might play an important role in *in vivo* cytotoxic properties of misltletoe grown on pears extracts. In addition, applied mistletoe extract might also possess beneficial effects on restoration of impaired oxidative balance in normal tissues as an efficient antioxidant. Our data suggest that "pear's" mistletoe may be potentially useful in the prevention of the tumour development, but emphasise the need of further elucidation of mechanisms of its action.

In vivo antioxidant activity of herbal formulations: Amala mus and Amala plus in ethanol induced oxidative stress

P 272

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Pharmaceutical preparations derived from natural sources often contain compounds that contribute to the antioxidant defense system and apparently play a role in the protection against degenerative diseases. Amala Mus and Amala Plus are the herbal formulations containing various constituents from the traditionally used medicinal plants. Present work evaluated the in vivo anti oxidant activity of these formulations in ethanol induced oxidative stress. Wistar rats (150-200g) were used for the study and treated with Amala Mus: 2.7 g/kg body weight and Amala Plus: 105 mg/kg body weight p.o for 21 days. Immunace tablets: 110 mg/ kg body weight served as the positive control. The rats were concomitantly administered 10 % ethanol: 20 ml/kg body weight p.o. since ethanol enhances generation of oxygen free radicals during its oxidation in liver. Liver damage was manifested by elevated plasma ALAT and γ-GT levels and test formulations significantly ($p< 0.001$) countered this rise. The antioxidant enzyme status in liver and kidney was also hampered in alcohol control group with decrease in superoxide dismutase, catalase, glutathione peroxidase; glutathione-S-transferase and glutathione reductase levels whereas test formulations ($p< 0.01$) restored the depleted enzymes. Reduced glutathione has a direct antioxidant function and plays a major role in eliminating large number of exogenous toxicants and its depletion enhances lipid peroxidation. The significant ($p< 0.01$) reduction in reduced glutathione with enhanced lipid peroxidation estimated by TBARS assay was observed in alcohol control group and these effects were ameliorated by the test formulations. The histopathological studies of livers showed marked diffuse vacuolar and moderate diffuse granular degeneration with moderate fatty infiltration and mild multifocal periportal leucocyte infiltration in alcohol control group. The test formulations treated groups showed only minimal diffuse lymphocytic infiltration depicting recovery of liver. In conclusion, Amala Mus and Amala Plus exhibited significant anti-oxidant activity in rats.

Studies on in vitro antioxidant and in vivo hepatoprotective activity of *Nyctanthus arbortristis* in carbon tetrachloride induced acute hepatic injury

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Reactive oxygen species (ROS) generated endogenously or exogenously are associated with the pathogenesis of various diseases, which can be managed with the use of agents having antioxidant activity. The leaves of *Nyctanthus arbortristis* Linn. *(*Family: Oleaceae) [NA] are used in Indian folklore medicine in the treatment of liver disorders hence the present study was undertaken to evaluate its *in vitro* antioxidant and *in vivo* hepatoprotective activities against carbon tetrachloride (CCl_4) induced hepatic injury. Dried powdered leaves of NA were sequentially extracted with petroleum ether, ethyl acetate and methanol respectively. *In vitro* antioxidant activity of these extracts was evaluated by using Di-Phenyl Picryl Hydrazyl radical scavenging assay; $FeSO_4$ induced lipid peroxidation in rat liver homogenate, nitric oxide, and superoxide radical scavenging assay. Methanolic extract of *Nyctanthus arbortristis* (MNA) revealed highest *in vitro* antioxidant potential, hence further evaluated for hepatoprotective activity. Wistar rats (150-200 g) used for the study were pretreated with 100, 250 and 500 mg/kg body weight of MNA respectively for 14 days. On 14th day CCl_4 (1.25 ml/kg, p.o.) was administered to induce acute hepatic injury, manifested by significant ($p<0.01$) rise in plasma ASAT, ALAT, ALP and LDH levels compared to respective control values. Administration of CCl_4 also caused alterations in plasma total protein, albumin, cholesterol and total bilirubin levels. Pre-treatment of rats with MNA significantly ($p<0.01$) inhibited the alterations in these biochemical levels in a dose dependent manner. MNA also countered the increase in liver weights and the severity of histological lesions in liver caused by CCl_4 with aversion of variations in catalase activity, glutathione levels, TBARS levels and DNA content of liver. These results indicate that MNA exhibited significant anti-oxidant and hepatoprotective activity against CCl_4 induced acute hepatotoxicity, comparable with the standard silymarin (100 mg/kg).

P 274 Antioxidative and hepatoprotective effect of *Equisetum* L. species

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Among several *Equisetum* species, only *E. arvense* L. has been investigated and used in herbal medicine mainly as a diuretic and a haemostyptic. Pharmacological activity of *E. arvense* mostly comes from the presence of the wide range of flavonoids, which are known as strong antioxidant and hepatoprotective agents (1). This report shows the *in vitro* antioxidant and hepatoprotective activities of some *Equisetum* sp., growing in Serbia: *E. arvense* L., *E. palustre* L., *E. hiemale* L., *E. maximum* L. and *E. ramosissimus* L. Antioxidant activities were examined following the DPPH and NO scavenging capacity, as well as, inhibition of lipid peroxidation (LP) in liposome. The highest scavenging capacity was shown by EtOAc extract of *E.arvense* both to the DPPH (EC_{50}= 2.37μg/ml) and NO (EC_{50}= 153.12μg/ml) radicals. On the other hand, EtOAc extract of *E.palustre* , was most potent inhibitor of Fe^{2+}/ascorbate induced LP (EC_{50}= 12.9μg/ml). Hepatoprotective effect was studied in the rat liver homogenate before and after intoxication with CCl_4. Addition of CCl_4 produced significant impairment in hepatic antioxidant status, decreasing GSH content and stimulating LP. Only incubation with extracts of *E. palustre* and *E. arvense* significantly reduced CCl_4 -mediated harmful alteration of LP and GSH. In summary, the obtained results show that among examined *Equisetum* species *E. arvense* and *E. palustre* possess highest antioxidative and hepatoprotective effects.

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P 275 Antioxidant capacities in spices and medicinal herbs

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Whole black peppercorn, nutmeg, rosehips, cinnamon, and oregano leaf samples were extracted with 50% acetone and 80% methanol, and evaluated for their antioxidant capacities. The antioxidant capacities were examined as their radical-scavenging activities against cation ABTS, DPPH and peroxyl (ORAC) radicals, and Fe^{2+} chelating capacity. The total phenolic content (TPC) was also determined for each extract since TPC contributes to the overall antioxidant ability. All botanical extracts showed significant radical- scavenging activities and TPC. The 50% acetone extract of cinnamon exhibited the greatest $ABTS^{\bullet+}$ scavenging capacity of 1243 μmoles TE/g and the strongest ORAC of 1256 μmoles TE/g, whereas the lowest ED_{50} value of 30 μg/mL was detected in the 80% methanol extract of cinnamon. In addition, the greatest Fe^{2+} chelating ability was detected in the 50% acetone extract of rosehips. Finally, the 50% acetone and 80% methanol extracts differed in their antioxidant properties, suggesting the potential effects of extraction solvent on antioxidant activity estimation of botanicals. The results from this study indicate that spices and medicinal herbs may serve as potential dietary sources of antioxidants for disease prevention and health protection.

Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds

P 276

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Antioxidant activity of hydro alcoholic extract of *Nelumbo nucifera* seeds (HANN) was studied both by *in vitro* and *in vivo* models. The HANN exhibited strong antioxidant activity as evidenced by the low IC_{50} values in both 1, 1-diphenyl-2-picryl hydrazyl (6.12 $\pm$ 0.41µg/m1) and nitric oxide (84.86 $\pm$ 3.56µg/m1) methods. The values were found to be less to those of rutin, the standard used. Administration of HANN at 100 and 200 mg kg^{-1} body weight given for four days prior to carbon tetrachloride (CCl_4) treatment caused a significant increase ($p<0.05$ to $p<0.001$) in the level of superoxide dismutase (SOD) and catalase and a significant decrease ($p<0.05$ to $p<0.001$) in the level of thiobarbituric acid reactive substances (TBARS), when compared to CCl_4 treated control in both liver and kidney. These changes observed at 100 mg kg^{-1} body weight treatment were comparable to those observed for standard vitamin E at 50 mg kg^{-1} treatment. The results support significant antioxidant nature of HANN.

Flavanolignans from Milk Thistle May Prevent Athersclerosis

P 277

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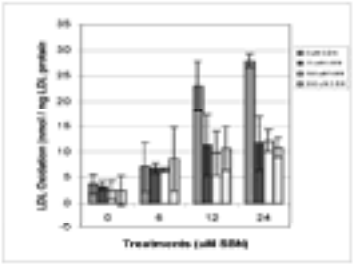
Milk thistle (*Silybum marianum* L.), used in the treatment of liver disorders, may also be useful in the treatment of atherosclerosis. In this study the ability of milk thistle flavanolignans silychristin (SCN), silydianin (SDN), and silybinin (SBN) to inhibit chemically-mediated oxidation of low-density lipoprotein (oxLDL) was determined. Native LDL was treated with $CuSO_4$ in the absence or presence of flavanolignans (at 0, 75, 150, and 300 µM). Generation of oxLDL was determined by TBARS and monocyte adhesion assays. SBN inhibited the generation of oxLDL at 24h time period (Fig 1). Similarly, SCN, SDN, and SBN showed about 68.7, 73.5, and 61.1 % reductions in LDL oxidation, respectively. Monocytes adhered (52,625 $\pm$ 2,441 cell number) to LDL incubated with $CuSO_4$ in the absence of SBN . However, the monocyte adhesion was reduced to 475 $\pm$ 361 cells when LDL and $CuSO_4$ were co-incubated with SBN. These results showed that the silibinin inhibited the generation of oxLDL and subsequent oxLDL-mediated moncotye adhesion, a primary event in the development of atherosclerosis. Milk thistle may offer a protective effect against LDL oxidation.



Acknowledgments: Arkansas Agricultural Experiment Station for funding

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P 278 Aroma precursors and scavenger activity of *Allium* species from Central Asia

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The very diverse genus *Allium* L. shows a nearly exclusive distribution across the northern hemisphere with a main centre of diversity in Southwest and Middle Asia. Since 8000 years common onion has been probably used by mankind as medicinal drug, spice and vegetable. Currently, rhizomes or bulbs, extracts of those or green parts of several species like *A. stipitatum* Regel, *A. macleanii* Baker, *A. rosenorum* R.M. Fritsch are intensively used by the native population of Central Asia. However, only a few wild growing Alliums were chemically analysed until now. To close this cleft of knowledge, samples from the Central Asian republics Uzbekistan and Tajikistan were analysed. Investigations were focused on the subgenera *Allium*, *Rhizirideum* and *Melanocrommyum* analyzing the pattern of cysteine sulphoxides as well as the radical scavenger activity of crude extracts. Remarkable high scavenger activity was reported for *A. giganteum* Regel, *A. alaicum* Vved. and *A. komarowii* Lipsky, all belonging to the subgenus *Melanocrommyum*. Because the amount of cysteine sulphoxides of these species is rather low (about 0.1 %), radical scavenger activity must be related to still unknown substances of this subgenus. In contrast investigated samples obtained from the subgenera *Allium* and *Rhizirideum* also giving a significant radical scavenger activity displayed high amounts of cysteine sulphoxides (*e.g.*, *A. turkestanicum* Regel, subg. *Allium*, 0.54 %).

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P 279 Comparative antioxidant activity of *Amaranthus seeds* (Amaranthaceae)

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The *Amaranthus* plants are spread throughout the world and are able to produce grains and leafy edible vegetables (1). Nutritionally, amaranth grain, has a 2 to 3 times higher biological value than common cereal grains (2) so is presently being exploited by food manufacturers (3) and has shown potential for the use in infant formulas (4). In the present study two varieties of *Amaranthus caudatus* (Oscar blanco and Victor red) were investigated with the aim of characterizing the plants for their antioxidant activity and oil, squalene and total phenolic contents. Plant materials were sun dried, ground to 0.2 mm mesh and extracted with MeOH through maceration. The resultant extracts were dried under reduced pressure using a rotary evaporator. In order to obtain unsaponifiable fraction and phenolic fraction, the methanolic extract was dissolved in MeOH/H_2O (9:1) mixture and partitioned with *n*-hexane and AcOEt. Squalene contents in unsaponifiable fractions were determined using Column Chromatography on a silica gel. The qualitative data of the unsaponifiable chromatographic fractions have been determined through GC-MS. Seeds of both investigated varieties were found to possess very different levels of squalene (2.2% in Oscar blanco and 7.5% in Victor red). The in vitro antioxidant activity tests were carried out using the TBA test (5). Ethyl acetate extracts of both varieties of *A. caudatus* showed significant antioxidant activity (IC_{50} of 0.50 mg/ml, var. Oscar blanco, and 0.62 mg/ml, var. Victor red). Isolated squalene showed potent ability to inhibit lipid peroxidation with an IC_{50} of 0.023 mg/ml.

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Additive and supraadditive effects of the different components contribute to the antioxidative properties of STW 5 (Iberogast®)

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The efficacy of the herbal drug STW 5 (Iberogast®) in the treatment of functional dyspepsia is proved in several clinical studies, but it is still open whether STW 5 and its nine herbal components interact in an additive or supraadditive way. Due to the fact that inflammation reaction and reactive oxygen species determine functional gastro-intestinal diseases (1) we selected experimental models in order to demonstrate antioxidative effects of STW 5 and its nine constiutent herbal extracts in vitro. First the spontaneous radical decay of 2,2'azobis (2amidinop ropan)dihydrochloride was used to determine antioxidative, radical scavenging characteristics of the extracts. It is seen that STW 5 (diluted 1:5000) is effective equally to 10µM trolox and that the components had significant radical scavenging activity in increasing order: bitter candy tuft, liquorice root, milk thistle, greater celandine, caraway, angelica root, camomile, STW 5, melissa and peppermint. Here we find additive action of the extracts. In further experiments oxidative burst reaction of alveolar macrophage obtained from pig lung and leukocytes obtained from human buffy coat was mesured using luminol-enhanced chemiluminescence. STW 5 and all constituent extracts showed antioxidative properties, however, in both systems, the effects of the whole mixture were stronger by 30 to 50% than the expected values when calculated in an additive manner from the different extracts, indicating a supraadditive cooperation of the different components. STW 5 (diluted 1:100) is significantly more effective than the strong antioxidant nordihydroguaretic acid (25 µM/l). It is reasonable to assume that the therapeutic effectiveness of STW 5 (Iberogast®) is due to additive and supraadditive actions of its constituents, which might be responsible for its effects in multifactorial gastrointestinal diseases.

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P 281 Leucanthoside A, a new triterpenoid saponin with microtubule-stabilizing activity from aerial parts of *Cephalaria leucantha*

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The plant material of *Cephalaria leucantha* was collected nearby Danilovgrad (Montenegro) in July 2003. Ground, air-dried aerial parts of the plant (150g) were extracted with 90% MeOH. The crude extract was fractioned on Sephadex LH-20 CC, silica gel CC, dry-column flash chromatography and lobar column chromatography, yielding 15 mg of triterpenoid saponin, 3-O-[β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-28-O-[β-D-allopyranosyl(1→6)-β-D-glucopyranosyl]-hederagenin, named Leucanthozide A. Its structure was elucidated using ESI MS, 1D and 2D NMR spectroscopy. Structures of sugar and aglycon moiety were confirmed after acid hydrolysis by TLC comparison with authentic samples. Leucanthozide A represents a new compound containing extremely rare sugar allose. Tubulin test (1) performed for this saponin showed microtubule-stabilizing activity (IC_{50} = 50 μM). Under the same conditions paclitaxel showed a hundred times higher activity (IC_{50} = 0.5 μM). Leucanthoside A showed no cytotoxic activity on *Artemia salina* assay (2) in concentrations less then 1 mg/ml.

Acknowledgements: The authors are grateful to the Ministry of Science and Environmental protection, Republic of Serbia (Project No. 1755) for financial support
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P 282 New Steroidal Glycosides from Indian *Caralluma* Species

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The genus *Caralluma* (Asclepiadaceae) consists of 56 species occurring from the mediterranean region to Eastern Asia as well as to Eastern Africa. The species occurring in Southern India are thick, succulent, almost leafless perennial herbs which grow wild e.g. in the region of Andhra Pradesh and Karnataka. They are a rich source of steroidal glycosides. The plants are known for their bitter taste and extracts of these plants were reported to possess anti-inflammatory activity (1). Although in general chemical investigation of Carallumas are rare, several pregnane glycosides have been reported from a few *Caralluma* species in recent years.
This study is a continuation of our work on chemical constituents of Carallumas of the Indian subcontinent. Here, we present the isolation and structural elucidation of new steroidal glycosides from *Caralluma stalagmifera*, *C. indica*, *C. umbellata* and *C. adscendens* var. *fimbriata*. The aglycones of these saponins are usually based on polyhydroxylated pregnane skeletons such as the 5α-dihydrosarcostin skeleton or the 3β,8β,12β,14β-tetrahydroxypregnane-20-one skeleton. Their structures were elucidated by extensive NMR spectroscopic studies without recourse to any derivatization.

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Triterpenoid Saponins from the Roots of *Ferula harmonii L.*

P 283

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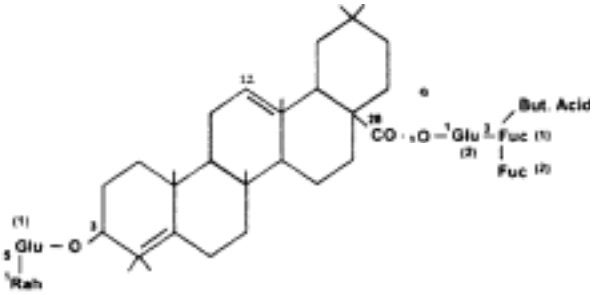

A new acylated triterpenoid saponin, ferharmoside A (1) of the aglycone oleanolic acid, was isolated from the roots of *Ferula harmonii* L. family *Apiaceae*. The structure of its aglycone and sugar parts has been elucidated by a general strategy involving thin layer chromatography (HPTLC, TLC) after acidic hydrolysis, NMR spectroscopy (^{1}H and ^{13}C NMR). The aglycone part was 3-hydroxyolean-12-en-28-oic acid and the sugar parts were two molecules D-glucopyranosyl, tow molecules D- fucopyranosyl and one molecule L- rhamnopyranosyl

Acknowledgements: Thanks to Prof. Dr. W. Blascheck for his scientific invitation to do some experiments in Institute of Pharmacy at CAU in Kiel, and Dr. Girreser from CAU in Kiel, Institute of Pharmacy, for his help by taking the ^{1}H and ^{13}C NMR-spectrums.

Cytotoxic Triterpenes with a New Rearranged Carbon Skeleton from the Bark of *Garcinia speciosa*

P 284

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In the continuation of our search for bioactive secondary metabolites from *Garcinia speciosa* Wall. we have isolated, besides the new friedolanostane, methyl (24*E*)-9α, 23α-dihydroxy-3, 15-dioxo-17, 15-friedolanostan-8(14), 24-dien-26-oate (**1**)**,** four novel triterpenes containing a new rearranged carbon skeleton, the 11(10→8)-abeolanostanes:, 14β,15β-epoxy-3β-hydroxy-9-oxo-11(10→8)-abeolanosta-22-*cis*, 24-*trans*-dien-26-oic acid (**2**), 14β, 15β-epoxy-3β-hydroxy-9-oxo-11(10→8)-abeolanosta-24-*trans*-en-26-oic acid (**3**), 14β, 15β-epoxy-3α-hydroxy-9-oxo-11(10→8)-abeolanosta-24-*trans*-en-26-oic acid (**4**), 14β, 15β-epoxy-3α., 23α-dihydroxy -hydroxy-9-oxo-11(10→8)-abeolanosta-24-*trans*-en-26-oic acid (**5**). The structures of these compounds were elucidated by ^{1}H, ^{13}CNMR, COSY, HSQC, HMBC, NOESY, HRMS and X-ray crystallography. The 11(10→8)-abeolanostanes **2**, **3** and **5** were evaluated, together with the friedolanostanes and lanostanes, previously isolated from the same material (1), for their effect on the *in vitro* growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS). The compounds showing strong cytoxicity were evaluated for their capacity to induce apoptosis in the MCF-7 cell line by TUNEL assay. The results showed that two of the friedolanostanes were more effective in inducing apoptosis than the lanostanes and the abeolanostane.

Acknowledgements: FCT (Unidade de I&D nº 226/94), FEDER, POCTI (QCA III), NCI (USA).
Reference: 1. Vieira, L. et al. (2004) Phytochemistry 65, 393.

P 285 Cytotoxic Physalins from *Physalis angulata*

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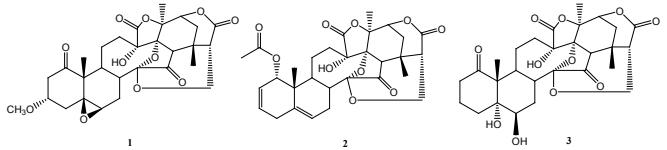
Bioassay-guided fractionation and purification of the chloroform layer of methanol extract of *P. angulata* (1) leads three new physalin-type steroids, physalin T (**1**), U (**2**), and V (**3**) and six known ones, physalin B (**4**), F (**5**), J (**6**), D (**7**), G (**8**), and I (**9**). Among them, **4**, **5**, and **7** exhibited strong cytotoxicity toward both HONE-1 and NUGC-3 tumor cell lines whereas **1** moderately cytotoxic toward both HONE-1 and NUGC-3 tumor cell lines (2). The co-occurence of nine physalins in *P. angulata* was of high interest with respect to the biogenetic origin of the typical steroids of this genus and implied a close biogenetic link between them. Their plausible biogenetic interrelationship is presented.



1 2 3

Acknowledgements: The authors are grateful to the National Science Council, Taiwan, R. O. C. (NSC 93-2113-M-006-001) for financial support for this research.

References: 1. Lindley, J. (1883) Flora Medica, Green and Longman. London. **2.** Rubinstein, L. V., Shoemaker, R. H., et al. (1990) J. Natl. Cancer Inst., 82, 1113.

P 286 Unusual steroidal compounds from *Selaginella chrysocaulos* and *S. bryopteris*

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The genus *Selaginella* (spikemoss, Selaginellaceae) consists of over 700 species with a world-wide distribution mainly in warm and moist climate. Very few species extend to higher northern or southern latitudes. On the Indian subcontinent, around 62 species of *Selaginella* are known (1). In general, reports on the medicinal usage of plants from the genus *Selaginella* are rare. So far, the genus is well-known for the occurence of biflavonoids, sugars (trehalose). Reports on alkaloids as well as on steroidal compounds are very rare. In our investigation, two new steroidal glycosides by name chrysocauloside A and B were isolated from *S. chrysocaulos* and identified by two-dimensional NMR techniques and MS spectrometry.

Chrysocauloside A and B are C_{28}-spirostene glycosides which were found in the ethyl acetate fraction of a whole plant extract of *S. chrysocaulos*. Their structures were established by extensive NMR spectroscopy. This is the first report of a C_{28}-spirostene skeleton bearing a methyl group at C-24.

In addition, four steroidal compounds showing carboxylation at C-21 were found and their structures elucidated in a similar way from the ethyl acetate fraction of a whold plant extract of *S. bryopteris*.

References: 1. Dixit, R. D. *Selaginellaceae of India*; Bishen Singh Mahendra Pal Singh, Dehra Dun (India), 1992.

Isolation of a new polihydroxylated sterol from the roots of *Myrtilocactus geometrizans* (Martius)

P 287

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Continuing with our systematical study of the Mexican medicinal flora, we decided to study *M. geometrizans*, an arborescent cactus from central areas of Mexico which is used as anti-inflamatory remedy (1). The aim of this work is to isolate the probable anti-inflammatory principles of *M. geometrizans*. From the anti-inflammatory roots methanolic extract the known penicerol and macdougallin (2, 3), as well as the new 3β, 6α, 9α, 22-tetrahydroxy-cholest-(8-14)-ene [**1**] were isolated. While, from the aerial parts, the known oleanane chichipegenin, previously isolated from this specie (4), and the new triterpenoid lactone [**2**] were isolated. The identification of **1** and **2** was achieved by spectral methods (EIMS, IR, ^{1}H, ^{13}C, and 2D NMR). The new sterol **1** was evaluated in the TPA-induced mouse ear edema model, showed inhibition of the edema of 50.79 ± 0.35 % at 0.31 μmol/ear.

References: 1. Luna-Morales, C., et al. (2001) Interciencia 26(1): 18-24., **2.** Djerassi c., et al. (1965) J. Chem. Soc. 1160-1165. **3**. Knight J.C**.** (1966) J. Am. Chem. Soc. 88(4): 790-798. **4**. Djerassi C., et al.. (1957) J. Am. Chem. Soc. 79: 3525-3528.

Six New Components from the Leaves of *Chamaecyparis obtusa* var. *formosana*

P 288

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C. obtusa var. *formosana* is a huge tree and can live for over two thousand years. With distinguished purple-pink coloring and strong resistance against termite and fungi caused it as an economic building material. The heartwood was investigated by us, and many novel compounds were elucidated.[1-3] In this time, we studied its leaves, and found one novel dimer of monolignol and totarol, 12-*p*-(*E*)-coumaroyltotarol, and five new sempervirenes, (19-acetoxysempervirol, 7β-hydroxysempervirol, 7α-hydroxysempervirol, 7-oxosempervirol and 9α-hydroxysempervira-8(14),11-dien-13-one). Two known lignans, yatein and formosalactone, were also isolated, they exhibited cytotoxic activity to KB cell with IC_{50} values 0.31 and 0.04 μM, respectively.

References: 1. Y. H. Kuo *et al*. (2001), *Tetrahedron Lett.*, *42*, 2985-2986. **2.** Y. H. Kuo *et al*. (2001), *Tetrahedron Lett.*, *42*, 6731-6735. **3.** Y. H. Kuo *et al*. (2002), *Helv. Chem. Acta*, *85*, 2657-2663.

P 289 New antiproliferative pregnane glycosides from *Leptadenia pyrotecnica*

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Leptadenia pyrotechnica (Forsk.) Decne. (Asclepiadaceae) is a plant, occurring on the sahelian region of West Africa, used in Malian folk medicine[2] to prepare antispasmodic, antinflammatory, and antibacterial remedies.[3] Previous study on the aerial parts of the plant led to the isolation of alkaloids, while pregnane glycosides, of which some showed antitumoral activity[1], have been reported from the genus *Leptadenia*.[3] The present report deals with the isolation and characterization from the aerial parts of *L. pyrotecnica* of twelve new polyhydroxy pregnane glycosides esters. The structures of these compounds are based on the skeleton of sarcostin, penupogenin, and plexigenin.[4] In addition, all compounds possesses an oligosaccharide chain at C-3 of the aglycon consisting of two to six sugar units. The structures were determined mainly through the use of 1D and 2D NMR, as well as by ESI-MS analyses. All isolated compounds were tested for their antiproliferative activity on J774.A1, HEK-293, and WEHI-164 cell lines. Moderate to high potency of cytotoxicities were found in almost all tested compounds, confirming the significant cytotoxic activity of pregnane glycosides.

References 1: Pan, W. et al. (2003) *J. Nat Prod*. 66:161-8. **2:** Baumer, M. (1995) *Arbres, arbustes et arbrisseaux nourriciers en Afrique occidentale*, Enda Tiers-Monde..**3**: Oliver-Bever, B. (1984) *Medicinal plants in tropical West Africa*, Cambridge University Press. **4:** Aquino, R. et al. (1996) *J. Nat Prod.* 59 : 555-64.

P 290 Tetra- and pentahydroxy – 5β - spirostanes and their glycosides from *Convallaria majalis* L.

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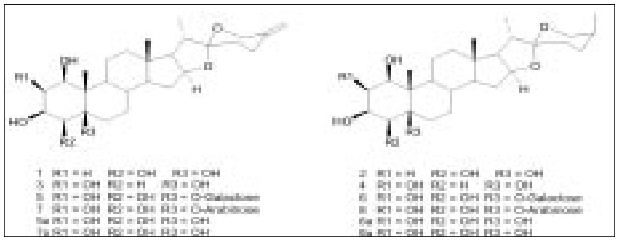
C. majalis L. from the family *Liliaceae* is widely distributed in Europe whereas *C. keiskei* Miq. grows in East Asia. Convallasaponins with several OH groups were isolated from the flowers of *C. keiskei* [1] and from the roots and rhizomes of *C. majalis* [2, 3]. Polyhydroxy steroidal saponins have been found in the family *Liliaceae* [4]. Our further investigation of the polar (n-BuOH / $CHCl_3$) fraction of the extract from the roots and rhizomes of *C. majalis* has led to the isolation of new convallasapogenins and convallasaponins.



The structures of the new compounds were deduced from ^1H and ^{13}C NMR spectral data and by the interpretation of two-dimensional COSY, HMQC, HMBC and ROESY correlations. The new steroidal sapogenins are tetra- or pentaols with β - orientation of all hydroxy groups, as illustrated above. The 5-O – substitution of sugar moieties seems to be rather unique.

References: **1.** Kimura M., Tohma M., Yoshizawa I. (1996) Chem. Pharm. Bull. 14:50-55. **2.**Tschesche R., Tjoa B., Wulff G., Noronha R.V. (1968) Tetrahedron Lett.49:5141-5144 **3.** Nartowska J., Strzelecka H. (1983) Acta Pol. Pharm.15 649-644. **4.** Pan W.B., Chang F.R., Wu Y.C. (2000) Chem.& Pharm. Bull.:1350-1353

Cytotoxic Furostanol Saponins and a Megastigmane Glycoside from *Tribulus parvispinus*

P 291

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Among the almost 25 species of *Tribulus*, only *T. terrestris*, *T. cistoides*, and *T. pentandrus* have been chemically studied. *T. terrestris* is a well known pharmaceutical herb which has recently received more attention due to its aphrodisiac properties [1]. As a part of our ongoing research for new bioactive compounds from medicinal plants of the Egyptian desert we have studied the aerial parts of *T. parvispinus* Presl (Zygophyllaceae). Three new furostanol saponins (25*R*)-26-*O*-β-D-glucopyranosyl-5α-furostan-2α,3β,22α,26-tetraol 3-*O*-{β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**1**), (25*R*)-26-*O*-β-D-glucopyranosyl-22α-methoxy-5α-furostan-2α,3β,26-triol 3-*O*-{β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**2**), (25*R*)-26-*O*-β-D-glucopyranosyl-22α-methoxy-5α-furostan-3β,26-diol 3-*O*-{β-D-galactopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**3**), and one new megastigmane glycoside (6*S*,7*E*,9ξ)-6,9,10-trihydroxy-4,7-megastigmadien-3-one 10-*O*-β-D-glucopyranoside (**4**) were isolated. Their structures were established by extensive spectroscopic methods including 1D and 2D NMR experiments. The antiproliferative activity of **1**–**3** was evaluated against HepG2 and U937 cell lines. All the tested compounds displayed cytotoxic effects (1 –50 μM). Results also suggested that the cytoxicity appeared to be tumor-specific. DNA fragmentation followed by cell exposure to the tested compounds was also observed indicating that **1**-**3** exerted their cytotoxic effect through necrosis and apoptosis. Finally, the antioxidant and pro-oxidative activities of **1**–**3** were evaluated. Production of oxygen reactive species in Fe/ascorbate-stimulated cells was strongly inhibited by **1**–**3**. Interestingly, their antioxidant activity resulted to be inversely related to their cytotoxic activity.

References: 1. Hamed, A.I., Piacente, S. (2004) Phytochemistry 65: 2935-2945.

A New 24-nor-Ursane Triterpenoid from the Stems of *Rumex japonicus*

P 292

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A new 24-nor-ursane triterpenoid was isolated from the stems of *Rumex japonicus* (Polygonaceae). The structure of the new triterpenoid was elucidated as 2α,3α,9α-trihydroxy-24-nor-4(23),12-ursadien-28-oic acid by spectroscopic methods, particularly by extensive 1D and 2D NMR studies.

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P 293 Isolation, Characterization and Biological Activities of Friedelane Triterpenes from M*aytenus chiapensis*

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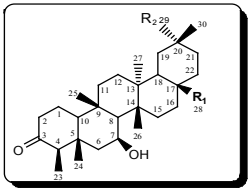
The present communication describes the isolation and structural elucidation of ten friedelane triterpenes (1) from *Maytenus chiapensis.* Three of the compounds were news and their structures were determined by spectroscopic methods, including ^{1}H-^{13}C heteronuclear correlation (HSQC), long-range correlation with inverse detection (HMBC), and ROESY NMR experiments. The compounds were tested for antimicrobial activity against Gram-positive and Gram-negative bacteria and the yeast *Candida albicans,* while the cytotoxic activity was assayed against HeLa, Hep-2, and Vero cell lines. Also their xanthine oxidase and ß-glucuronidase inhibition activities were assayed. Compound **1** exhibit moderated antimicrobial and cytotoxic activities, while compound **3** showed significant xanthine oxidase inhibitory activity.

1 $R_1 = COOH$ $R_2 = CH_3$
2 $R_1 = COOMe$ $R_2 = CH_3$
3 $R_1 = CH_3$ $R_2 = CH_2OH$



Acknowledgements: Gobierno Autonono de Canarias-IUBO for a fellowship, and DGES (BQU2003-09558-C02-01) project for financial support.

References: **1**. Xu, R.; Fazio, G. C.; Matsuda, S. P. (2004) Phytochemistry, 65: 269.

P 294 New terpenoids from *Maytenus magellanica* and *Maytenus chubutensis* and their MDR reversal activity

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We have undertaken a systematic survey of the Chilean genus *Maytenus* (Celastraceae) (1). The phytochemical analysis of the root bark extracts of *M. chubutensis* and *M. magellanica* led to the isolation of three new secondary metabolites, one phenolic *nor*-triterpene and two diterpenes with a *nor*-kaurene and an isopimaradien skeleton, respectively. Their structures were determinated by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear and heteronuclear correlation experiments (COSY, ROESY, HMQC and HMBC), and chemical correlations.

These compounds have been tested on a multidrug-resistant *Leishmania tropica* line overexpressing a P-glycopratein-like transporter to determine their ability to revert the resistance phenotype and to modulate intracellular drug accumulation (2).

Acknowledgements: DGES (BQU2003-09558-C02-01) project for financial support .

References: **1.** Kennedy M. L., Cortés –Selva F., Pérez-Victoria J. M., Jiménez I. A., González A. G., Muñoz O. M., Gamarro F, Castanys S. and Ravelo A.G. (2001) J. Med. Chem. 44/26: 4668-4676. **2.** Cortés –Selva F., Campillo M., Reyes C. P., Jiménez I. A. , Castanys S., Bazzocchi I. L., Pardo L., Gamarro F. and Ravelo A. G. (2004) J. Med. Chem. 47: 576-587.

New Lupane Triterpenoids from *Maytenus* species

P 295

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Species of the genus *Maytenus* (*Celastraceae*) are being extensively investigated for bioactive compounds since they are widely used in tradicional medicine and agriculture in North Africa, South and Central America, and Central and East Asia (1). Triterpenoids are widely occurring and structurally diverse compounds which have always attracted attention, and whose pharmacological activities have often been evaluated. Triterpenoids from the Celastraceae belong to the lupane, oleane, fridelane, taraxerane, glutinane, ursane, dammarane, and baccharane series.
In our continuing work on *M. cuzcoina* and *M. chiapensis,* we report herein on the isolation and structural elucidation of five new lupane triterpenoids, whose structures were determined by application of 1D and 2D NMR techniques, including COSY, HSQC, HMBC, and ROESY experiments. In addition, twenty-four known lupane triterpenes were isolated and their structures were identified by comparison of their spectral data with values reported in the literature. The compounds were assayed for antimicrobial and cytotoxic activities. Correlation between the structures and the cytotoxic activity is discussed in detail.

Acknowledgements: DGES (BQU2003-09558-C02-01) project for financial support.

References: **1.** González A. G.; Bazzocchi I. L., Moujir L. M., Jiménez I. A. (2000) In Studies in Natural Products Chemistry, Bioactive Natural Products (Part D), Atta-ur Rahman, Ed.; Elsevier Science Publisher: Amsterdam.

Phytochemical study of the roots of *Eryngium campestre*

P 296

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From a crude saponin mixture obtained from a methanolic extract of the roots of *Eryngium campestre* (Apiaceae), two new triterpene saponins were isolated by successive MPLC on silica gel and RP-18. Their structures were elucidated mainly by 2D NMR techniques (600 MHz, COSY, TOCSY, NOESY, HSQC, HMBC) and mass spectrometry (HR-ESI-MS and FAB-MS).
The first molecule presented R1-barrigenol as aglycon, esterified by an angeloyl residue at the position 22. Its complete structure was established as 3-*O*-*ß*-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*ß*-D-glucuronopyranosyl-22-*O*-angeloyl-R1-barrigenol (**1**). The second saponin was elucidated as 3-*O*-*ß*-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-*ß*-D-glucuronopyranosyl-22-*O*-*ß,ß*-dimethylacryloyl-A1-barrigenol (**2**). The differences between these two molecules were located at the aglycon moiety, which is A-1 barrigenol, esterified by a *ß,ß*-dimethylacryloyl residue at the position 22 in compound **2**.

P 297 Novel seco-Cycloartane Triterpenes from *Gardenia aubryi*

R. Grougnet [a], *P. Magiatis* [a], *S. Mitaku* [a], *A.-L. Skaltsounis* [a], *S. Michel* [b], *F. Tillequin* [b] *and P. Cabalion* [c]

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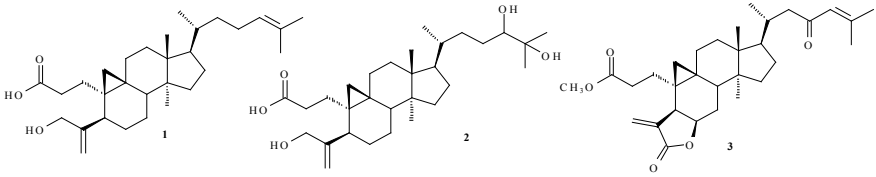
Six endemic species of the genus *Gardenia* (Rubiaceae) occur in New Caledonia. The leave and flower buds of two of them, *Gardenia aubryi* Vieill. and *Gardenia urvillei* Montrouz., are covered with a gum which is locally used for chewing. Three novel 3,4-seco-cycloartanes, secaubryenol (1), secaubrytriol (2), and secaubryolide (3) were isolated from the gum collected on the aerial parts of *Gardenia aubryi* Vieill. Their structures were established by mass spectrometry and NMR experiments. Terpenoids (24R)-cycloartane-24,25-diol-3-one, coccinetane A and the known 3-methoxyflavones herbacetin 3,8-dimethylether, hibiscetin 3,8,3',4'-tetramethylethyer, and conyzatin were also obtained from the plant material.



P 298 Isolation of a new triterpenoid saponin from the root of *Anchusa strigosa l.* , Family Boraginaceae

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It was isolated a new triterpenoidsaponin from the root of plant *Anchusa strigosa* L. family *Boraginaceae*.
The plant *Anchusa* is widely spread in different places in Syria, and is used in treatment of some diseases like oedema, and fever and pulmonary diseases.
The isolation and purification procedures was perfumed using thin layer chromatography TLC and column chromatography CC and high performance liquid chromatography HPLC and by using the methods of nuclear magnetic resonance chromatography NMR it was determined the chemical structure of the aglycon as triterpenoid linked by glycoside pond with three sugar parts.

Acknowledgemenst **;** We thank Prof Dr. Anwar Alkhatieb from the department of botany, Damascus University, who identified the Plant, and We would thank DAAD, that they make our work easier and covered the finance of our visit in Germany.

New 26-Hydroxylated Ecdysteroids from *Silene viridiflora*

P 299

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Ecdysteroids represent a large family of polyhydroxylated steroids. They are widely distributed both in invertebrates and plants. There is a growing interest in ecdysteroids due to their important human pharmacological effects (1). Large amounts of ecdysteroids are presently being produced for human use (2). *Silene viridiflora* has been considered as a good source of ecdysteroids, including new hitherto never identified compounds. We now report the isolation and structural identification of five minor ecdysteroids from this plant. The isolation procedure started with extraction. The crude extract was purified using simple separation techniques, such as fractionated precipitation and solvent-solvent partition. Further purification include flash chromatography on C_{18} and cyano, and HPLC. Structural identification was done by using mass spectrometry and NMR spectroscopy. Our sophisticated isolation procedure led to the discovery of some new 26-hydroxylated ecdysteroids, such as 20,26-dihydroxyecdysone 20,22-monoacetonide, 2-deoxy-26-hydroxy-polypodine B, 26-hydroxy-polypodine B 20,22-monoacetonide in addition to 2-deoxy-20,26-dihydroxyecdysone and 20,26-dihydroxyecdysone.

Acknowledgements: This work was sponsored by Hungarian National Research Fund (OTKA T035054).
References: 1. Báthori, M. et al. (2005) CMC 12: 153-172. **2.** Lafont, R. et al. (2003) J. Insect Sci. 3: 7.

Molecular research on endemic plants of Sardinia

P 300

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Chemistry of natural organic products regards studies of structural character devoted to the identification of new molecular architectures and to search new compounds present in natural matrices characterized by biological useful activity. More recently particular attention was directed to the phytochemical studies of plants growing in restricted areas, characterized by narrow limits. A wide variety of climates, environmental, geological, geographical and biological conditions has led to the diversification of species. Here we report the results on the molecular composition of endemic plants of Sardinia. The plant extracts were separated on the bases of their polarity: low, medium and high polar fraction. In the *Teucrium marum* L. a new neoclerodane was isolated. Loganic acid and loganin were for the first time isolated respectively from *Galium corsicum* Sprengel and from *Galium schmidii* Arrigoni. *Stachys corsica* Pers. and *Stachys glutinosa* L. showed, together with the presence of known iridoids, the occurrence of a new iridoid containig allose as glycosidic component of the molecule. *Gentiana lutea* L., *Verbascum conocarpum* Moris and *Scrophularia trifoliata* L., present in the Gennargentu area, were examined also and the content in bitter compounds was also determined. In the end, *Ephedra nebrodensis* Tineo., an endemic plant present in a restricted area of the Supramonte mountain, was investigated and the presence of pseudo-ephedrine as main alkaloid was demonstrated. The anti oxidizing, anti micotic and anti microbic activity of crude extracts and pure compounds was also determined, confirming some traditional use of the described plants. In particular significative anti oxidizing activity was showed to be present in the high polar extracts of *T. marum*, and in the medium polar extracts of *E. nebrodensis*.

P 301 A novel benzoylated iridoid glycoside derivative from *Gentiana lutea*

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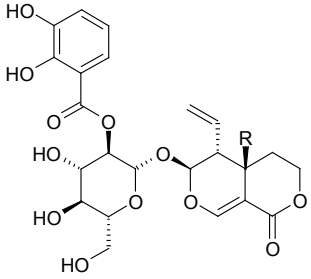

1 R = OH
2 R = H

Roots of *Gentiana lutea* (Gentianaceae) have a long history of use as a herbal bitter in the treatment of digestive disorders and are an ingredient of many proprietary medicines. It contains some of the most bitter compounds known e.g secoiridoid glycosides and is used as a scientific basis for measuring bitterness (1). In the course of our ongoing investigations, two iridoide glycoside derivatives with unusual spectroscopical features were isolated from a EtOH/H_2O extract by repeated preparative RP-HPLC. HPLC-DAD/MS and NMR based structure elucidation allowed to identify these metabolites as 2'-(2,3-dihydroxybenzoyl)-swertiamarin (**1**) and 2'-(2,3-Dihydroxybenzoyl)-swerosid (**2**). Both constituents have not been described from *G. lutea* yet. Whereas sweroside derivative **2** has been found in two related taxa – namely - *G. algida* (2) and *G. scabra* (3), the swertiamarin derivative **1** has not been described yet and therefore can be considered as a novel secondary metabolite.

References: 1. Hänsel R. et al. (1994) Hagers Handbuch der Pharmazeutischen Praxis – Vol. 5, Springer, Berlin. **2.** Tan R.X. et al. (1996) *Phytochem.* 41: 111-116. **3.** Tan H.Q., Tan R.X. (1997) Planta Med. 63: 388.

P 302 Iridoid glucosides from *Galium tortumense*

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The genus *Galium* L. (Rubiaceae) is represented in Turkey by 101 species gathered in 10 sections (1). *Galium tortumense* is one of the endemic *Galium* species. Some of the *Galium* species are used as diuretic, choleretic, tonic, sedative and antidiyareic in folk medicine (2). Iridoids are the most common substances in the genus *Galium*. They might play an important role for the chemotaxonomic classification. In this study, we report the isolation and structure elucidation of eight iridoid glucosides from the methanolic extract of the aerial parts of the plant. Their structures were identified as scandoside methyl ester, daphylloside, geniposide, geniposidic acid, loganin, 7-ketologanin, loganic acid and deacetyl-asperulosidic acid. The structures of the compounds were elucidated by high field one (1D) and two dimensional (2D) NMR techniques and EI-MS spectroscopies.

References: **1**. Ehrendorfer, F., Schönbeck-Temesy, E. (1982) Galium L., "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 767-849, University Press, Edinburgh (edited by P.H. Davis). **2.** Baytop, T. (1999) "Therapy with Medicinal Plants in Turkey (past and Present)", 2nd ed. p. 419, Nobel Tıp Kitabevleri, İstanbul

Chemical constituents of *Galium humifusum*

P 303

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Galium L. genus (Rubiaceae) is represented by 101 species in Turkish flora (1). *Galium* species are traditionally used to coagulate milk because of an enzyme in their chemical composition. For this reason this plant is known as "yoğurt herb" (2). Some of the *Galium* species are used as diuretic, choleretic, tonic, sedative and antidiyareic in folk medicine (3). In this study, we report the isolation and structure elucidation of 3 iridoid glycosides (1 new, 2 known), humifusumoside, scandoside methyl ester and daphylloside, a naphthohydroquinon, 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone 1,4-di-O-β-glucoside, three anthraquinone glycosides, rubiadin 3-O-β-primeveroside, rubiadin-1-methylether 3-O-β-primeveroside and anthragallol-2-methoxymethyl 3-O-β-primeveroside, two oleanane-type triterpene, 3-acetoxy olean 12-en 28 oic acid and 3- acetoxy olean 12-en 28 al from the roots of *Galium humifusum* Bieb. The structures of the compounds were elucidated by high field 1D and 2D NMR and EI-MS spectroscopies.

References: **1**. Ehrendorfer, F., Schönbeck-Temesy, E. (1982) Galium L., "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 767-849, University Press, Edinburgh (edited by P.H. Davis). **2.** Ergun, F. et al. (1999) Antimicrobial activities of Galium species, GUEDE J Fac Pharm Gazi, 16:7-11. **3.**Baytop, T. (1999) "Therapy with Medicinal Plants in Turkey (past and Present)", 2nd ed. P. 419, Nobel Tıp Kitabevleri, İstanbul

Iridoid and flavonoid glycosides from *Wiedemannia orientalis*

P 304

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Wiedemannia orientalis (Lamiaceae) is an endemic species in Turkey and widely grows in Anatolia (1). In this study, we decided to investigate *W. orientalis* collected from Sivrihisar (Eskişehir). Powdered dried aerial parts of this plant were extracted with methanol. After evaporation of the solvent the crude residue was suspended in water and successively extracted with petroleum ether, chloroform and n-butanol, respectively. The organic layers were evaporated to dryness. As a result of the chromatographical studies, six iridoid and four flavonoid glycosides were isolated from the n-butanol extract. The structures of the compounds were elucidated by high field one (1D) and two dimensional (2D) NMR techniques and EI-MS spectroscopies. Their structures were identified as lamiide, ipolamiide, 6β-hidroksiipolamiide, ipolamiidoside, deacetyl-asperulosidic acid, 5-hidroksiloganin, isorhamnetin 3-O-rutinoside, apigenin 7-O-β-glucoside, luteolin 7-O-β-glucoside and apigenin 7-O-(6"-O-trans-p-coumaroyl) β-glucoside. These compounds were first isolated from *Wiedemannia orientalis.*

References: **1**. Mıll, R.R. (1982) Wiedemannia Fisch. & Mey., "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 148-149, University Press, Edinburgh (edited by P.H. Davis)

P 305 New iridoid glucosides from *Scrophularia desertii*

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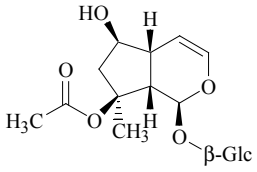

[**1**]

The flora of Kuwait consists of approximately 400 naturalised plants, many of which are xerophytic and halophytic (**1**, **2**). During the months of July and August, temperatures regularly exceed 50° centigrade and the major taxa present at this time are plants of the Chenopodiaceae and Zygophyllaceae. During the months of March and April, conditions are favourable for flowering of many plants belonging to the Asteraceae and Scrophulariaceae families. During the early spring, a collection of *Scrophularia desertii* was made from the Wadi-Al Batin, a dry river bed that runs along the border with Iraq. This area is a repository for many interesting species in Kuwait, presumably as during the winter and early spring rains, there is a greater presence of water in this region. A phytochemical study of the chloroform and methanol extracts of *S. desertii* led to the isolation of seven iridoid glycosides, typified by compound **1.** The structure of this new compound was assigned on the basis of NMR spectroscopy and mass spectrometry as 6-*epi*-ajugoside and is described here for the first time.

Acknowledgement: The University of London School of Pharmacy is thanked for a PhD scholarship to M. Stavri

References: **1.** Daoud, H.S. (1985) Flora of Kuwait. KPI Ltd. London. **2.** Shuaib, L (1995) Wildflowers of Kuwait. Stacey International. London.

P 306 Isolation and characterization of a new biological active cyathane diterpene from *Sarcodon cyrneus*

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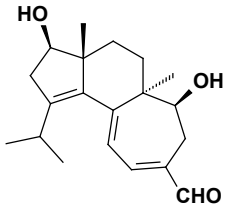
As part of our ongoing research into bioactive compounds from *Sarcodon* species, the mushroom *Sarcodon cyrneus* Maas Geest was investigated. From related mushroom species were previously isolated some diterpenes possessing a cyathane skeleton, that showed biological activities, such as antibacterial activity (1) and a strong inductive activity of nerve growth factor (NGF) synthesis. (2) Two new cyathane diterpenes, Glaucopine A and B, were isolated from *S. glaucopus*. These demonstrated anti-inflammatory activity similar to that induced by the reference compound indomethacin (NSAID). (3) These results enabled us to carry out an activity-guided isolation and characterization of a new cyathane exctracted from *S. cyrneus*, Cyrneine A. Cyrneine A was isolated by column chromatography purification of the hexane extract. The structure reported here was elucidated by spectroscopic analysis. The inhibition of the Croton oil-induced ear oedema in mice was used as the experimental model of inflammation. (4) Cyrneine A induced (1 μmol/cm^2) 46% reduction in oedema, compared with 61% reduction caused by indomethacin (0.3 μmol/cm^2). We also evaluated the activity of Cyrneine A on PC12 cells, as the model system of neuronal differentiation. The molecule stimulated neurite outgrowth in PC12 cells when tested at a dose of 100 μM, using dbcAMP as a positive control.



References: 1. Anke, T.et al. 1977, J. Antibiot. 30: 221-225. **2**. Kenmoku, H. et al. 2002, Biosci. Biotechnol. Biochem. 66(3): 571-575. **3**. Marcotullio, M.C. et al. 2005, Planta Med. 71: 194-196. **4**. Della Loggia, R. et al. 1985, Agents Actions 17: 347-349.

C-19 terpenolides from *Thapsia garganica*. Structure elucidation, biogenetic proposal and serca-inhibiting activity

P 307

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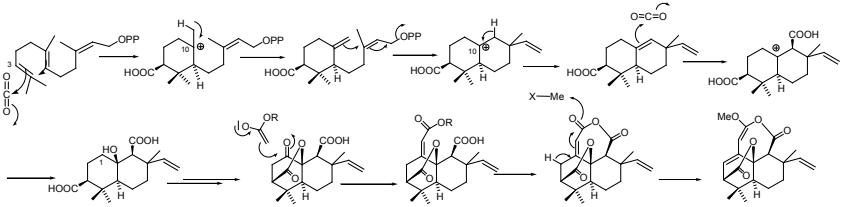
The biomedical relevance of the Mediterranean umbelliferous plant *Thapsia garganica* L. can hardly be overestimated, since the guaianolide thapsigargin (TG), its major constituent, has become an indispensable tool in cell physiology and in the study of calcium homeostasis (1, 2). Along with TG, an investigation of *T. garganica* from Sardinia afforded a series of unusual tetracyclic C-19 dilactones. A possible biogenetic derivation for these compounds is proposed, suggesting a tetrahomo-sesquiterpenoid nature, and formation via a carbon dioxide-triggered electrophilic polyolefin cyclization. Despite the structural diversity with thapsigargin, these compounds showed SERCA inhibiting properties.



References: 1. Thastrup, O.et al. 1990 Proc. Natl. Acad. Sci. (USA) 87: 2466-2469. (2) (Jakobsen, C. M. et al 2001 J. Med. Chem. 44: 4696-4703.

Chemical constituents from *Croton insularis*

P 308

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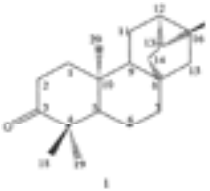
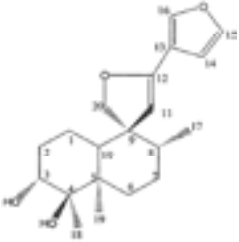
[b]Lab. de Pharmacognosie de l'Université René Descartes, Fac.de Pharmacie, Paris, France
[c]ICSN du CNRS, F-91190 Gif sur Yvette, France





The genus *Croton* L. (Euphorbiaceae), is a rich source of bioactive diterpenes, possessing wellknown anti-ulcer, anti-tumor, or co-carcinogenic properties (1). From the aerial parts of the plant, it has been isolated the novel trachylobane diterpene: ent-trachyloban-3-one [1], four new clerodane terpenoids: crotinsularin [2], furocrotinsulolide A, furocrotinsulolide B, crotinsulactone and the new phenolic disaccharide: 1-(α-L-rhamnosyl(1-6)-β-D-glucopyranosyloxy)-3,4-dimethoxy benzene (2,3). Besides, 13 known compounds, were also isolated and identified as: 3-β-hydroxy-trachyloban-19-oic acid, ent-trachyloban-19-oic acid, 3α,19-dihydroxy--trachylobane, ent-trachyloban-3β-ol, 19-acetoxy-ent-trachylobane, ent-13-epi-manoyl oxide, isokaempferide, 3α,4β-dihydroxy-15,16-epoxy-12-oxoclerodan-13(16),14-diene, 3α,4β-dihydroxy-15,16-epoxy-12-oxo-clerodan-13(16),14-dien-9-al, 1-(α-L-rhamnosyl(1-6)-β-D-glucopyranosyloxy)-3,4,5-trimethoxybenzene, 1-(β-D-apiofuranosyl (1-6)-β-D-gluco pyranosyloxy)-3,4,5-trimethoxybenzene, as well as ferulic and vanillic acids. All structures have been established by modern spectral data (NMR. MS). The absolute configuration of the isolated trachylobane diterpenes was deduced from chemical correlations.

References: 1. Kitazawa, E. et al. (1980) Chem. Pharm. Bull. 28: 227-234. **2.** Graikou, K. et al. (2004) J. Nat. Prod. 67:685-688. 3. Graikou, K. et al. (2005 submitted).

P 309 New Cytotoxic Polyester Sesquiterpene from the Root of *Microtropis fokienensis*

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Microtropis fokienensis Dunn (Celastraceae) is a small shrub growing in high altitude forests throughout southern China and Taiwan (1). Polyester sesquiterpenes and their derivatives are widely distributed in plants of the family Celastraceae. Many of the isolates exhibit diverse biological activities, including insecticidal, antifeedant, anti-inflammatory, and antitumor properties. However, the chemical constituents and biological activities of this plant have never been studied. Approximately 1000 species of Formosan plants have been screened for cytotoxic test and *M. fokienensis* was shown to be one of the active species. Investigation on EtOAc-soluble fraction of the root of *Microtropis fokienensis* has led to the isolation of two new polyester sesquiterpenes with dihydro-β-agarofuran skeletons, fokienagarofuran A (**1**) and fokienagarofuran B (**2**), together with eight known compounds. The structures of new compounds were determined through spectral analyses including extensive 2D NMR data. Among the isolates, fokienagarofuran A (**1**) showed cytotoxic activitiy with ED_{50} value = 2.7 μg/mL against P-388 cell line *in vitro*. The structural elucidation of **1** and **2** and the cytotoxic activities of the isolates will be discussed in this congress.

Acknowledgements: This work was supported by a grant (NSC 93-2323-B-127-003) from the National Science Council of the Republic of China.

References: 1. Lu, S.U., Yang, Y.P. (1993) Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan. Vol. 3: 640-656.

P 310 Secondary metabolites from the aerial parts of *Anthemis auriculata* (Asteraceae) and their antimicrobial activity

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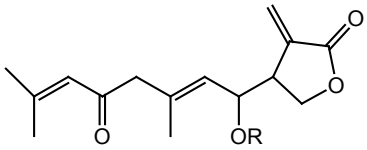
[b]Department of Plant Physiology, Mycological Laboratory, Institute for Biological Research, 29 Novembar 142, 11000 Belgrade, Serbia-Montenegro



1 R = H
2 R = $COCH_3$

The genus *Anthemis* comprises about 130 species predominately distributed around the Mediterranean, but species are also found in southwest Asia and South Africa (1).Continuing our research on the chemical constituents from the aerial parts of Greek *Anthemis* sp., we report here the isolation and identification of sesquiterpene lactones 1-3, flavonoids 4-8, a sterol 9 and a phenolic ester 10, from *A. auriculata* Boiss.The isolation was proceeded according to the Bohlmann isolation method (2). The structures of the isolated compounds were established by means of 1D and 2D NMR [^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HSQC, HMBC, NOESY] spectral analyses. Compounds 1 and 2, are new naturally occurring linear sesquiterpene lactones. The compounds 3-10 are known as anthecotuloide (3), apigenin (4), luteolin (5), luteolin-7-glucoside (6), eriodictyol (7), pectolinaringenin (8), taraxasterol (9) and vanillic acid methylester (10). The isolated compounds were tested for their antimicrobial activity against several gram (+) and gram (-) bacteria, as well as *Candida albicans* and showed moderate activity.

References: 1. Heywood V. H. et al. (1978), Biology and Chemistry of the Compositae. Heywood V. M., Harborne J. B., Turner B. L. (Eds). Academic Press. London. **2.** Bohlmann F. et al. (1984), Phytochemistry 23: 1979-88.

Antimycobacterial and 12(S)-HETE inhibitory activities of sesquiterpenes isolated from *Warburgia ugandensis* stem barks

P 311

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The East African pepper-bark tree, *Warburgia ugandensis* Sprague (Canellaceae), has been used as a folklore remedy for a wide spectrum of ailments, such as cough, fever, muscle pains, weak joints and general body pains (1). The Shinasha people in Ethiopia use the stem bark for the treatment of tuberculosis, asthma and malaria. Previous phytochemical reports revealed drimane sesquiterpenes as the characteristic metabolites of this species (2, 3), which have been shown to possess insect antifeedant antimicrobial, antiulcer, molluscicidal and antifungal properties (4).In this study, two new and nine known sesquiterpenes were isolated from the stem bark of *W. ugandensis* and evaluated for their antimycobacterial property against rapidly growing strains of mycobacteria, such as *Mycobacterium aurum*, *M. fortuitum*, *M. phlei* and *M. smegmatis*; as well as inhibition of 12(*S*)-HETE formation using human platelets. Among the compounds tested muzigadial displayed significant antimycobacterial activity with MICs ranging from 16-64 µg/ml, whereas muzigadiolide showed moderate activity compared to the antibiotic drugs ethambutol and isoniazid. At 20 µg/ml, some of the sesquiterpenes tested for inhibition of 12(*S*)-HETE formation showed pronounced activity with percentage inhibition ranged from 30 – 80 % compared to the positive control, baicalein which showed 41 % inhibition at 5 µg/ml.

Acknowledgement: A. A. Wube would like to acknowledge the Austrian Academic Service (ÖAD) scholarship.
References: 1. Kokwaro, J.O. (1976) Medicinal plants of East Africa, East African Literature Bureau, Nairobi. **2.** Kioy, D., Gray, A. I., et al. (1990) Phytochemistry 29: 3535-38. **3.** Rajab, M. S., Ndegwa, J. M. (2000) Bull Chem Soc Ethiop 14: 45-9. **4.** Kubo, I., Taniguchi, M. (1988) J Nat Prod 51: 22-9.

Phytochemical Studies on *Origanum acutidens* (Hand.-Mazz.) Iestwaart

P 312

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Origanum acutidens (Lamiaceae) is an endemic species and grows mainly in calcareous and non-calcareous rocks, slopes and screes (1000-3000 m) in Central and East Anatolia (1). Many *Origanum* species known as "Kekik, kekik otu, keklik otu" are used by the public especially as a spice and for its several medicinal effects in Turkey (2). In this study, phytochemical studies was performed on aerial parts of plant. The isolation of the compounds was carried out using several and repeated chromatographic techniques from chloroform, ethyl acetate and water phases that partitioned from methanolic extract obtained from plant. Ursolic acid and oleanolic acid were isolated from chloroform phase, rosmarinic acid from ethyl acetate phase, protocatechuic acid, 2 monoterpenoid glucosides -betulalbuside A and 2(*E*)-2,6-dimethyl-2,7-octadien-1,6-diol-6-*O*-β-D-glucopyranoside- from water phase. The structures of the compounds were elucidated by means of spectral analysis (^{1}H-NMR, ^{13}C-NMR and EI-MS) .

References: 1. Ietswaart, J.H. (1982) Origanum L., "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 297-313, University Press, Edinburgh (edited by P.H. Davis). **2.** Baytop, T. (1999) "Therapy with Medicinal Plants in Turkey (Past and Present)", 2nd ed. p. 283, Nobel Tıp Kitabevleri, İstanbul

P 313 A New Neuroprotective Compound of *Ligustrum japonicum* leaves

K. Y. Lee, E. S. Kim, M. K. Lee, C. J. Ma, S. H. Sung, Y. C. Kim
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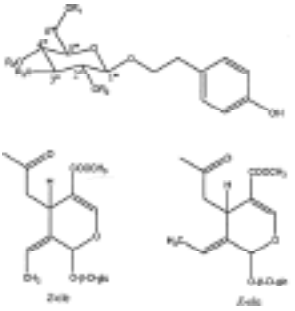
A new secoiridoid glycoside characterized as (8*Z*)-nuezhenide A (**1**) with five known glycosides, (8*E*) - nuezhenide (**2**), (8*Z*) - nuezhenide (**3**), oleonuezhenide (**4**), osmanthuside B (**5**), osmanthuside D (**6**) were isolated from the *n*-BuOH fraction of *Ligustrum japonicum* leaves. All six compounds significantly protected human neuroblastoma SH-SY5Y cells from 6-hydroxydopamine-induced neurotoxicity.



1	R_1 = H	R_2 = *Z*-ole	R_3 = H	R_4 = H
2	R_1 = *E*-ole	R_2 = H	R_3 = H	R_4 = H
3	R_1 = *Z*-ole	R_2 = H	R_3 = H	R_4 = H
4	R_1 = *E*-ole	R_2 = *E*-ole	R_3 = H	R_4 = H
5	R_1 = H	R_2 = H	R_3 = α-L-rham	R_4 = *trans*-*p*-coumaroyl
6	R_1 = H	R_2 = H	R_3 = α-L-rham	R_4 = *cis*-*p*-coumaroyl

Acknowledgements: This research was supported by a grant (M103KV010019-04K2201-01940) from Brain Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology, Korea.
References: 1. Kudo, K. et al. (1980) Planta Med 40: 250-61. **2.** Inoue, K. et al. (1982) Phytochemistry 21: 2305-11. **3.** Machida, K. et al. (2002) Chem Pharm Bull 50: 493-7.

P 314 Chemosensitization of a Multidrug-Resistant *Leishmania tropica* Line by New Sesquiterpenes from *Maytenus magellanica*

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Parasite resistance to drugs has emerged as a major problem in current medicine, and therefore there is great clinical interest in developing compounds that overcome these resitances. In an intensive study of South American medicinal plants, herein we report the isolation, structure elucidation and biological activity of 13 dihydro-ß-agarofuran sesquiterpenes from the roots of *Maytenus magellanica* (1)**.** The structures of the new compounds were determined by means of ^{1}H and ^{13}C NMR spectroscopic studies, including homonuclear and heteronuclear correlation experiments, and their absolute configurations were determined by means of CD studies. The compounds have been tested on a multidrug-resistant *Leishmania tropica* line to modulate intracellular drug accumulation. From this series, three of the compounds, showed potent activity. The structure-activity relationships is discussed.

Acknowledgements: DGES (BQU2003-09558-C02-01) project for financial support.
References: 1. Kennedy M. L., Cortés –Selva F., Pérez-Victoria J. M., Jiménez I. A., González A. G., Muñoz O. M., Gamarro F., Castanys S. and Ravelo A.G. (2001) J. Med. Chem. 44/26: 4668-4676.

New guaianolides from *Pulicaria crispa*

P 315

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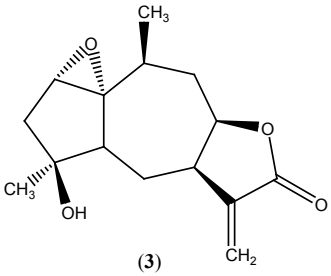

(**3**)

The asteraceous herb *Pulicaria crispa* Sch. Pip. was studied phytochemically and biologically as part of a project to assess the flora of Kuwait. This species, as with the genus in general, has been shown to be a rich source of sesquiterpenes, particularly of the guaianolide, xantholide and eudesmanolide class (**1**,**2**). The phytochemical investigation of this herb led to the characterisation of three new guaianolide sesquiterpenes. These were elucidated as 2α,4α-dihydroxy-10β-methyl-guaia-1(5),11(13)-dien-8β,12-olide [**1**], 4-*epi*-1α,2α-epoxy-1,10α-dihydropseudoivalin [**2**] and 5,10-*epi*-2,3-dihydroaromatin [**3**] and are reported here for the first time. **3** was the only guaianolide to exhibit antimycobacterial activity against *Mycobacterium phlei* with a minimum inhibitory concentration of 128 μg/ml.

Acknowledgement: The University of London School of Pharmacy is thanked for a PhD scholarship to M. Stavri
References: 1. Al-Yahya M.A. et al. (1984) J. Nat. Prod. 47: 1013-1017. **2.** Dendougui H. et al. (2000) Fitoterapia 71: 373-378.

A New *Ent*-trachylobane Diterpenoid from the Roots of *Sapium sebiferum*

P 316

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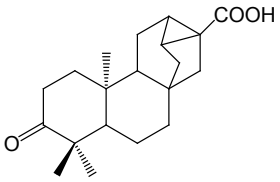
Bioactive study for antitumor agents from Chinese herbs, the acetone extracts of *S. sebiferum* (1) showed a significantly cytotoxic activity on A 549 and AT12 lung cancer cell lines. Bioassay-guided fractionation and purification of the acetone extracts, one new *ent*-trachylobane diterpenoid, saposebonic acid (**1**), together with thirteen known compounds, were isolated from the roots of *Sapium sebiferum*. Four of them, 5,7,8-trimethoxycoumarin, baccatin, fattyl ferulate, and 2,6-dimethoxyquinone, were reported the first time from this plant. Their structures were established on the basis of spectral evidence.



1

Acknowledgements: The authors are grateful to the National Science Council, Taiwan, R. O. C. (NSC 92-2323-B-016-002) for financial support for this research.
References: 1. Kee, C.H.(1999) The pharmacology of Chinese herbs, 2nd,CRC Press LLC.

P 317 Biological activity of diterpenes from *Crossopetalum gaumeri*

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In the search for active compounds from natural sources, we study the roots of *Crossopetalum gaumeri* (Celastraceae) (1), a scrub tree of the Yucatecan coast, used in Mexican folk for the treatment of stomach illness. From this study we isolated nine diterpenes whit an abietan skeleton, tree of which were new in the bibliography and their structures were determined by spectroscopic methods, including homonuclear and heteronuclear NMR experiments. The compounds were tested for their antimicrobial activity against fourteen selected microorganisms using the 96 microwell method (2).

Rerferences: 1. González, A. G., Bazzochi, I., Moujir, l., Jimenez I. (2000) Studies in natural products chemistry. Bioactive Natural Products (Part D)*;* Elsevier Science Publisher. Amsterdam. **2.** Charaïbi, D., Giron, S., Michel, G. J. (1990) J. Per. Res. 25: 201-206.

P 318 Antibacterial cassane diterpenes from *Mezoneuron benthamianum*

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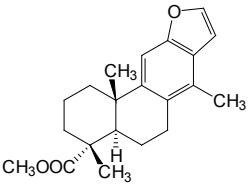
New cassane-type diterpenoids eg **1** were isolated from the air dried roots of *Mezoneuron benthamianum* (Caesalpinaniaceae).The structures of these compounds were elucidated by spectroscopic studies using a combination of 1D and 2D NMR Spectroscopy and Mass Spectrometry (ESI).



1

Tests for MIC using serial dilution were carried out for each compound. Four of the compounds were active against several bacteria. **1** was the most active compound and gave MIC values of 15.6 µg/ml, 31.2 µg/ml, 15.6 µg/ml, 125 µg/ml, 125 µg/ml, 32 µg/ml and 32 µg/ml against *Micrococcus flavus (*NCTC7743), *Bacillus subtillis* (NCTC10073), *Staphylococcus aureus (* NCTC4263), *Streptococcus feacalis* (NCTC775), *Pseudomonas aeruginosa* (NCIMB1042), Methicillin-resistant *S.aureus* (MRSA) (SA1199B) and tetracycline-resistant *S. aureus* (XU212) respectively.

Acknowledgements: Commonwealth Scholarship Commission, UK for financial support.

Cytotoxic labdane diterpenes from *Marrubium velutinum* and *Marrubium cylleneum*

P 319

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[b]Institute of Pharmacy, Department of Pharmaceutical Biology, University of Regensburg, Universitätsstrasse 31, D-93040, Regensburg, Germany
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In continuation of our phytochemical investigations into *Marrubium* species of the Greek flora (1), we report on the isolation and identification of secondary metabolites from the dichloromethane extracts from the aerial parts of *Marrubium velutinum* and *M. cylleneum*. Five new labdane diterpenes, velutine A, 15-epi-velutine A, velutine B, 15-epi-velutine B, velutine C have been isolated together with seven known diterpenes and four known flavones. The structures of the isolated compounds were established by means of NMR [^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HMQC, HMBC, HMQC-TOCSY, NOESY] and MS spectral analyses. Complete NMR assignments are reported for known compounds. Selected compounds 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. Effector PBMC were further assayed for enhancement of their lytic ability against ^{51}Cr-labeled target cells (K562, Daudi and Jurkat) at effector to target ratios varying between 10-80:1. Preliminary data show that some of these compounds could be potentially used to enhance PBMC anticancer activity.

References: 1. Karioti, A. et al. (2003). Acylated and flavonoid and phenylethanoid glycosides from Marrubium velutinum. Phytochemistry 64: 655-660. **2.** Tsavaris, N. et al. (2004). The effect of edrecolomab (Mo17-1A) or fluorouracil-based chemotherapy on specific immune parameters in patients with colorectal cancer. Oncology 67: 403-410.

Chemical constituents of *Inula pseudolimonella* (Asteraceae) endemic to Greece - Antimicrobial activity

P 320

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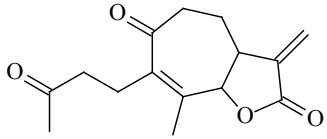
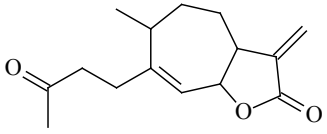
Inula pseudolimonella (Rech. f.) belonging to Asteraceae family is one among four endemic species of Inula in Greece. The plant has been collected from Mountain Dikti of Eastern Crete. From the crude CH_2Cl_2 extract of each aerial parts yielded a new xanthanolide [1] together with the known one, inusoniolide [2] (1), as well as the triterpenoids dammara-20,24-dien-3-ol and dammara-20,24-dien-3β-yl-acetate (2). All compounds were structurally elucidated by ^{1}H and ^{13}C NMR spectroscopy, MS techniques. The CH_2Cl_2 extract and the isolated compounds were tested for their antimicrobial activity. Through antimicrobial screening, the extract proved to be active against all nine human pathogenic tested bacteria - fungi, while the pure isolated compounds showed an interesting profile mostly against Gram (+) bacteria.



[1]



[2]

Acknowledgments: Dpt. of Pharmacognosy - Chemistry of Natural products, School of Pharmacy, University of Athens, Mr Kalpoutzakis El.
References: 1. Bloszyk, E. et al. (1990), Czech. Chem. Commun., 55, 1562. 2. González, A. et al. (1982), Phytochemistry, 21, 7, 1826-1827.

P 321 New Cytotoxic Sesquiterpenes from the Leaves of *Neolitsea buisanensis* f. *buisanensis*

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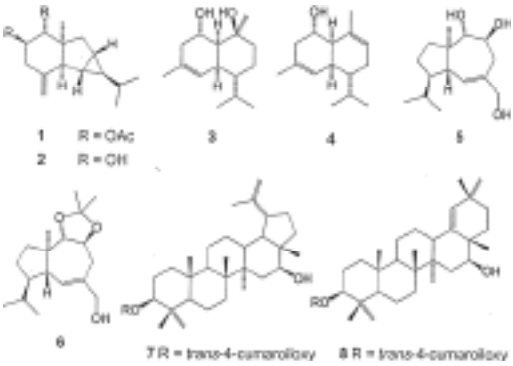
Neolitsea buisanensis Yamamoto & Kamikoti f. *buisanensis* (Lauraceae) is a small evergreen trees with much branched toward apex of branchlets, which is distributed in Kwangtung and Kwangsi of China, Vietnam and Taiwan (1). Five new sesquiterpenes, including neobuisanolides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), together with seven known compounds of spathulenol (**6**), glechomanolid (**7**), neolitacumone B (**8**), litseacassifolide (**9**), (-)-*ent*-4β-hydroxy-10β-methoxyaromadendrane (**10**), (6β,7β)-4β-hydroxy-10α-methoxyaromadendrane (**11**), and (6α,7α)-4β-hydroxy-10α-methoxyaromadendrane (**12**) were obtained from the cytotoxic $CHCl_3$-soluble fraction of the leaves of this plant. The structures of these isolates were determined by spectroscopic analyses. Compounds **7** and **8** exhibited significant cytotoxicity against P-388 and marginal cytotoxicity against HT-29 cancer cell lines in *vitro*. Compounds **1** and **4** showed marginal cytotoxicity against P-388 cancer cell line.

Acknowledgements: This work was financially supported by a grant from the National Science Council of the Republic of China (NSC 91-2320-B-037-029).
Reference: 1. Liao, J.-H. (1996) Lauraceae in Flora of Taiwan, 2nd ed., Editorial Committee of the Flora of Taiwan, Taipei, Taiwan, Vol. II: 487-489.

P 322 Uncommon Sesquiterpenoids and New Triterpenoids from *Jatropha neopauciflora*

A. García and G. Delgado
Instituto de Química, Universidad Nacional Autónoma de México. Ciudad Universitaria, Circuito Exterior, Coyoacán 04510. México, D. F.



This work deals with the phytochemical study of *J. neopauciflora* (Euphorbiaceae), an endemic Mexican plant used traditionally for the treatment of infections (1). Two new cycloaxene-type sesquiterpenes (**1**,**2**), two new cadinane-type sesquiterpenes (**3**,**4**), two new isodaucane-type sesquiterpenes (**5**,**6**), and two new pentacyclic triterpenoids (**7**,**8**), together with nine known compounds, were isolated from the dried bark of *J. neopauciflora*. **4** and **6** were isolated as artifacts from **3** and **5**, respectively. The structures were established by extensive 1D- and 2D-NMR spectral analyses and by X-ray crystallographic analysis. This is the first evidence of the presence of *cis*-fused sesquiterpenoids in the chemistry of Euphorbiaceae family.

Acknowledgements: A. G. acknowledges to CONACyT and DGAPA-UNAM for PhD scholarships and PAEP-UNAM for support. The authors are indebted to P. Guevara-Fefer and J. Jiménez-Ramírez for providing and identifying plant material.
References: 1. Canales M., Hernández T., Caballero J., Romo de Vivar A., Avila G., Duran A., Lira R. (2005) *J. Ethnopharm.* 97: 429-39.

Sesquiterpene Lactones and Flavonoids from the aerial parts of *Anthemis melanolepsis* L. and their cytotoxic/cytostatic activity against human cell lines *in vitro*

P 323

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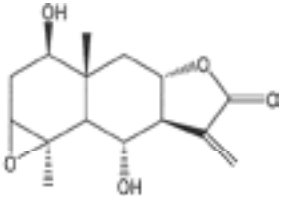
[b] Foundation for Biomedical Research of the Academy of Athens, Athens, Greece



Continuing our research on the chemical constituents from the aerial parts of Greek *Anthemis* sp., we report here the isolation and identification of sesquiterpene lactones **1**-**6** and flavonoids **7**-**10** from *A. melanolepsis* L, a species belonging to the section Cota (1). 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. Compound **1**, namely melanolepsin A, is a new naturally occurring eudesmanolide. Besides compound **1**, five known sesquiterpene lactones and four flavonoids were isolated, namely desacetyl-β-cyclopyrethrosine (**2**), being the major constituent of the plant, eginselolide (**3**), tatridin A (**4**), 1-epitatridin B (**5**), sivisanolide (**6**), quercetin **(7)**, 3, 6, 3′,4′ trimethoxy quercetin **(8)**, isorhamnetin **(9)** and 8-hydroxyquercetagenin **(10)**. Cytotoxic/cytostatic activity of the fractions, as well as of the isolated compounds was tested against the human cancer cell lines SF268, H460, MCF7, OVCAR3, BLD1 (3). Compound **2** was found to be the most active.

References: 1. Davis P. H. (1975), Flora of Turkey and the East Aegean islands, Edinburgh, 5, 174-221. **2.** Bohlmann F. *et al.* (1984), Phytochemistry 23, 1979-88. **3.** Maswadeh H. *et al*. (2000) Anticancer Research 20:4385-4390.

Oxygenated diterpenes from bark of *Juniperus brevifolia*

P 324

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Juniperus brevifolia, the unique conifer tree endemic to the Azorean archipelago, is locally known as "cedro-domato", and classified as belong to cupressaceae family. This species is a typical component of the primitive *laurisilva* forest and occurs in almost all of the islands, except S. Maria and Graciosa. Old stands of some size are now rare, since *J. brevifolia* has been widely used in shipbuilding and carved work due to the high quality of its wood. Apart from the papers published by Adams (1) and Silva (2), which includes the chemical composition of essential oils of *J. brevifolia* there are no phytochemical reports about this taxon.

We become interested in the constituents of this plant due to the biological activity of related species in the same genus and their spetifacient (3, 4). The dichloromethane extract of leaves and core of *J. brevifolia* exhibited a significant citotoxic activity against the human tumour cell line Hep-2 (larynx cancer) (5). We wish to report here the investigation of the hexane extract from *J. brevifolia* bark which has resulted, for the first time in the Juniperus genus, in the isolation of several oxygenated diterpenes like totarol-1,3-dione, 11-hydroxi-6,7-dehydroferruginol, 6,7-dehydrohinokiol, 6,7-dehydroferruginyl methyl ether.

The experimental procedures and detailed mass spectra studies of these diterpenes and of their TMS derivatives will be presented and discussed.

Acknowledgements: This work was funded by University of Aveiro and FCT-Lisbon for funding the Research Unit "Química Orgânica, Produtos Naturais e Agroalimentares". and Fundação Calouste Gulbenkian.

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P 325 Phenolics from *Brahea aramata* Fruits

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The genus *Brahea* (Arecaceae) includes the famous species, *Brahea serrulata* (synonym : *Serenoa repens*) that have a long folk history of use for improving the signs and symptoms of benign prostatic hyperplasia (BPH) (**1**). The berry of another *Brahea* species, namely, *Brahea aramata*, cultivated in Egypt, has not been investigated previously for its biological activity or constitutive phenolics. The present study proved the antibacterial activity and the interesting inhibitory effect against 5-α-reductase (**2**) of the aqueous alcoholic extract of this berry berry. Also, we were able to isolate five phenolics **1**-**5**, including the new chalcone, 4′,6′-dimethoxy β,4,2'-trihydroxy chalcone **1**. The structures were established by conventional methods and confirmed by ESIMS and NMR analysis. **1** exhibited in its ESIM spectrum a molecular ion at m/z 316. In the ^{1}H NMR spectrum of **1**, the absence of the β-chalcone proton was concluded from the singlet resonance located at δ 7.8 ppm attributable to an α-chalcone proton lacking a vicinal olefinic proton at the β-position. In this spectrum the relatively low field location of the resonances of the H-3' and H-5' (δ 5.95 & 6.08) together with the presence of two methoxyl proton resonances at δ 3.72and 3.85 proved methyl etherification at the chalcone positions 4′and 6′. The resonances in the ^{13}C spectrum at δ 124..2, 168.2 and 192.6 ppm were assigned to the α-, the hydroxylated β-carbons and the chalcone carbonyl carbon, respectively, thus proving, together with other present resonances the achieved structure of **1**.

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P 326 Antitumor activity of flavonoids from *Pteleopsis suberosa* leaves

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Pteleopsis suberosa Engl. et Diels (Combretaceae) is a tree widely distributed in the sahelian savanna, from Mali to Nigeria. In Malian folk medicine the decoction of the plant is used for the treatment of jaundice, asthenia, and disentery (1), while the decoction of the bark is popularly used especially for antiulcer properties, confirmed by pharmacological studies (2,3). In this work we describe the chemical and biological studies of the plant leaves. Sixteen flavonoids, including gallocatechin and flavonols having kaempferol, quercetin, and myricetin as aglycons, were isolated and identified. Among myricetin derivatives, myricetin 3-*O*-(4″-acetyl)-α-L-arabinopyranoside and myricetin 3-*O*-(3″-acetyl)-α-L-arabinopyranoside are now reported for the first time. The biological activity of the extracts and their active components was tested with androgen-insensitive prostate cancer cells (DU-145) testing cell viability (MTT assay), genomic DNA fragmentation (COMET assay) (4),and cell membrane integrity (LDH release) (4,5). The data obtained evidenced that the methanol extract of the leaves significantly ($p<0.001$) inhibited the growth of DU-145, probably triggering an apoptotic process.

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Sarcolobin and sarcolobone – new isoflavonoids from *Sarcolobus globosus*

P 327

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Sarcolobus globosus (Asclepiadaceae) is a liana growing in the mangrove forests in Asia. The plant has been used in traditional medicine to treat rheumatism, dengue and fever. Natives have also utilized the plant for poisoning animals. The chemical composition of *S. globosus* has not previously been described in the literature; therefore our aim was to identify its constituents. *S. globosus* was collected in Sundarbans mangrove forest in Bangladesh, and the dried stems were milled and extracted with 80 % methanol (crude extract). The crude extract was further partitioned with liquid-liquid extraction, giving diethyl ether, ethyl acetate and n-butanol extracts, and an aqueous residue. Fractionation of the diethyl ether and the ethyl acetate extracts resulted in the isolation of a new rotenoid (sarcolobin) and a new isoflavone (sarcolobone), along with ten rotenoids, two isoflavones and one chromone. The chromone (6,7-dimethoxy-2,3-dihydrochromone) has not previously been reported as a natural product. The structure elucidations of the isolated compounds were performed using MS, CD, 1D and 2D NMR techniques. Antioxidant (DPPH-radical scavenging and 15-lipoxygenase inhibition) and antimicrobial activities of selected compounds have been examined.

Biflavonoids from Indian *Selaginella* species and their biological activities

P 328

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The genus *Selaginella* (spikemoss, Selaginellaceae) consists of over 700 species with a world-wide distribution mainly in warm and moist climate. Very few species extend to higher northern or southern latitudes. In general, reports on the medicinal usage of plants from the genus *Selaginella* are rare. The Traditional Chinese Medicine lists *S. tamariscina*, *S. pulvinata* and *S. doederleinii* to promote blood circulation and to stimulate menstrual discharge as well as a bactericidal and anti-cancer drug. The genus produces a variety of compound classes such as hordenin-type alkaloids, lignans, biflavonoids, steroidal compounds and sugars (e.g. trehalose). However, the chemistry of the genus has not been sufficiently studied yet. In India, where about 62 species occur, only two species, *S. tamariscina* and *S. rupestris*, have been phytochemically investigated (1,2).
The chemical investigation of *Selaginella bryopteris* collected from southern India and *Selaginella chrysocaulos* collected from north-eastern India yielded three new and twelve known biflavonoids mainly of the amentoflavone and hinokiflavone type. The structures of the compounds have been elucidated using 2D NMR experiments. The absolute configuration of the hydrogenated biflavonoids was determined using circular dichroism. The anti-inflammatory activities was evaluated in 5- and 12-LOX inhibitory assays.

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P 329 New and cytotoxic compounds from *Disporopsis aspera* Engl. (Liliaceae)

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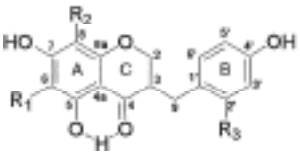

	R_1	R_2	R_3
1 Disporopsis A	H	H	OH
2 Disporopsis B	CH_3	H	H
3 Disporopsis C	CH_3	OCH_3	H
4 Disporopsis D	CH_3	CH_3	H

Disporopsis aspera Engl. (Liliaceae) is a perennial herb, 20 – 50 cm high, well known in Vietnam with the name of "Ngoc Truc Hoang Linh". Its rhizome in decoction is prescribed as tonic in asthenia, persistent fever, night sweats, spermatorrhea, dry cough, polyuria and is used for cancer treatment [1]. In a preliminary communication we reported the cytotoxicity of different extracts *i.e.* CH_2Cl_2, EtOAc, MeOH, MeOH-H_2O and H_2O from *Disporopsis aspera* rhizome [2]. The most bio-active EtOAc extract has been investigated leading to the isolation and structure elucidation of a new homoisoflavanone named disporopsis A (**1**), three rare methyl-homoisoflavanones, disporopsis B (**2**), disporopsis C (**3**) and disporopsis D (**4**) together with six known compounds, *N-trans*-feruloyl tyramine (**5**), adenine (**6**), 5-(hydroxymethyl)-2-furfural (**7**), β-sitosterol (**8**) and β-sitosterol glucopyranoside (**9**). As disporopsis A, B, C and D showed significantly different cytotoxic activities against a panel of human cancer cell lines (HCT15, T24S, MCF7, Bowes, A549 and K562) in MTT assay, a structure-activity relationship is discussed.

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P 330 Isolation and structure elucidation of two novel flavonoids from *Belamcanda chinensis* with antiproliferative effect on prostate cancer

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Extracts of the rhizomes of *Belamcanda chinensis* contain remarkable amounts of isoflavonoids like tectorigenin and irigenin. Isoflavonoids have been shown to exert antiproliferative effects on cancer cells by steroid receptor signalling. Recently we demonstrated the antiproliferative effect of the isolated isoflavonoids tectorigenin and irigenin on prostate cancer cells in vitro through cell cycle regulation. In this study we present for the first time the structure elucidation of the flavonoids hispidulin (5,7,4'-trihydroxy-6-methoxyflavone) and 7-methylaromadendrin (3,5,4'-trihydroxy-7-methoxy-flavanone (2R,3R)) isolated from Belamcanda chinensis and their potential as anti-cancer drugs by down-regulating the aberrantly up-regulated expression of genes relevant in proliferation, invasion and immortalisation. Subsequent separation of the aqueous ethanolic extract by liquid-liquid partition and Sephadex-LH-20 chromatography led to a mixture of both substances. Final separation was achieved by HSCCC. Structures were elucidated by MS and 2D NMR. LNCaP prostate cancer cells were treated with hispidulin/7-methylaromadendrin in combination and mRNA expression was quantified by using real time RT-PCR. In addition, PSA secretion from LNCaP in conditioned media was measured with the Elecsys® System 2010. Hispidulin/7-methylaromadendrin down-regulated androgen receptor (AR), prostate-derived Ets transcription factor (PDEF), prostate specific antigen (PSA), IGF-1 receptor (IGF-1R) and telomerase (hTERT) mRNA expression in vitro: downregulation mRNA expression to [%]: AR / 6.2, PDEF / 6.9, PSA/ 14.3, IGF-1R / 15.0, hTERT / 7.1. Furthermore, PSA secretion of LNCaP prostate cancer cells was diminished to 22.8% after hispidulin/ 7-methylaromadendrin treatments. The down-regulation of the androgen receptor, PDEF, PSA, hTERT and IGF-1 receptor gene expression by hispidulin and 7-methylaromadendrin demonstrates the antiproliferative potential of these agents. hTERT and IGF-1R are established targets for cancer therapies. As new insights into the importance of AR and PDEF in prostate cancer progression caused a demand for new therapeutic strategies targeting these structures, hispidulin and 7-methylaromadendrin may be useful for the prevention or treatment of human prostate cancer.

Flavonoids and Andrographolides from *Andrographis paniculata* and Their Antiplatelet Aggregatory and Vasorelaxing Effects

P 331

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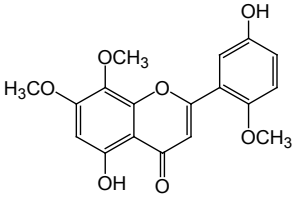

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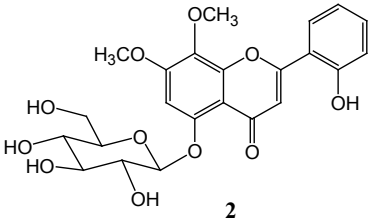

2

Andrographis paniculata Nees (Acanthaceae) is an erect herb found widely in Subtropical Asia, Southeast Asia, India and China. It has been traditionally used in China as an antiinflammatory, hepatoprotective, antiviral, antioxidant, immune enhancement herbal medicine. Two new flavones andropaniculosin A (**1**) and andropaniculoside A (**2**) and thirty known compounds were isolated and characterized from the methanol extract of the *A. paniculata* collected from Taiwan. Among them, four flavonoids showed potent inhibition of collagen, arachidonic acid, thrombin, and platelet activation factor induced platelet aggregation. Furthermore, a diterpenoid demonstrated moderate vasorelaxing effect in isolated rat thoracic aorta.

Acknowledgements: The authors are grateful to the National Science Council, Taiwan, R. O. C. (NSC 93-2113-M-006-001) for financial support for this research.

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Flavonoid Glycosides from *Cephalotaxus koreana*

P 332

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Three new flavonoids, 6-methylaromadendrin 3-*O*-β–*D*-glucopyranoside, apigenin 5-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside, and apigenin 5-*O*-(2-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside-6‴-*O*-actate, were isolated from the aerial parts of *Cephalotaxus koreana*, along with five known compounds, quercetin-3-*O*-β--glucpyranoside, quercetin 3-*O*-a-L-rhamnopyranoside, quercetin-3-*O*-β-D-glucpyranoside-6‴-*O*-acetate, quercetin 3-*O*-(6-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside, quercetin 3-*O*-α-L-rhamnopyranoside, and apigenin 8-*O*-β-D-glucopyranoside. Their structures were elucidated using spectral evidences and comparison with literature data.

P 333 Anti-Trypanosomal secondary metabolites from two Cameroonian medicinal plants

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In the search of our course for antitrypanosomal compounds from natural source, two plants used in central and western part of Cameroon to treat several diseases were investigated : *Xymalos monospora* Baill (Monimiaceae), *Millettia griffoniana* Bail (Leguminosae). From the stem bark of *Xymalos monospora,* five alkaloids: mollinedine, 1-(*p*-methoxybenzyl)-6,7-methylenedioxyisoquinoline, doryafranine, *N* –methyllaurotetanine and the new *Epi*-temuconine were isolated. From the seeds of *Millettia griffoniana*, five new isoflavonoids namely 7-methoxyebenosin, griffonianone (E-H) along with the known calopogonium isoflavone B, 7,2′-dimethoxy-4′,5′-methylenedioxy isoflavone, prebarbigerone, pseudobaptigenin, pseudobaptigenin methyl ether, tephrosine, dipterixine, odorantine , 2′, 4′, 5′,7-tetramethoxyisoflavone and isojamaicin were isolated. The structure of compounds were established by MS, 1D and 2D spectroscopy including DEPT, COSY, HMQC and HMBC experiments. Crude extracts as well as pure compounds have been tested for growth inhibitory activity in vitro vs. Bloodstream forms of African trypanosomes. IC50 values in the range of 1-6μg/ml were found for many of pure compounds isolated.

Acknowledgements: The authors gratefully acknowledge financial support from the international program in the chemical Sciences (IP-ICS), Uppsala, Sweden.
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P 334 Phytochemical and Antimycobacterial Properties of *Chlorophytum inornatum*

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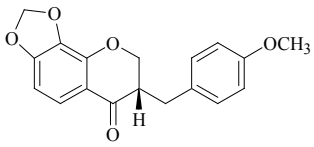

(**1**)

The emergence of multi-drug resistant tuberculosis has led to an urgent need for the discovery of new anti-TB drugs. As part of an investigation into the antimycobacterial properties of the Liliaceae, we report here the isolation of two new compounds from the roots of *Chlorophytum inornatum*, [**1**] 3-(4′-methoxybenzyl)-7,8-methylenedioxy)-chroman-4-one and 7-2′,3′-dihydroxypentyl-benzofuran [**2**]. Compound [**1**] was the most active with minimum inhibitory concentration (MIC) values ranging from 16-256 μg/ml against a panel of fast growing *Mycobacterium* species. The structure of [**1**] was elucidated by 1D and 2D NMR and by comparison with similar compounds from *Ophiogogon japonicus,* (Liliaceae) (**1**). There is no literature specific to the phytochemistry of *C. inornatum,* however *C. comosum* is of use in Africa, India and China (**2**) and the roots of *C. arundinaceum* are a constituent of Aryuvedic formulations (**3**).
Homoisoflavonoids such as compound **1** have not been characterised as antimycobacterial agents previously, and the simplicity of this class highlights the potential for further synthetic analogue generation.

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Flavonolignans from *Avena sativa* L.

P 335

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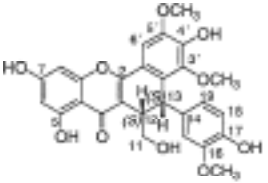
As a part of our ongoing phytochemichal and pharmacological studies on *Avena sativa* L. (oat), we isolated a new tricin derived flavonolignan with an unusual skeleton from the oat herb dichloromethane extract [1] along with the two rare diastereomeric tricin derivatives salcolin A and B [2, 3]. This is the first report on the presence of flavonolingnans in *A. sativa*. Isolation was performed by size exclusion chromatography using Sephadex LH20®, followed by RP18 column chromatography and semipreparative HPLC. Structures were elucidated by LCESIMS^n and 1D and 2D ^{13}C and ^{1}H NMR spectroscopy. Enantiomeric purity of salcolin A and B was examined by capillary electrophoresis using the method of Schmid et al (1). The absolute stereochemistry of the new compound [1] was determined to be 12*S*, 13*S* from NOE experiments and circular dichroic data (2).



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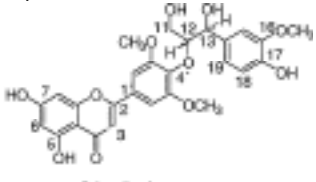

2 (erythro)
3 (threo)

Acknowledgements: We thank W. Keller (Department of Physical Chemistry, Institute of Chemistry, University of Graz, Austria) for his assistance during the CD measurements.

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Antibacterial galloylated phloroglucinol glucosides from myrtle

P 336

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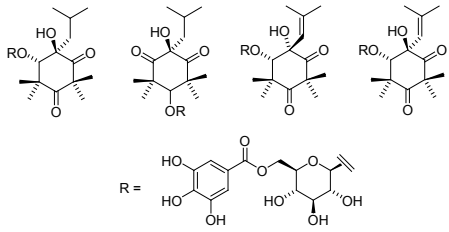

Myrtle (*Myrtus communis* L.) is nowadays better known as a culinary herb rather than a medicinal plant, but holds an important place in Western culture because of its mythological associations and its medicinal use as an antiseptic agent. The leaves of myrtle contain unique oligomeric phloroglucinol derivatives that show remarkable antibacterial and antioxidant activities (1,2). From this source, we have now characterized a mixture of non prenylated monomeric phloroglucinols characterized by a core structurally related to the G-hormones (3) and by glucosidation with a galloylated sugar. We have named these unique compounds gallomyrtucommulones A-D, and report their structure elucidation and evaluation as antibacterial agents.

References: 1. Appendino, G. et al. 2002 J. Nat. Prod. 65: 334-338. **2.** Rosa, A. et al. 2003 Free Rad. Res. 37: 1013-1019. **3.** Ghisalberti, E. L. 1996 Phytochemistry 41: 7-14.

P 337 An anti-staphylococcal acylphloroglucinol from *Hypericum foliosum*

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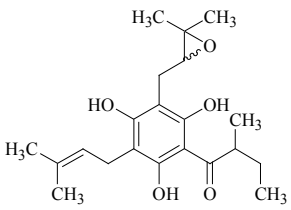

[**1**]

As part of a continuing project to characterise antibacterial constituents of the Guttiferae we have been evaluating members of the genus *Hypericum,* which are prolific producers of antibacterial acylphloroglucinols (**1**-**3**). This was initially driven by the activity of the acylphloroglucinol hyperforin, from *Hypericum perforatum,* which is exceptional in its activity against multidrug-resistant (MDR) and methicillin-resistant (MRSA) strains of *Staphylococcus aureus*. This prompted us to extract and screen a number of *Hypericum* species for antibacterial activity (**4**). In the current study, large-scale collections of *Hypericum foliosum* Aiton. (Guttiferae), a plant that is endemic to the Azores (**5**) were made. Bioassay-guided isolation of an anti-staphylococcal hexane extract has led to a new acylphloroglucinol, which by NMR spectroscopy and mass spectrometry was characterised as 1,3,5-trihydroxy-6-[2‴,3‴-epoxy-3‴-methyl-butyl]-2-[2″-methyl-butanoyl]-4-[3′-methyl-2′-butenyl]-benzene [**1**]. This metabolite is described here for the first time and was evaluated against a panel of MDR and MRSA strains of *S. aureus* and MIC values ranged from 16-32 μg/ml.

References: **1.** Gibbons, S. (2004) Nat. Prod. Rep. 21, 263-277. **2.** Winkelmann, K. et al. (2000) J. Nat. Prod. 63, 104-108. **3.** Winkelmann, K. et al. (2001) J. Nat. Prod. 64, 701-706. **4.** Gibbons, S. et al. (2002) Fitoterapia 73, 300-304. **5.** Robson, N. K. B. (1977) Bulletin of the British Museum (Natural History) Botany 5, 118-119.

P 338 Isolation and Identification of New Phloroglucinol Derivatives from *Hypericum* L. (Clusiaceae) Species from the Southeastern United States

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As part of our continuing phytochemical investigation of members of the medicinally valuable plant genus *Hypericum* L. (Clusiaceae) occurring in the Southeastern United States, fractionation of the non-polar extracts of the aerial parts of three species led to the isolation of new phloroglucinol derivatives. Phloroglucinol derivatives, the most well-known of which is hyperforin from *H. perforatum* L., are of considerable interest due to their chemical diversity and interesting bioactivities[1]. The three species presented are taxonomically assigned to the Section *Myriandra*, are native to the Southeastern United States, and had not been previously phytochemically investigated. Investigation of the dichloromethane extract of *H. cistifolium* Lam. (Roundpod St. John's Wort), the hexane extract of *H. lissophloeus* P. Adams (Smoothbark St. John's Wort), and the hexane extract of *Hypericum galioides* Lam. (Bedstraw St. John's Wort) led to the isolation of interesting three new phloroglucinol derivatives. Structures were elucidated on the basis of 1D and 2D NMR experiments, and where possible, x-ray diffraction analysis. Potential chemotaxonomic implications of these findings are discussed.

References: 1. Hoetzel, J., Petersen, M. (2003). Chemical constituents of *Hypericum* spp. In E. Ernst (Ed.), *Hypericum: The genus Hypericum* (pp. 77-93). New York, New York: Taylor and Francis.

New Biphenyls and Antitubercular Constituents from the Root of *Garcinia linii*

P 339

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Garcinia linii C. E. Chang (Guttiferae) is a small endemic evergreen tree which grows on Lanyu Island and Green Island of Taiwan (1). The plants of genus *Garcinia* are rich in xanthonoids, depsidones, and benzophenones, some of which have demonstrated cytotoxic activities. In preliminary screening, the methanolic extract of the root of this species showed cytotoxic and antitubercular activities *in vitro*. Previously, we have reported three new xanthones, linixanthones A–C, two new biphenyls, garcibiphenyl A, garcibiphenyl B, and a new benzopyran, garcibenzopyran, together with thirteen known compounds, including several cytotoxic agents (2) from the root of this plant. Continuing investigation of the minor constituents and the antitubercular principles led to the isolation and characterization of three new biphenyls, garcibiphenyl C (**1**), garcibiphenyl D (**2**), garcibiphenyl E (**3**), and a new benzopyran, 3-hydroxygarcibenzopyran (**4**), together with thirteen known compounds. The structures of these new compounds were determined through spectral analyses. Among the isolates, 1,7-dihydroxy-3-methoxyxanthone and 1,5-dihydroxy-3-methoxyxanthone showed antitubercular activities with MICs of 3.13 and 6.25 μg/mL against *Mycobacterium tuberculosis* 90-221387 *in vitro*, respectively.

Acknowledgements: This work was supported by a grant (NSC 91-2320-B-127-006) from the National Science Council of the Republic of China.

References: 1. Robson, N.K.B. (1996) Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan. Vol. 2: 694-714. **2.** Chen, J.J., Chen, I.S., Duh, C.Y. (2004) Planta Med 70: 1195-1200.

Three new bibenzyl derivatives from *Tragopogon orientalis* L. and their radical scavenging activity

P 340

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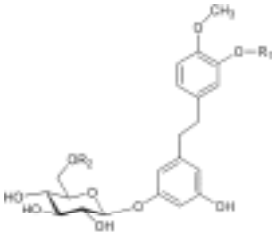

The new natural products α,β-dihydrorhaponticin (**1**), its 6´´-7,8-dihydrocaffeoyl derivative (**2**) and 3´-O-methyl-α,β-dihydrorhaponticin (**3**) were isolated from the methanolic extract of subaerial parts of *Tragopogon orientalis* L. (Asteraceae, Lactuceae). Structure elucidations were based on mass spectrometry as well as extensive 1D- and 2D-NMR spectroscopy. The discovery of bibenzyles is in congruence with the close phylogenetic relationship of the genera *Tragopogon* and *Scorzonera*, because related structures, bibenzyles with a benzofuran-2-carboxylic acid carbon skeleton – so called Tyrolobibenzyls – were found in *Scorzonera humilis* L. (1-3). Compound **1a** (the aglycon of **1**) was obtained via enzymatic hydrolysis. The free radical scavenging activity of compounds **1**, **1a** and **2** was assessed using the DPPH assay, showing potent radical scavenging activity with IC_{50} values of 21.3 μM (± 1.24 μM) for **1,** 18.6 μM (± 1.20 μM) for **1a**, and 15.4 μM (± 0.37 μM) for **2**, respectively. The presented data demonstrated the influence of the number of free phenolic hydroxyl groups and their positions on radical scavenging activity.

Acknowledgements: This work was supported by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF, P15594).

References: 1. Zidorn, C. et al. (2000) Helvetica Chimica Acta 83: 2920-2925. **2.** Zidorn, C. et al. (2002) Zeitschrift für Naturforschung C 57: 614-619. **3**. Zidorn, C. et al. (2003) Phytochemistry 63: 61-67.

P 341 Cytotoxic phenanthrenes from the rhizomes of *Tamus communis* L.

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In recent years the cis stilbene combretastatins have attracted great interest because of their potential use in the cancer chemotherapy. The most potent member, combretastatin A-4 in phosphate pro-drug form is currently tested in clinical trials against different solid tumors, including multidrug resistant cancers (1). The aim of our work was the antitumor evaluation of structurally similar compounds, namely phenanthrenes, which are the conformationally restricted congeners of cis stilbenes.
Previous phytochemical examination of *Tamus communis* (Dioscoreaceae) has shown the presence of alkoxy-substituted phenanthrenes (2, 3), but no data were reported about their antitumor potency. The present paper deals with the bioguided isolation, structure elucidation and cytotoxic activity of phenanthrenes from the fresh rhizomes of T. communis. Five compounds were obtained from the $CHCl_3$ fraction of the MeOH extract using VLC, preparative TLC, HPLC and gelfiltration. The compounds were identified by means of various NMR techniques (^{1}H-NMR, JMOD, NOESY) as new and known hydroxy- and methoxy-substituted phenanthrenes. The cytotoxic assay on HeLa cells using MTT test revealed that four of the isolated phenanthrenes possess remarkable cell growth inhibitory activity compared to that of the positive controls cisplatin and doxorubicine.

Acknowledgement: The authors thank Prof. Kálmán Szendrei (Department of Pharmacognosy, University of Szeged) for the cooperation in this study and for his valuable suggestions.
References: 1. Cirla, A., Mann, J. (2003) Nat. Prod. Rep. 20: 558-564 **2.** Reisch, J. et al. (1973) Phytochemistry 12: 228-229. **3.** Aquino, R. et al. Biochem. Syst. Ecol. 13: 251-252

P 342 Structure elucidation of two new bicoumarins from the leaves of *Murraya exotica*

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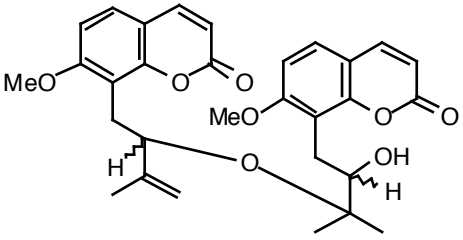
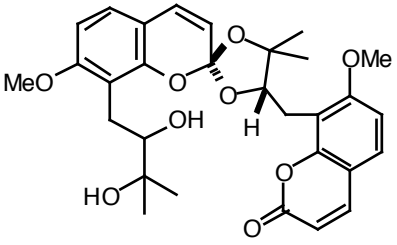


1



2

New bicoumarins, named murradimerin A (1) and murramarin B (2) were isolated from *Murraya exotica* (Rutaceae). Their structures were elucidated on the basis of spectroscopic data especially using 2D NMR spectrum. Naturally occurring bicoumarin connected two coumarin moieties by *orthoester* structure as in murramarin B (2) is unusual type and murramarin B (2) is the first example not possessing furanocoumarin moiety.

Phytochemical study of *Tephrosia deflexa*

P 343

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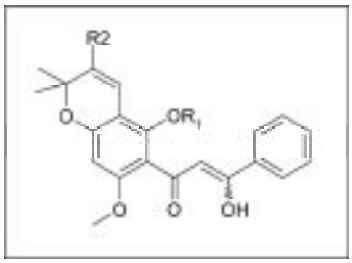
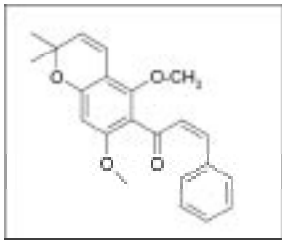
From the ethereal extract of *Tephrosia deflexa* Baker (Leguminosae) seeds and pods, three rotenoids and six chalcones were isolated, including four reported for the first time : deflexachalcone (**1**) and its 6′-*O*-methylated derivative (**2**),3″-chloro-6′-*O*-methyldeflexachalcone (**3**) and cis-6′-*O*-methylpongachalcone (**4**). From the ethyl acetate extract of dried aerial parts, only usual polyphenols and rotenone were identified (1).



	R_1	R_2
(**1**)	H	H
(**2**)	CH_3	H
(**3**)	CH_3	Cl



(**4**)

References: 1. *Niassy, B. et al. (2005) Biochem. Syst. Ecol.33 :309-312.*

New dihydrobenzofuran glucosides form the roots of Edelweiss (*Leontopodium alpinum* cass.)

P 344

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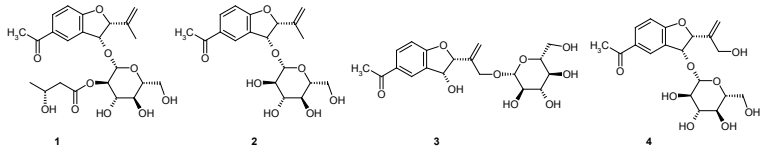
Investigations of polar extracts of the roots of Edelweiss (*Leontopodium alpinum* Cass.) yielded three additional dihydrobenzofuran glucosides [**1**,**2**,**3**]. The isolation was performed by silica gel CC, Sephadex® CC, semi preparative HPLC as well as 2D-preparative TLC. The structure elucidation was carried out by means of 1- and 2D-NMR and mass spectrometry. Reinvestigation of the previously described dihydrobenzofuran glucoside **4** (1) together with the new compounds **1** and **2**, as well as the previously described compound **3** (2), lead to a stereo chemical reassignment of the position 3*S** to 3*R** (3) of compound **4**. Thus, the novel constituents **1** and **2** were determined as 1-{(2*R**,3*R**)-3-(2-[(3*R*)-3-hydroxy-1-oxobutyl]-β-D-glucopyranosyloxy)-2,3-dihydro-2-[1-(methyl)vinyl]-1-benzofuran-5-yl}-ethanone and 1-{(2*R**,3*R**)-3-(β-D-glucopyranosyloxy)-2,3-dihydro-2-[1-(methyl)vinyl]-1-benzo-furan-5-yl}-ethanone, substance **3** as 1-{(2*R**,3*R**)-2,3-dihydro-3-hydroxy-2-{1-[(β-D-glucopyranosyloxy) methyl] vinyl}-1-benzofuran-5-yl}-ethanone and compound **4** as 1-{(2*R**,3*R**)-2-[1-(hydroxymethyl)vinyl]-3-(β-D-gluco-pyranosyloxy)-2,3-dihydro-1-benzofuran-5-yl}-ethanone.



References: 1. Dobner, M.J. et al. (2003) Helv. Chim. Acta 86 : 733-8. **2.** Gongora, L. et al. (2002) Phytochemistry 59: 857-60. **3.** Zalkow, L.H. et al. (1972) Tetrahedron Lett. 28: 2873-6.

P 345 New lignans from sesame perisperm

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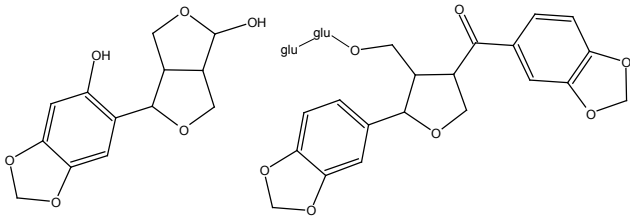

The process used by the food industry for the purification of sesame seeds, *Sesamum indicum* (Pedaliaceae), produces big quantities of the sesame coat (perisperm) which is an agricultural waste. The present study concerns the development of a methodology for the efficient obtainment of extracts rich in polyphenols from the sesame perisperms and the chemical investigation of the contained lignans.

The first step of the followed procedure was the extraction of the dried perisperms using solvents of increasing polarity: cyclohexane and CH_2Cl_2 in order to remove the oil efficiently and then methanol for the extraction of polypehenols. In order to enrich the polyphenol concentration, the dried methanol extract was diluted with warm water and this aqueous solution passed through a column containing XAD-4 resin, which selectively adsorbed the polyphenolic compounds. Then, the polyphenols were recovered , furnishing an enriched extract.

This dry extract was submitted to several chromatographic separations that finally led to the isolation of 9 known lignans: sesamin, pinoresinol, lariciresinol, olivil, matairesinol, todolactol, sesaminol diglucoside, pinoresinol glucoside, 5′-methoxylariciresinol and 2 new lignans: 1, 2, identified using NMR and MS spectroscopy.

P 346 A New Cytotoxic Pentamethoxyflavone Isolated from the Root Barks of *Casimiroa pubescens*

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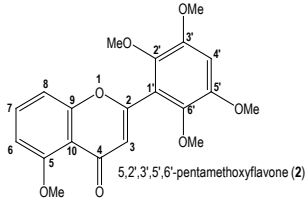
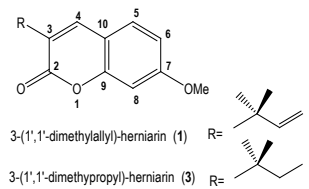
[b]Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México D. F.
[c]Colegio de Posgraduados, Montecillo, Edo. de México, México





Casimiroa pubescens known as "zapote de rata" and "zapote cimarrón", is a small tree which grows in Central Mexico. The new cytotoxic 5, 2′, 3′, 5′, 6′-pentamethoxyflavone (**1**) together with the known coumarin 3-(1′,1′-dimethylallyl)-herniarin (**2**) were isolated from the root barks of *C. pubescens*. Their structures were determined by spectroscopic data, and X-Ray analyses. Additionally the dihydroderivative (**3**) from **2** was prepared. All compounds were evaluated for cytotoxic effects against five human cancer cell lines in the SBR model (1). The isolates showed a moderate cytotoxic activity while the dihydroderivative **3** was inactive.

References: 1. Monga, M., Sausbille, E. (2002) Leukemia 16, 520-526.

Chemical Constituents and Their Radical Scavenging Activities from *Taxillus liquidambaricolus*

P 347

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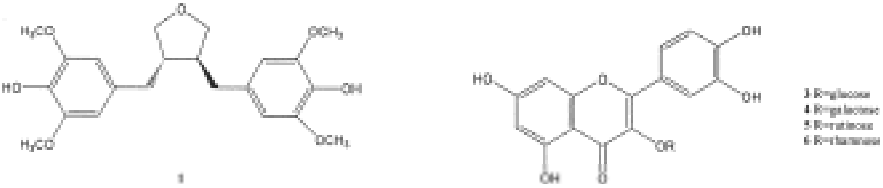
Taxillus liquidambaricolus is a specific Loranthaceae plant in Taiwan. It has commonly been used in Chinese medicine as a curative for a number of ailments such as hemorrhage, gout, epilepsy, arthritis and rheumatism. One new lignan (**1**), *trans*-3,4-(bis-3,5-dimethoxy-4-hydroxy-benzyl)tetrahydrofuran, and thirty-two known compounds, include seven triterpenoids, ten benzenoids, three chlorophylls, one lignan and eleven flavonoids, were isolated and characterized from *T. liquidambaricolus*. Their structures were identified by spectral analyses and compared with authentic samples. Three categories of flavonoids, catechins, kaempferols and quercetins, were afforded from this study and they were subjected to DPPH radicals scavenging activity assay. The results showed that quercetins (**3**~**6**) exhibited significant scavenging activity with the $IC_{0.2}$ values of 4.13, 4.20, 5.22 and 5.24 μM, respectively, compared to the reference compound vitamin E ($IC_{0.2}$, 8.34 μM).



Acknowledgements: The authors are grateful to the National Science Council, Taiwan, R. O. C. (NSC 93-2113-M-182-001) for financial support for this research.

References: 1. Shimada, K. *et. al.* (1992), *J. Agric. Food Chem.*, **40**, 945-948.

Two Norneolignaneglucosides from *Chione venosa*, the Carribean aphrodisiac Bois bandé

P 348

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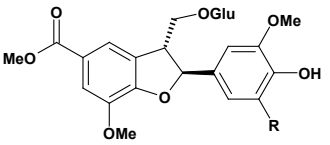
Bois bandé is a popular Carribean aphrodisiac originating from different plant sources. The investigated material was purchased in Grenada and consists of the stem bark and the roots of *Chione venosa* (SW.) URBAN var. *venosa*. So far the isolation of three iridoids and two acetophenone-derivatives has been described (1, 2). In continuation of our investigation we now present two new norneolignaneglucosides which were isolated from the drug´s bark. The plant material was extracted with methanol-water (1+1 v/v) and fractionated by repeated column chromatgography on sephadex LH-20, C-18 material and silicagel. Afterwards the two substances were isolated by preparative TLC and purified by column chromatography both on silicagel. The structures were elucidated by LC-MS and one- and two-dimensional NMR experiments, the stereochemistry was confirmed by CD spectroscopy. The two presented structures are reported for the first time.



1...R = H
2...R = OMe

References: 1. Lendl A. et al. (2002) Revista de Fitoterapia 2 (S1): 309 (B190). **2.** Lendl A. et al. (2005) Phytochemistry, submitted for publication.

P 349 New Cytotoxic Tetrahydrofuran-type Lignan from the Leaves of *Beilschmiedia tsangii*

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Beilschmiedia tsangii (Lauraceae) is an evergreen tree, distributed in forests at low altitudes throughout southern Taiwan (1). The chemical constituents and biological activities of this plant have never been studied. As an extention of our continuing studies on the cytotoxic constituents of Formosan plants, approximately 1000 species have been screened for *in vitro* cytotoxic test and *Beilschmiedia tsangii* was shown to be one of the active species. Investigation on chloroform-soluble fraction of the leaves of *Beilschmiedia tsangii* has led to the isolation of two new tetrahydrofuran-type lignans, beilschmin A (**1**) and beilschmin B (**2**), together with ten known compounds. The structures of new compounds were determined through spectral analyses including extensive 2D NMR (NOESY, COSY, HSQC, and HMBC) data. Among the isolates, beilschmin A (**1**) and α-tocopheryl quinone showed cytotoxic activities with ED_{50} values = 1.21 and 2.67 μg/mL against P-388 cell line *in vitro*, respectively**.** In this congress, the structural elucidation of **1** and **2** and the cytotoxic activities of the isolates will be discussed.

Acknowledgements: This work was supported by a grant (NSC 92-2320-B-127-004) from the National Science Council of the Republic of China.

References: 1. Liao, J.C. (1996) Flora of Taiwan. 2nd edition. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan. Vol. 2: 433-437.

P 350 Benzofurans: a new class of phytoestrogens isolated from *Onobrychis* sp. - Protective effect of the extract of Onobrychis ebenoides in osteoporosis

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Five species of the genus Onobrychis are at the present under chemical investigation. All found to be very rich in isoflavonoids (isoflavons, flavonols, coumestans, pterocarpans) and especially in 2-arylbenzofurans. Previously we reported the isolation and structural determination of three new 2-arylbenzofurans ebenfuran I, II and III from Onobrychis ebenoides, Leguminosae [1]. In a continuing study of the genus, a new benzofuran named ebenfuran IV and sainfuran were also isolated and structurally characterized on the basis of spectroscopic evidence. Their ability to bind to the estrogen receptor together with their capability to promote growth of estrogen-depended MCF7 cells in culture was evaluated. Estrogenic activity for this group of natural compounds haven't been published before. In the course of the present study was also investigated the possible protective effect of the aqueous solution of the methanolic extract of the plant Onobrychis ebenoides, with proven in vitro phytoestrogenic action, on bone mass loss of the ovariectomized (OVX) rat model of osteoporosis.

References: 1. M. Halabalaki, N. Aligiannis, Z. Papoutsi, S. Mitaku, P. Moutsatsou, C. Sekeris, A.L. Skaltsounis, (2000) J. Nat. Prod., 63: 1672- 1674.

New coumarins from the fruits of *Seseli devenyense*

P 351

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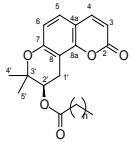
[2] Dept. of Pharmacognosy, Faculty of Pharmacy, Skubiszewski Medicinal University of Lublin, Lublin, Poland



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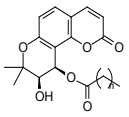
3: n = 8
4: n = 10



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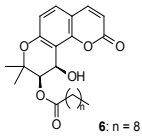

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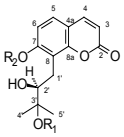

7: $R_1 = R_2 = H$
8: R_1 = glu, R_2 = H
9: $R_1 = H$, R_2 = glu
10: $R_1 = R_2$ = glu

The genus *Seseli* (Apiaceae) is a well known source of the pyranocoumarins. Having as target the investigation of the chemodiversity of the natural pyranocoumarins and their related metabolites, the fruits of Seseli devenyense, have been studied, not previously studied. Eight new coumarins have been isolated and their structures have been established from NMR and MS data and their absolute stereochemistry from chemical correlation reactions.

The new structures are: the decanoic and dodecanoic esters of (+)-Iomatin (3,4), the decanoic esters of (+)-cis-khellactone at positions 4′ (5) and 3′ (6) as well as the 2S epimer of 8-(2,3-dihydroxy-3-methylbutyl)-7-hydroxy-chromen-2-one (7) named devenyol, its two O-monoglucosides at positions 3′ and 7 named devenyoside A (8) and B (9) and the corresponding 3′ and 7 O-diglucoside named devenyoside C (10). This plant is an interesting example of chemodiversity based on biodiversity given that other members of the Apiaceae family produce exclusively the 2R epimers of compounds 7-9.

A new furanocoumarin from *Anethum graveolens*

P 352

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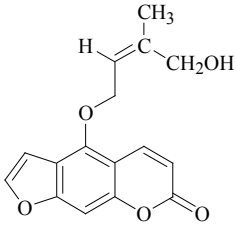

[**1**]

Anethum graveolens (Apiaceae) is a widely used culinary and medicinal herb and is known as dill. This herb is used particularly for its oil in gripe water formulations and is presumably of utility due to its calming effects. During a project to study the antibacterial activity and phytochemistry of Kuwaiti desert plants (**1**,**2**), extracts of *Anethum graveolens* were screened. From the hexane extract the antimicrobial principle falcarindiol was characterised (**3**). Compound **1** was isolated from an inactive fraction and the structure was elucidated by using extensive 1 and 2-dimensional NMR spectroscopy and mass spectrometry. HMBC spectroscopy was used to confirm the position of the prenyloxy substituent at position C-5 and NOESY spectra elucidated the stereochemistry of the prenyl group as *cis*. Compound **1** was assigned as 4″-hydroxyisoimperatorin and is described here for the first time.

Acknowledgement: The University of London School of Pharmacy is thanked for a PhD scholarship to M. Stavri

References: **1.** Stavri, M. et al. (2003) Planta Med. 69: 956-959. **2.** Stavri, M. et al. (2005) Phytochemistry 66: 233-239. **3.** Lechner, D. et al. (2004) Phytochemistry 64: 331-335.

P 353 Phytochemical Analysis and in vitro Evaluation of Estrogenic Activity of *Lathyrus ochrus*

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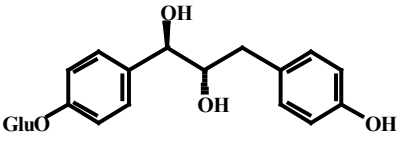
Many plant species of the Leguminosae family are important constituents of Cretan diet (1). The young shoots of *Lathyrus ochrus* (Leguminosae) participate in human diet and its seeds are used in feeding of animals. The aerial parts and seeds were collected in village 'Zaros' of the island 'Crete' and extracted successively with CH_2Cl_2, MeOH and H_2O.



1

Metanolic extracts were the most active, when evaluated in vitro in MCF-7 cell line by E-screen test. Phytochemical investigation of extract obtained from aerial parts revealed the presence of seven glycosides of the flavonols isorhamnetin and quercetin and the new natural product lathyrocroside [1]. On the other hand, two phenolic derivatives (p-hydoxybenzoic acid and its 4-O-β-D-glucopyranoside), roseoside (an ionone glucoside), 3-O-β-D-glucopyranosyl-β-sitosterol and esters of glycerol were found to be constituents of the methanolic extract obtained from seeds. The evaluation of all fractions obtained from above mentioned extracts by VLC chromatography, showed that flavonoids are mainly responsible for the increase of proliferation of MCF-7 cells.

References: 1. Kafatos, A. et al. (2000) J. Amer. Dietetic Assoc. 100:1487-1493.

P 354 Phytochemical Investigation of *Trifolium uniflorum*

S. Fakas, M. Halabalaki, N. Aligiannis and A. L. Skaltsounis
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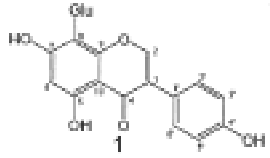
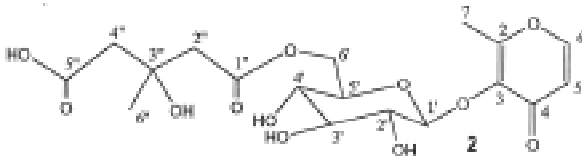
The present study concerns *Trifolium uniflorum* (Leguminosae), which has to date, not been investigated. Past studies of the genus Trifolium have revealed the presence numerous constituents which possess antimicrobial activity many of which belong to the phytoalexins group. The phytoalexin medicarpin, common to Leguminosae family [1] and a standard constituent of the *Trifolium* genus [2] has been isolated. In addition the kaempferol 6''-acetyl-glucopyranoside and its analogous galactopyranoside have been isolated together with the 8-C-genistein glucoside (1) which has been reported to the genus for the first time. Furthermore, simple phenolics, nucleosides as well as a maltose glucoside named licoagroside (2) were isolated. This is the second report of the presence of licoagroside in nature [3]. Lastly, both the methanolic extract and the pure compounds thereof have undergone antimicrobial evaluation and shown interesting activity.



1



2

References: 1. Bolland G., & Donelly D.,(1998), Natural Product Reports, 241-259. **2**. Ingham J., (1990), Biochemical Systematics and Ecology, 18,5:329-343. **3**. Li W., Asada Y. and Yoshikawa T., (2000), Phytochemistry, 55: 447-456.

Inophyllum F, a new coumarin from *Calophyllum inophyllum* grown in French Polynesia

P 355

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[b] JE TRACES and LVCF UMR 6178, Facultés des Sciences de Saint-Jérôme, Case 511, 13397 Marseille Cedex 20, France.
[c] LVCF UMR 6178, Facultés des Sciences de Saint-Jérôme, 13397 Marseille Cedex 20, France.

Calophyllum inophyllum, had been widely used in Polynesian traditional medicine for many centuries (1). Different parts of this plant contain bioactive secondary metabolites such as coumarins (2).
A new compound, named Inophyllum F, had been isolated from *Calophyllum Inophyllum* grown in French Polynesia as a minor component with regard to inophyllums B, P and C obtained from the same source. This compound was detected by normal-phase liquid chromatography – tandem mass spectrometry using a special electrospray source configuration and isolated from leaf extracts by preparative HPLC using UV detection (3). The structure was determined from 1D and 2D NMR techniques, HR-ESI-MS and tandem mass spectrometry.
Inophyllum F contains not only a fused dimethylcyclopropyldihydrofuran ring as well as the rare inophyllums G1 and G2, but in addition a chromanol ring having the three sequential (R, S, S) stereochemistries at the 10, 11 and 12 positions which has been suggested to be mostly responsible for the antiviral activity such as anti-HIV-1 and a potent inhibitor of HIV-1 reverse transcriptase (4,5).

References: 1. P.Pétard (1986) Plantes utiles de Polynésie Raau Tahiti, Édition Haere Po No Tahiti. **2.** Ishikawa, T (2000) Heterocycles, 53, 2 : 453. **3**. Charles, L. et al (2005) J. Mass Spectrom., 40 :75. **4.** Patil, A. D. et al (1993) J. Med. Chem., 36 : 4131. **5.** Sekino, E. et al (2004) J. Org. Chem., 69 : 2760.

Benzocyclic compounds from *Kigelia pinnata*

P 356

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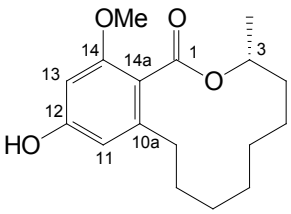
Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, United Kingdom



Kigelia pinnata DC (Bignoniaceae) is native to the drier tropical regions of Africa with several traditional uses, some of which have been investigated (**1**). The fruits have not received much attention as regards their chemistry so dried, ripe fruits of *Kigelia pinnata* were cut, and reduced to a powder. The powder was extracted with dichloromethane using a Soxhlet apparatus. The extract was taken to dryness under reduced pressure and subjected to normal phase flash chromatography with a light petroleum:dichloromethane gradient. The fractions obtained after analysis (TLC with visualisation under UV [254 and 365nm] and with acidic anisaldehyde spray), were grouped into 5 main fractions: B, C, D, E, F. B showed the presence of zones giving an unusual orange colour. A portion of fraction B was chromatographed on a silica gel column using a light petroleum:dichloromethane:methanol gradient.
The compounds giving the orange colour were purified and the two major compounds were identified as 3,4,5,6,7,8,9,10-octahydro-12-hydroxy-14-methoxy-3-methyl-1H-2-benzoxacyclododecin-1-one and 3,4,5,6,7,8,9,10-octahydro-12,14-dihydroxy-3-methyl-1H-2-benzoxacyclododecin-1-one (**2**).These macrolide compounds are more commonly encountered as fungal metabolites.

Acknowledgement: Stiefel Laboratories (Maidenhead) Limited financial support for GM
References: 1 Houghton, P J, 2002, *S. Afr. J. Bot.*, 68:14-20; **2** Aldridge, D C, *et al*, 1971, *J.Chem. Soc. C*, 1623

P 357 New metabolites from a Formosan soft coral *Nephthea chabrolii*

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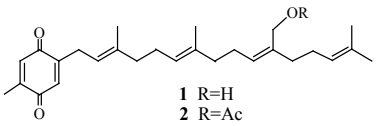
We have previously isolated nine meroditerpenes from a Formosan soft coral *Nephthea chabrolii*.[1] Our further investigation of this soft coral also has led to the discovery of six new meroditerpenes, including three tetraprenyltoluquinone–related metabolites, chabrolobenzoquinones E–G (**1**–**3**), and three tetraprenyltoluquinol–related metabolites, chabrolohydroxybenzoquinones E–G (**4**–**6**). The structures of **1**–**6** were elucidated on the basis of extensive spectroscopic analyses and by comparison of the spectral data with those of the related metabolites. Furthermore, cytotoxicity screening of the above compounds revealed that **6** exhibited 98% inhibition toward the growth of MCF–7 cancer cell line at 20 μg/mL.



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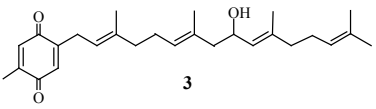

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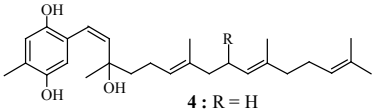
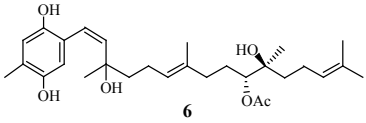


4 : R = H
5 : R = OH



6

References: 1. Sheu J.-H. et al. (2004) J. Nat. Prod. 67: 2048–2052.

P 358 Anthranoyl-substituted norditerpene alkaloids from *Aconitum vulparia* Reichenb.

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Plants of the family Ranunculaceae are rich source of diterpene and norditerpene alkaloids. Many compounds of lycoctonine-type C_{19} alkaloids have shown high toxicity and interesting pharmacological effects, including anti-inflammatory, analgesic and local anesthetic potencies (1). Furthermore, norditerpene alkaloids with N-substituted anthranilate ester side chain, e.g. methyl-lycaconitine, display extreme affinity and high selectivity to the α7 nicotinic acetylcholine receptor (nAChR) subtype, and therefore are prime lead compounds for the design and synthesis of new drugs for the therapy of Alzheimer's disease, epilepsy and schizophrenia (2, 3). In continuation of our studies on the alkaloids of Hungarian *Aconitum* species, we now investigated the alkaloidal constituents of *A. vulparia* Reichenb. The present paper reports the isolation of two new norditerpene alkaloids together with two known ones: septentriodine and delectinine. The alkaloids were obtained from the methanolic extract of the dried whole plants using pH-gradient solvent-solvent partitioning, vacuum liquid-chromatography, preparative TLC and gelfiltration. The structures were established by detailed NMR studies (^{1}H-NMR, ^{1}H-^{1}H COSY, NOESY, JMOD, HSQC and HMBC) and HRMS. The isolated new alkaloids and septentriodine are the members of the small group of norditerpene alkaloids containing N-substituted anthranoyl group. Regarding the structural similarity with methyllycaconitine, these compounds may also be potent ligands of α7 nAChR.

Acknowledgements: This work was supported by the Hungarian National Research Fund Agency (OTKA grant T038390).
References: 1. Bello-Ramyrez, A. M., Nava-Ocampo, A. A. (2004) Fund. Clin. Pharmacol. 18: 699-704. **2.** Hardick, D. J. et al. (1996) J. Med. Chem. 39: 4860-4866. **3.** Barker, D., Brimble, M. A., McLeod, M. D. (2004) Tetrahedron 60: 5953-5963.

Alkaloids from Leucojum vernum and anti-HIV-1 effect of some *Amaryllidaceae alkaloids*

P 359

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[b] Department of Organic Chemistry, University of Szeged, Szeged, Hungary
[c] Johan Béla National Center for Epidemiology, Microbiological Research Group, Budapest, Hungary

Biological screening of Amaryllidaceae species have resulted in the discovery of several new promising antiviral alkaloids. Many compounds have strong inhibitory activities against flaviviruses, bunyaviruses, poliovirus and various oncogenic viruses, and are promising candidates for drug development programmes (1-3). In our previous paper the HIV-1 inhibitory activity of lycorine, haemathamine, trisphaeridine and homolycorine obtained from Leucojum vernum L. and other Amaryllidaceae species were reported (4).
As a part of our ongoing interest in this field, we report here the isolation and antiviral evaluation of further compounds from L. vernum. The alkaloid-containing chloroform extract of the fresh bulbs afforded after multistep chromatography seven alkaloids. The structures were established by HREIMS and advanced 1D and 2D-NMR methods as new galanthamine-type alkaloids, named leucovernine and acetylleucovernine, and the known N-demethylgalanthamine, hippeastrine, 9-O-demethylhomolycorine, 5α-hydroxyhomolycorine and 11-hydroxyvittatine. All compounds are described for the first time from this species.
2-O-Acetyllycorine, 11-hydroxivittatine and N-demethylgalanthamine were tested in vitro for HIV-1 growth inhibitory activity on MT4 human T cell line. First the cytotoxicity was controlled in uninfected cells by means of MTT assays, and then the antiviral activities were determined by means of solid-phase reverse transcriptase test. The results demonstrated that 2-O-acetyllycorine and 11-hydroxivittatine possess remarkable antiretroviral activity.

Acknowledgements: This work was supported by the Hungarian National Research Fund Agency (OTKA grant T038390).
References: 1. Gabrielsen, B. et al. (1992) J. Nat. Prod. 55: 1569-1581 **2.** Ieven, M. et al. (1983) Planta Med. 49: 109-114 **3.** Zee-Cheng, R. K. et al. (1978) J. Med. Chem. 21: 199-203 **4.** Szlávik, L. et al. (2004) Planta Med. 70: 871-873

Alkaloids and amides from *Triclisia sacleuxii* (Pierre) Diels

P 360

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[b] Laboratoire de Biotechnologie Végétale, Faculté des Sciences, ULB C.P. 320, 1160 Bruxelles, Belgium
[c] Centre d' Instrumentation de Résonance Magnétique, Faculté des Sciences, Université ULB, C.P. 160 / 8, 1050 Bruxelles, Belgium

Triclisia sacleuxii (Menispermaceae) is used in traditional medicine to treat kidney, venereal (gonorrhea) and parasitic (schistosomiasis, ascariasis) diseases and as a snakebite antidote. Earlier phytochemical investigations on *Triclisia sp*. showed the presence of bisbenzylisoquinoline alkaloids. These compounds usually display numerous properties among which antiplasmodial, antitrypanosomal and cytotoxic activities. In the search for antiparasitic agents, a phytochemical analysis of *T. sacleuxii* was undertaken by column and thin-layer chromatography. Here we report the first results of this analysis : Four known (phaeanthine, 1,2-dehydroapateline, *N*-methylapateline, *O*-methylcocsoline and one new (gasabiimine or 2H-22,26-Epoxy-1,24:12,15-dietheno-6,10-metheno-16H-pyrido[2',3':17,18][1,10] dioxacycloeicosino [2,3,4-ij] isoquinoline,3,4 dihydro-9,21-dimethoxy-) bisbenzylisoquinoline alkaloids were isolated from the roots, together with two known amides *: cis-trans N* -(4-hydroxyphenethyl) ferulamides from the stems.

Acknowledgements : The authors are grateful to the Coopération Universitaire au Développement (Belgium) for the support through a scholarship granted to S. M. They also thank Mr F. Mbago for plant identification and collection, and Mr C. Moulard for MS measurements.

P 361 A new Pyridium Alkaloid from *Abrus precatorius* seeds

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Abrus precatorius seeds commonly known as "rosary beads" is a widely spread genus of the family (fabaceae). Various preparations of the extracts from the *Abrus* seeds have been used as expectorant, antimicrobial, antimalaria, Abortifacient, anti-inflammatory and oxytocic (1, 2). Previous studies have resulted in the isolation of precatorine, sugar ester of trigonelline, polygalacturonic acid, N-methyl L. tryptophan and some others (3).
We in this study report the isolation and structural elucidation of the aqueous methanol portion of the extract of chlorofom – methanol [2:1], which was fractionated using combination of gel filtration on sephadex LH_{20} eluted and further separated with TLC on silica gel F_{254} in MEOH-$CHCl_3$-NH_3 [8:1:1] and Butanol-Acetic acid-water [65:13:22](4). This afforded I-(N-Methyl nicotinyl)-7-(N-amino-nicotinamido)-D-heptulose (ABS1) and Nicotinic acid Hydrazinamide (ABS2). The structures of the compounds were established by means of spectral (UV, IR, NMR and MS) evidence. Among the isolated compounds ABS1 exhibited anti-trypanosomal activity.

Acknowledgements: Hans-Knoll-Institute for natural product research, Jena, Germany.
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P 362 Amides with Anti-platelet Aggregation Activity from *Piper taiwanense*

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Piper taiwanense Lin & Lu (Piperaceae) is a climber, endemic in Taiwan, and distributed in forests at low to medium altitudes throughout the island (1). The Paiwan aborigines use the leaves, older stems and pistillate inflorescences as material for betel quid, in the same way they use the common *P. betle*. The methanolic extract of *P. taiwanense* showed more potent inhibitory activity on platelet aggregation *in vitro* than any other *Piper* species in Taiwan. Previously, we have reported a new compound, piperolactam E, along with fourteen known compounds, including several anti-platelet aggregation agents (2) from the stem of this plant. Continuing investigation of the minor constituents and the anti-platelet aggregation principles led to the isolation and characterization of five amides, including three new taiwanamides A-C (**1**-**3**), together with two cinnamoylpyrrolidines, 1-cinnamoylpyrrolidine (**4**) and 1-(*m*-methoxycinnamoyl)pyrrolide (**5**). They exhibited inhibitory activity of platelet aggregation *in vitro* and **2**, **3**, **5** owned more potent antiplatelet aggregation activity induced by collagen with IC_{50} values of 17.3, 8.9 and 17.4 μM, respectively.

Acknowledgements: This work was supported by a grant (NSC 89-2314-B-037-044) from the National Science Council of the Republic of China.
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Phytochemistry of *Cordia platythyrsa*

P 363

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In the genus *Cordia* the presence of cordiaquinones (1) and cordiachromes (2) was already described but in our study, from the stem bark of *Cordia platythyrsa* (Boraginaceae), only know compounds were isolated by VLC and successive MPLC on silicagel, as allantoin, widely distributed in the Boraginaceae family, β–sitosterol and its glucoside, balanophonin, precesterol. Moreover, with these compounds, a sphingosine-type shingolipid was identified as (2*S*,3*S*,4*R*,8*E*)-2*N*-[(2′*R*)-2′-hydroxy-tetracosanoyl]-8(*E*)-octadecene-1,3,4-triol (**1**), together with its corresponding β-D-glucopyranoside derivative 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*S*,4*R*,8*E*)-2*N*-[(2′*R*)-2′-hydroxy-tetracosanoyl]-8(*E*)-octadecene-1,3,4-triol (**2**). Their structures were elucidated mainly by 2D NMR techniques (600 MHz, COSY, TOCSY, HSQC, HMBC) and mass spectrometry (FAB-MS). This is the first time that the isolation of sphingolipids and cerebrosides has been reported from *Cordia*.
As sphingolipids and cerebrosides were shown to possess antifungal activities (3), **1** and **2** were tested against *Candida albicans* and *C. glabrata.* However, no significant effect could be found in this bioassay with the two compounds (MIC>200 μg/ml).

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Isolation of an enzyme with catecholoxidase activity of medicinally used *Allium* subgenus *Melanocrommyum* species growing in Central Asia

P 364

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Different species of the genus *Allium* (more than 700 species worldwide) are widely used as spices, vegetables and medicinal plants. About 140 Allium species belong to the subgenus *Melanocrommyum*. All fresh plant parts of some species of this subgenus produce a red dye directly after wounding, which was identified for *A. giganteum* as a dithio-dipyrrole. The formation seems to be catalyzed by an enzyme, which also exhibits a catecholoxidase activity. The now presented investigation included *A. giganteum* Regel, *A. macleanii* Baker, *A. rosenorum* R.M. Fritsch, *A. jesdianum* Boiss. et Buhse, *A. alexeianum* Regel and *A. akaka* Gmelin ex Schult. et Schult. collected in Central Asia. SDS-PAGE of the protein extracts showed species-specific enzyme structures. The enzymes of *A. macleanii* and *A. giganteum* were characterized by two subunits with a molecular weight of approx. 25 and 31 kDa in a 1:1 ratio. Other species like *A. alexeianum*, *A. akaka* or *A. jesdianum* additionally had one, respectively two, smaller protein bands between these two main subunits. The ratios between the subunits differed from species to species. The enzyme of *A. macleanii* was partially purified by using GPC. The native enzyme had a molecular weight of approx. 100-110 kDa. The active fraction had two main features: after incubation of a low-molecular weight extract of the same species, the red dye was formed. It also showed a catecholoxidase activity, a fast reaction with certain ortho-polyphenols, like pyrogallol, dopamine or dopa, as well as a slower reaction with meta-diphenols like resorcinol. The resulting oxidation products were deeply brown. Further purification and structural elucidation of the enzyme is required.

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P 365 Sulphurpyrroles – a new class of substances of medicinally used *Allium* species growing in Central Asia

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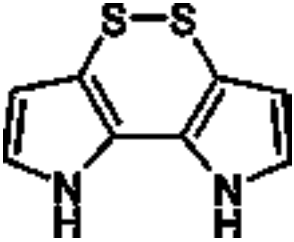

Figure. Chemical structure of the dithio-dipyrrole isolated from *A. giganteum*.

About 200 *Allium* species occur naturally in the mountain areas of Central Asia. A large part of these belong to the subgenus *Melanocrommyum*. Several impressive members of this group like *A. giganteum* Regel, *A. rosenorum* R.M. Fritsch, *A. stipitatum* Regel and *A. macleanii* Baker, the so-called drumstick-onions, are grown as ornamentals in european gardens. However these plants are also collected in natural habitats and used as vegetable and medicinal plants. The use of *A. rosenorum* as greens for soups is very popular in Tajikistan. This species and also related ones used as vegetables exude a red or partially orange ichor after wounding of the green parts of the plant. It was possible to isolate and to characterize the red dye from *A. giganteum* as dithio-dipyrrole (Figure). This compound was only de- tectable in freshly wounded tissue. It was assumed that the formation of the red dye is catalyzed by enzymes. An enzyme consisting of two subunits with molecular weights of 24 kDa and 31 kDa could be isolated and partially purified. Incubation with a low-molecular weight extract of the same plant resulted in the formation of the red dye.

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P 366 Advances in the Chemistry and Bioactivity of Royal Jelly

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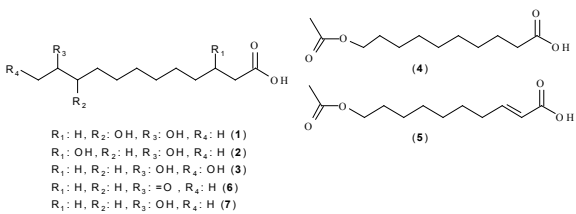

Twenty five compounds were identified, from the dichloromethane and methanol extracts of royal jelly from Greece. Among them, sixteen compounds are reported for the first time as royal jelly constituents, while seven of them are isolated for the first time as natural products. The new seven compounds were fatty acid derivatives: 10(R),11(R)-dihydroxydodecanoic acid (1), 3,11-dihydroxydodecanoic acid (2), 11(S),12-dihydroxydodecanoic acid (3), 10-acetoxydecanoic acid (4), 10-acetoxy-2-decenoic acid (5), 11-oxododecanoic acid (6) and 11(S)-hydroxy-dodecanoic acid(7). The structures of the isolated compounds were determined by spectroscopic methods, 1D, 2D NMR techniques (HMQC, HMBC) and mass spectrometry. The isolated compounds were studied for their antimicrobial activity against six Gram(+) and Gram(-) bacterial strains, two oral pathogens and 3 pathogen fungi. The results of these tests showed interesting and promising antimicrobial activity.

Seed oil composition of *Allophylus natalensis*

P 367

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Seed oils from some species of *Sapindaceae* have been shown to contain acylglycerols (AG) and a rare class of plant lipids, the cyanolipids (CL), derived from the aminoacid leucine (1). Presence of cyanogenic compounds has long been recognized in plants and their insufficient removal from food or forage plants may constitute a health hazard. As a continuation of our studies (2) on *Sapindaceae* plants, we have analyzed the seed oil composition of *Allophylus natalensis* (Sonder) De Winter, commonly known as the "dune false currant"

Soxhlet extraction with petroleum ether gave an oil product which was separated into three main fractions corresponding to 49 % of AG and 51% of CL. CL consisted in 50% of type I cyanolipids (1-cyano-2-hydroxymethylprop-2-en-1-ol diesters) and 1% of type III cyanolipids (1-cyano-2-hydroxymethylprop-1-en-3-ol diesters).

Structural investigation of the oil components was accomplished by chemical, chromatographic and spectroscopic means. Saponification and *tert*-butylation allowed the identification of oleic, gondoic (*cis*-11-eicosenoic), arachidic, *cis*-vaccenic (*cis*-11-octadecenoic) and paullinic (*cis*-13-eicosenoic) as the major fatty acids. Linoleic and linolenic acids were particularly abundant in the AG oil components.

A detailed NMR study on the cyanolipids of type III identified in this plant has also been carried out to improve previous investigations on CL from *Sapindaceae*.

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P 368 Characterisation of *Arabidopsis thaliana* metabolites at the microgram level with microflow LC/NMR, FIA/LC-MS/MS and GC/MS for a metabolomic study

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In plant metabolomic studies the amount of extract is often restricted and compounds responsible for key differences between plants sets might be found only in minute amounts. This is the case for the study of defence-induced compounds in the model plant *Arabidopsis thaliana* (Cruciferae) in which only a few rosette leaf are generally analysed in a given stress experiment. The detection of interesting key metabolome variations can be monitored by metabolite profiling with LC/MS, but the *de novo* characterisation of secondary metabolites of interest cannot rely on this approach alone in the absence of reference substances. The use of on-line complementary hyphenated techniques such as LC/NMR usually provides information on the main constituents of an extract but often fails to analyse efficiently minor constituents. Thus various strategies were developed *off-line* for the characterisation of small amount of metabolites collected at the microgram level based on a microfractionation triggered by LC/MS according to information provided by the metabolomic study. In this respect examples of datasets obtained on HPLC-microfractions with the combined use of a 5µl microflow capillary LC/NMR (CapNMR) , flow injection analysis TOF/ES-MS, ES-MS^n and GC/MS will be discussed.

Acknowledgements: Swiss National Science Foundation is thanked for supporting this work (grant 205320-100396 to JLW).

P 369 Polar constituents from the aerial parts of *Origanum vulgare* L. ssp. *hirtum*, growing wild in Greece and their characterization using the VolSurf procedure. Inhibitory effect on mushroom tyrosinase

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From the polar extracts of *O. vulgare* L. ssp. *hirtum* eighteen compounds were isolated: eriodictyol, apigenin, apigenin 7-O-β-D-glucopyranoside, luteolin, diosmetin, quercetin, chrysoeriol, vicenin-2, caffeic acid, rosmarinic acid, lithospermic acid B, p-menth-3-ene-1,2-diol 1-O-β-D-glucopyranoside, thymoquinol 2-O-β-glucopyranoside, thymoquinol 5-O-β-glucopyranoside, thymoquinol 2,5-O-β-diglucopyranoside, 12-hydroxyjasmonic acid, 12-hydroxyjasmonic acid 12-O-β-D-glucopyranoside and lithospermic acid. Lithospermic acid B, p-menth-3-ene-1,2-diol 1-O-β-D-glucopyranoside, 12-hydroxyjasmonic acid, 12-hydroxyjasmonic acid 12-O-β-D- glucopyranoside were isolated for the first time from *Origanum*. Chemoinformatics tools were applied in order to characterize the isolated compounds. From 3D molecular fields were calculated VolSurf descriptors and successively analyzed by Principal Component Analysis. The results highlight the compound's diversity space and the structural features for possible drug-receptor interactions. The evaluation of tyrosinase inhibitory activity was performed according to the procedure of Kubo et al. (2). Rosmarinic acid and lithospermic acid B were proved the most active.

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Metabolomic study of the effects caused by wounding on *Arabidopsis thaliana* with a rapid LC/TOF-MS analysis method

P 370

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Metabolomic strategies based on LC/MS have been developed in order to obtain the most comprehensive possible survey of the metabolome modifications that occur in the leaves of *A. thaliana* (ecotype Columbia) upon stress induced by wounding. LC/MS methods involving slow gradients on long columns on quadrupole instruments were compared to very rapid methods on short columns with high resolution time-of-flight (TOF) mass spectrometers. The LC/MS data were parsed in the form of filtered ion maps and compared by multivariate analysis after vectorization of the mass spectrometric and chromatographic information. The methods were evaluated for their potential of differentiation of *Arabidopsis* specimens stressed by wounding based on a global metabolome survey. The first results demonstrate that a clear discrimination of the sets of plant specimens is possible. This information was then used for the detection of discrete induced metabolite responsible for a given change. Such a strategy is applied for the non-targeted detection of putative new low molecular mass regulators involved in defense signaling.

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Metabolomics to birch genotype discrimination and pattern recognition

P 371

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Metabolomics is a set of analytical and bioinformatic methods for comprehensive and unbiased identification and quantification of all metabolites present in a biological sample. Metabolites are the end products of cellular regulatory processes and their levels can be regarded as the ultimate response of biological system to genetic and environmental changes. Therefore, subject of plant metabolomics is to define changes in the metabolome due to genetic modification or environmental stress. The main aim of the study was to test the power of metabolomics to woody plants genotype discrimination. Samples of leaves were taken from two genotypes (clones) of *Betula pendula* trees: clone 2 (tolerant to ozone) and clone 5 (sensitive to ozone). Metabolites were extracted, fractionated into polar and lipophilic compounds, transformed to TMS derivatives and quantified with GC-MS. Polar phenolics were analysed with HPLC-DAD. The metabolomic data of individual trees, that included 331 chemical traits, were compared using descriptive statistics, cluster, principle component, and correlation network analyses. Our results showed clearly that different genotypes of birch trees were discriminated. Among the most important metabolites that discriminated the tree genotypes were dammarane triterpenoids and polar phenolics. We assume that some of these secondary metabolites can be used as a biochemical markers associated with tolerance of birch trees to ozone.

P 372 Differentiation of *Hypericum perforatum* (St. John's Wort) lots by NMR based metabonomics

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Hypericum perforatum L. (Clusiaceae), also known as St. John's wort, is one of the best characterized phytotherapeuticals on the market. A broad variety of secondary metabolites with phloroglucinols, flavonoids, and naphtodianthrones as most prominent substance classes have been characterized. Their pharmacological activities have been evaluated thoroughly (1,2) and several HPLC based analytical methods have been developed to identify and quantify the bioactive entities. Within the last years, addressing biological diversity and metabolic time courses have become major topics of NMR based metabonomics (3-5). This methodological approach does combine 1D proton NMR spectroscopy with chemometrical data processing and has been proven as powerful tool to discriminate otherwise indistinguishable biological entities (6,7). Since only a limited number of metabonomics related contributions deal with the biodiversity encountered in secondary metabolite profiles of pharmaceutically used plants (8-10) we decided to investigate *H. perforatum* drug batches of different origin. A principal component analysis (PCA) carried out with 600 MHz 1D ^{1}H-NMR spectra allowed to discriminate the lots unambiguously in the scores plot. The correlation of the obtained differentiation with specific spectral regions was facilitated by the loading plots. Major discriminating metabolites were identified by the aid of 2D NMR experiments and literature data (11). NMR based PCA results were compared with HPLC-DAD/MS assay (12) derived PCA classifications obtained from the identical data set. This strategy allowed a critical comparison of two major analytical concepts addressing the complexity of secondary plant metabolite matrices.

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Supercritical Fluid extraction and interaction with beta-cyclodextrin of kavalactones

P 373

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The powder of Kava-Kava (*Piper methysticum,* G. Forster) and its commercially available extract (CE) are highly cohesive, thus negatively affecting the preparation of pharmaceutical dosage forms. Major components of Kava-Kava extracts are poorly water-soluble lactones having an arylethylene-a-pyrone skeleton (kavalactones). The aim of this work was the preparation of a Kava-Kava extract with supercritical CO_2, and the comparison with the CE in terms of kavalactones contents. To improve the technological and biopharmaceutical properties of kava-kava extracts the interaction with beta-cyclodextrin, as the final step of the supercritical extraction process, was investigated. Supercritical extractions were carried out at four different pressures, ranging from 10 to 40 MPa, and at different temperatures (40 and 60 °C). Kavalactones content was determined by HPLC-DAD. The supercritical extract (40 °C - 20 MPa) was expanded in an aqueous solution of b-cyclodextrin solution (1% w/v). A yellowish precipitate formed and was isolated by filtration. The resulting powder was characterised in terms of kavalactones contents, particle size distribution, Carr Index and repose angle. The highest kavalactones content (630 µg /mg extract) was obtained at 20 MPa, irrespective of temperature, while the content relevant to CE was lower (516 µg/mg). The relative abundance of each kavalactone was similar for CE and supercritical extracts. Supercritical treatment of CE at 60 °C and 40 MPa resulted in an enrichment of kavalactone contents (740 µg /mg). With respect to Kava-Kava powder, the precipitate with beta-cyclodextrin showed smaller particle size (30 and 6.4 µm , respectively) but similar repose angle (47° *vs* 46°) and Carr Index values (21 *vs* 20).

Studies on the Extractability of Devils Claw (*Harpagophytum procumbens*) at elevated Temperatures

P 374

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Devils Claw *(Harpagophytum procumbens)* is an important medical plant in southern Africa and has been established as a phytopharmaceutical in Western medicine over the past 50 years. Marker substance for its anti-inflammatory and antirheumatic effects is harpagoside, the main iridoid glycoside oft the plant (1). Industrial manufacturing processes include extraction, concentration and drying steps which influence the content of the active principle. The objective of this contribution is to examine systematically the role of extraction conditions and drying processes (freeze-, spray drying) on the recovery of harpagoside. In particular the extraction effectiveness at elevated temperatures has been the primary subject of this study. For this purpose static laboratory scale extractions were conducted at temperatures between 0°C and 200°C by means of an Accelerated Solvent Extraction (ASE) device (2). A reversed-phase HPLC method was set up to analyse liquid extracts and dried powders. It turned out that the content of harpagoside in dried extracts was slightly increasing with temperature reaching a maximum at approx. 100 °C. Above 150°C significant thermal degradation of harpagoside became noticeable and the content of degradation products raised. Temperatures above 175°C are unsuitable due to the formation of tar-like precipitates in extraction cells. In contrast spray drying of aqueous extracts did not result in reduced harpagoside yields even at inlet air temperatures higher than 200°C.

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P 375 Vegetable oil extracts of herbal drugs – an analytical approach regarding the transfer of biologically active components

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Vegetable oil extracts of herbal drugs, especially macerations with olive oil or arachis oil, are commonly used in herbal and alternative medicinal products and as ingredients in cosmetics. In order to assess the expected effects and the possible side effects of the oil extracts, we investigated the transfer of biologically active components from the herbal drug into the vegetable oils. As a first approach we studied the transfer of sesquiterpene lactones, essential oils, flavonoids and phenolcarboxylic acids with Arnica flowers (Ph. Eur.) and Matricaria flowers (Ph. Eur.) as exemplary objects. The oil extracts were prepared according to the regulations Nos. 12f (37°C for 7d; "W 10%") and 12d (65°C for 4h, "H 10%") of the German Homoeopathic Pharmacopoeia (HAB 2004) using olive oil (Ph. Eur.).
The sesquiterpene lactones from the Arnica oil extracts were isolated by liquid-liquid-partitioning and the sample was cleaned up using a silica gel column. Quantification of the helenaline and dihydrohelenaline derivatives was then accomplished by HPLC-DAD using santonin as an internal standard (λ=225 nm). 81,2 % (W 10%) and 70,9 % (H 10%) of the sesquiterpene lactones could be recovered in the oil extracts without quantitative discrimination of the individual sesquiterpene lactones.
The transfer of 96,3 % (W 10%) and 91,4 % (H 10%) of the essential oils from Matricaria was determined indirectly by hydrodistillation of the remains of the extracted flowers using a Clevenger-type apparatus (Ph. Eur.). The essential oil patterns of the herbal drug and the oil extracts were compared by means of GC-MS.
The phenolic compounds (flavonoids and phenolcarboxylic acids) from Matricaria were quantified by the method of *Folin-Ciocalteu*. Concerning the flavonoids only the aglycones were found in the oil extracts whereas the glycosides remained in the drug. The aglycone patterns of the flavonoid fractions of the herbal drug and the oil extracts were compared by means of HLPC-DAD (λ=340 nm) and HPLC-MS/MS.

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P 376 Application of adsorption resin technology and fcpc chromatography for the recovery of stilbenoids from grape pomace

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Grape pomace is an agricultural waste produced in huge amounts during the wine making procedure. It is a very rich source of bioactive compounds and especially stilbenoids, phenolic acids and flavonoids. A pilot scale system for the treatment of grape pomace with the aim to recover the contained stilbenoids/polyphenols and reduce the environmental problems was designed and developed. The treatment system consists of four main successive treatment sections: The first section includes the extraction of dried pomace with ethanol and water. The second section includes successive filtration stages that aim at the gradual reduction of the suspended solids. The second section includes the pass of the filtered extract through a series of specialised adsorbent resins in order to achieve the removal/ recovery of the polyphenols and stilbenoids content. The third section aims at the thermal evaporation and recovery of the organic solvents mixture, which has been used in the resins regeneration process, and finally the fourth section aims at the separation of the polyphenols and other contained organic substances using FCPC chromatography. The final outcome of the whole procedure is

- an extract rich in stilbenoids and polyphenols with high antioxidant activity and high added value
- pure resveratrol and viniferin

Dried Extract of *Centella asiatica* for Cosmeceutical and Nutraceutical Applications

P 377

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A solid pharmaceutical dosage formulation using a novel dry plant extract of *Centella asiatica* (CA) (Umbelliferae) is proposed. The botanical evaluation of plant material, through morphological and anatomical diagnosis, is presented. This evaluation permits to identify the herb to be used correctly. The analysis of the most extractive solvent mixture and the attainment of plant extract (fluid and dry) are reported. Several physical characteristics of a novel dry plant extract of CA for direct compression of CA are evaluated. The method described for loading PVP with ECA yields DECA with improved flow and compressibility properties. DECA exhibits satisfactory D_B and D_T values as well as improved angle of repose and compressibility. Release of asiaticoside from DECA was complete within a few minutes. Therefore, the method presented appears to be interesting to process liquid plant extracts and to form a product that can be incorporated as a constituent for cosmeceutical and nutraceutical applications.

Acknowledgements: We appreciate Faculty of Pharmaceutical Sciences and Prince of Songkla University for the finacial support.
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TLC and HPLC methods for determination of principal compounds of *Galeopsis ladanum* and *Galeopsis tetrahit* seeds using different extraction methods

P 378

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It has been described a myopathy outbreak after quail meat ingestion that contained *Galeopsis ladanum* seeds. It was postulated the implication of betaine alkaloids (stachydrine) as the mechanism of toxicity through interaction with homocysteine metabolism (1).
Two extraction methods for *Galeopsis ladanum* and *Galeopsis tetrahit* seeds were compared. The extraction with solvents of increasing polarity (dichloromethane, ethyl acetate and methanol) has been realized by cold maceration at 4ºC. The other extraction method used was by soxhlet. It is necessary to proceed to a previous defatted before the extraction with methanol. Soxhlet is a fast and suitable method for alkaloids extraction but not for other compounds, which degenerate for the heat.
The study of the composition of the two species of *Galeopsis* by TLC and HPLC-UV shows well-known differences. Different flavonoids are described in leaves of both species but not in the seeds (3,4). Principal compounds detected in *Galeopsis ladanum* are phenolic acids and alkaloids (stachydrine and derivates). Nevertheless in *Galeopsis tetrahit* the principal compounds are flavonoids (derivate from luteoline) and alkaloids (in minor proportion than *Galeopsis ladanum*).

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P 379 Biological activities and processing of *Hibiscus sabdariffa* extracts

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Dry calyx extracts of roselle (*Hibiscus sabdariffa* L.), used for health food product and medical herb was investigation the method of extraction and drying methods which showed high biological activities. Four extracts; ethanolic extract[**HSE**], two water extracts with boiling and dry by vacuum dry [**HSV**] , spray dry [**HSS**]and fresh calyx juice and dry by freeze dry [**HSF**]were studied on biological activities such as antioxidant, cytotoxic activity and antimicrobial activity. The antioxidant activity used with DPPH assay (1), cytotoxic assay against five types of cancer cell lines (liver[HepG2], prostate[PC3], lung [CORL23], colon[LS174T] and breast [MCF-7]cancer cell lines) by SRB assay (2)and antimicrobial by disc diffusion and agar dilution method(3). The results found that **HSS** and **HSV** showed high antioxidant activity with DPPH (EC_{50}=11.3 and 15.1μg/ml). All of extracts had no cytotoxic activity against breast, lung and colon cancer but the water extracts showed cytotoxic activity against liver and prostate cancer. **HSS** showed the highest cytotoxic against liver and prostate cancer (IC_{50}=30.1, 47.2 μg/ml respectively). All extracts exhibited great antibacterial against gram positive but no activity against gram negative and fungi . **HS-F** showed the highest antibacterial activity against *S.aureus, S. epidermidis* and *B. subtilis* (9.5,10.6 and 10.1 mm and MIC=5, 5, 5mg/ml respectively). These results concluded that the process of extraction and production method affected the biological activity. The water extracts showed higher biological activities than the ethanolic extract so this result could support used for the process of production roselle extract for health food.

Acknowledgements : National Research Center of Thailand for the financial support.
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P 380 Development of a new process to obtain green tea extract low in caffeine only using water as solvent

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Green tea is a beverage consumed worldwide, associated with longevity and health in Asia and considered a part of wellness lifestyle in the Western world. It contains many polyphenolic compounds and between them the flavanols commonly known as catechins. The most abundant and active catechin is (-)-epigallocatechin-3-gallate. In addition, caffeine is the principal alkaloid and theobromine and theophylline are present in minor content.[1]
Green tea extracts obtained from *Camellia sinensis* leaves have demonstrated significant antioxidant, antiinflammatory, anticarcinogenic, prebiotic and antimicrobial activity as well as stimulation efficacy in several studies. Stimulation effect is provided by caffeine and the other alkaloids and all the other activities are related to catechins.[2,3] Drinks and food products formulated for children and elderly people should not contain stimulating substances[4,5] and non-stimulating green tea is a request to achieve its antioxidant effects without the risks of sleeplessness. It results in an increasing market request for green tea extracts with low caffeine content. Answering to this necessity has been developed a green tea extract with a maximum caffeine content of 0.5%.
In described processes to reduce the caffeine content in natural sources are used organic solvents as methylene chloride, ethyl acetate and carbon dioxide.[6] A new process was developed to obtain a green tea extract with low caffeine content using only water as solvent. The new process consists in a treatment of an aqueous green tea extract with an ionic exchange resin resulting a reduction of caffeine content higher than 95% while maintaining the desired catechins.[7] This innovative development presents a natural and easy to apply alternative to the chemical methods guarantying the production of a suitable ingredient for application in dietary supplements and functional foods.

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Development of new controlled release formulations for delivery of bioactive molecules using DSCand raman spectroscopy

P 381

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Curcuma longa is a ginger-like plant that grows in tropical regions. The roots contain a bright yellow substance (turmeric) that contains curcumin and other curcuminoids that exhibit important anti-inflammatory, antioxidant, antimutagenic and anticarcinogenic activities (1). Drug delivery of lipophilic bioactive molecules is a difficult issue as these drugs demonstrate water insolubility and poor pharmacokinetic properties. The overall goal of this study was to incorporate dimethoxycurcumin (1), a highly lipophilic derivative of curcumin in DPPC (dipalmitoylphosphatidylcholine) lipid bilayers and to study its ability to interact and influence their properties. 1 has been incorporated in DPPC lipid bilayers in various concentrations and its thermal properties have been evaluated using Differential Scanning Calorimetry. Raman spectroscopy was used as well in an attempt to specify the exact location of the interaction of the lipid bilayer with 1. The results show an increase in the fluidity of the membrane as the concentration of the incorporated molecule increases. A decrease in the value of Specific Enthalpy was noticed at increasing encapsulated compound's concentrations while an abolition of the pretransition peak in all concentrations was observed. An increase in the bending of the Carbon-chain and the final methyl group of DPPC was noticed in all concentrations of 1. These results confirm the concentration dependent increase of the fluidity of the lipid membranes noticed at the thermal analysis results and they demonstrate the location of 1 to be at the acyl chains of DPPC and not with the polar group. The incorporation of bioactive molecules in lipid bilayers could lead to the development of new pharmaceutical formulations (i.e. liposomes and dendrimers) with improved properties in order to increase the effectiveness of lipophilic compounds.

References: 1. Hironori Ohtsu et al. (2002) J. Med. Chem. 45, 5037-5042

Preparation of Grapefrute oil concentrate by fractional distillation

P 382

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Grapefruit, *Citrus paradsi* M. (Rutaceae) the tree grows in north and south part of Iran with a hight about 3-5m. It's fruit is globuse, with nipple at apex, mostly big and bright yellow or lemone colored with a mild acid or slightly bitter taste.

Grapefruit oil was obtaind from peel of the fruits by hydrodistillation methed. GC and GC/Mass analysis performed on the sample and computer library and kovates index used to identify the compounds.

d- Limonene (96.11%), β-Myrcene (1.89%), α-Pinene (0.58%) detceted as the major components in the essential oil. 20 fold concentrate was prepared by a fractional distillation process from hydrodistilled oil and analyzed quantitivly by GC and GC/Mass(1). Major and minor constituents were identified by computer library and kovats index. The influence of the concentration process on oxygenated flavour compounds, primarilly aldehydes and alcohols and monoterpene hydrocarbons was evaluated by comparing the results.

d- Limonene content in 20 fold concentrate decreased 21.86%, wherase α-Pinene, Sabinene and β-Myrence were completely removed.

Concentration of decanal and linalool in the 20 fold oil increased 41.96 and 11.94 times respectively.

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P 383 Ex vivo skin permeation of a soy beans dry extract vs pure daidzein end genistein

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Transdermal administration can be considered as a suitable route of administration for genistein (GEN) and daidzein (DAI) because it can assure a prolonged release of active principles. Nevertheless, the ability of GEN and DAI to permeate the skin was scantily investigated.
Aim of this work was a preliminary ex vivo evaluation of the ability of these isoflavones to reach therapeutical steady state plasma concentrations following transdermal administration. The effect of the other components on the skin permeation of GEN and DAI contained in the dry extract was also evaluated by comparing their skin permeability to pure DAI and GEN.
The skin permeation studies were conducted by using modified Franz diffusion cell and the human stratum corneum and epidermis (SCE) as a membrane. Saturated solutions of a dry soy extract containing 11.7% w/w GEN and 12.3% w/w DAI were prepared in water, oleic acid, Labrasol®, Transcutol®, polyetilenglycol 400 (PEG400), and propylene glycol and used as donor phase. The amounts of GEN and DAI permeated through the SCE were not quantificable when water and OA were used as vehicles. The DAI and GEN fluxes determined by using PG resulted lower than those obtained by using PEG400 solutions. The GEN fluxes obtained by using LB and TR were less than 0.1 μg/cm^2/h; in the same solvents, the DAI amount permeated through the skin after 24h was lower than the quantification limit.
PEG400 was the most effective vehicle for both molecules (GEN permeated amount: 31.7 $\pm$ 8.7 μg/cm^2; DAI permeated amount : 23.9 $\pm$ 11.9 μg/cm^2). In the same solvent, the permeated amounts of pure GEN and DAI were 155.7$\pm$ 18.2 μg/cm^2 and 37.3 $\pm$ 16.8 μg/cm^2, respectively. The permeated amounts of pure isoflavones were respectively four-fold and two-fold higher than those of GEN and DAI contained in the extract.
These results cannot be attributed only to the higher solubilities of GEN and DAI in PEG400. Indeed, the Kp of the pure isoflavones resulted one order of magnitude higher than those determined for the dry extract. Thus, the flux reduction can be mainly attributed to the effect of other components of the dry extract which can inhibit and/or compete with the DAI and GEN partition in the stratum corneum.
On the bases of the ex vivo permeation results and the estimated therapeutical plasma concentration only pure GEN seems to be able to reach the therapeutical plasma concentration when administered by transdermal route.

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P 384 Tea tree oil patches: evaluation of oleic acid as skin permeation enhancer

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The antimicrobial, antifungal and anti-inflammatory properties of tea tree oil (TTO), the essential oil of *Melaleuca alternifolia* are well documented. In order to optimize its therapeutical activity TTO patches were designed. Moreover, oleic acid was selected as skin permeation enhancer on the basis of the results obtained by using TTO solutions (1).
The aim of this study was to evaluate the performance of oleic acid as skin permeation enhancer in TTO patches. Terpinen-4-ol, the main component of TTO, was the marker used to evaluate TTO skin permeation. The permeation profiles were determined through human epidermis by using Franz diffusion cells. The samples were assayed by a CGC/FID method. Monolayer patches were prepared by using a methacrylic copolymer, Eudragit E100 (EuE), or a silicon resin, BioPSA7-4602 (BIOPSA). TTO and oleic acid content were 10% w/w and 3% w/w, respectively. Control patches without oleic acid were also prepared. The patches were prepared by casting method.
The terpinen-4-ol content of the four types of patches was not significantly different (349$\pm$16 μg/cm^2). The permeated amount was higher in the case of BIOPSA patches (185$\pm$28 μg/cm^2) then in the case of EuE patches (86$\pm$11μg/cm^2). Furthermore the permeated amount of terpinen-4-ol from the patches containing oleic acid were not significantly different from those of control patches (BIOPSA: 217$\pm$28μg/cm^2; EuE: 98$\pm$21μg/cm^2).
In conclusion, oleic acid did not enhance the skin permeation of terpinen-4-ol contained in TTO patches.

Acknowledgements: This research is financially supported by a grant of MIUR (PRIN 2004).
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Application of Spray drying technology on Mountain tea decoctions-phytochemical investigation of *Sideritis euboea* and *Sideritis clandestina*

P 385

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Mountain tea is a very famous and "popular" beverage in Greece. It is prepared using plants of the genus Sideritis. In Greece there are many local species and subspecies that are used for the preparation of the corresponding mountain tea. Two widely used species are Sideritis euboea (Evia) and Sideritis clandestina subsp.clandestina (Taygetos, Parnon). The present study concerns the application of spray drying technology on mountain tea decoctions as well as the phytochemical investigation of the decoction of Sideritis euboea as the dichloromethane extract of Sideritis clandestina subsp.clandestina. Dried inflorescences of S. euboea were used for the preparation of the decoction. The decoction was submitted to spray drying and the resulting fine powder was submitted to several chromatographic separations. The purified compounds were identified by spectroscopic methods. The decoction of S. euboea was very rich in iridoids (ajugol, 8-acetylajugol) and in flavonoids especially allosides of isoscutellarein, constituents with strong antioxidant activity. Dried inflorescences of S. clandestina were pulverized and extracted successively with dichloromethane, methanol and water. The dichloromethane extract was also submitted to several chromatographic separations. The extracts contained mainly diterpenes that were identified as siderol, α-bisabolol and epoxy linearol.

Incorporation of curcumin derivative in liposomes and dendrimers and physicochemical characterization

P 386

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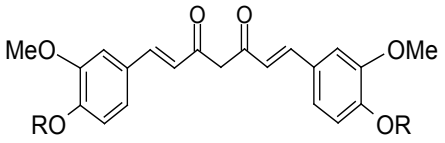
Curcumin (1), a natural phenol found as a major pigment in the Indian spice turmeric (Curcuma longa), possesses anti-inflammatory, anticarcinogenic, and anti-oxidant activity (1). The pharmacological application of lipophilic molecules is limited due to their water insolubility. The overall goal of this study was the dispersion of the lipophilic dimethoxycurcumin (2) in aqueous media using the technology of liposomes and dendrimers. 2 was incorporated in PAMAM dendrimers, generation G3.5 (polyanionic) or G4 (polycationic). Liposomes composed of DPPC (dipalmitoylphosphatidylcholine) and EPC/DPPG (9:0.1 molar ratio) were prepared using the lipid film hydration method and their physicochemical characteristics (size distribution and ζ-potential) were measured before and after incorporation of 2. The molar ratio of dimethoxycurcumin/lipids and dimethoxycurcumin/dendrimer were estimated by using UV-vis spectroscopy and High Performance Thin Layer Chromatography (HPTLC). The stability of the liposomal formulation was assessed measuring size distribution and ζ-potential, over time. The results showed a PAMAM/2 5:1 and 4:1 molar ratio, for PAMAM G4 and G3.5 respectively, while liposomes showed high incorporation efficiency.



1. R: H Curcumin (diferuloylmethane)
2. R: CH_3 Dimethoxycurcumin

References: 1. Hironori Ohtsu et al. (2002) J. Med. Chem. 45, 5037-5042

P 387 Micelles and mixed micelles with natural tensides to improve artemisinin solubility

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Malaria morbidity and mortality continue to increase across all the world and represent the most important parasitic disease of man. It causes over 1 million deaths, 300 million cases, and an economic loss of US$ 12 billion annually (1). This is largely as a result of the continued use of chloroquine and sulfadoxine-pyrimethamine, despite widespread resistance. Artemisinin represent an interesting molecule to treat multidrug-resistant *Plasmodium falciparum* malaria. It is extracted from the plant qing hao (*Artemisia annua* L. or sweet wormwood), a plant whose dried leaves have been used to treat fevers in China for over 2 millennia (2). However, due to its low solubility both in oil and water, it has a poor oral bioavailability. In order to develop a formulation to use for parenteral administration, micelles were investigated to improve its biopharmaceutical characteristics. In the present investigation the improvement of apparent solubility of artemisinin in the presence of colic acid sodium salt micelles was evaluated using a tenside concentration of 7, 11, 25, 50, 100, 150, 200, and 250 mM. A linear increase of artemisinin solubility was evidenced to reach at the maximum concentration of tenside a value of ca. 0.6 mg/ml, 13 times more than water solubility. In addition, mixed micelles of phosphatydilcholine/sodium dodecyl sulphate (SDS) and phosphatydilcholine/colic acid sodium salt were also investigated. The apparent solubility using micelles of phosphatydilcholine (10 mg/ml) and increasing concentration of SDS (from 8.1 to 200 mM) showed again a linear increasing and reached a maximum value of 1.5 mg/ml corresponding to ca. 33 times the value of solubility in water. If mixed micelles were prepared using phosphatydilcholine (10 mg/ml) and increasing concentration of colic acid sodium salt (from 7 to 250 mM) the maximum solubility value was 0.5 mg/ml. These results are encouraging in view of the possibility to use artemisinin for parenteral preparations.

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P 388 Improvement of water solubility and dissolution properties of silymarin by natural and semisynthetic ciclodextrins

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Milk thistle extracts are used for toxic liver damage and supportive treatment in patients with chronic inflammatory liver conditions and hepatic cirrhosis. They contain 40-80% of silymarin which consists of several flavolignans, mainly silibinin and isosilibinin (each of which is a pair of diastereoisomers), silidianin and silicristin (1). Pharmacokinetic studies of silymarin, using silibinin as the tested constituent, evidenced that its bioavailability is generally low and it is strongly affected by the preparation, as was shown for various silymarin products on the market (2). In order to increase the bioavailability of silymarin, inclusion complexes with β-cyclodextrin and two semisynthetic derivatives, namely trimethyl-β-cyclodextrin and dimethyl-β-cyclodextrin were investigated. The phase solubility studies performed using increasing concentrations of cyclodextrins in water (i.e. 10 and 20 mM solutions of β-cyclodextrin and 10, 20, 30, 40 and 50 mM solutions of the two derivatives) evidenced the improvement of water solubility of silymarin in the presence of cyclodextrins. Thus, water solubility of silymarin at room temperature, using silibinin as reference constituent was ca. 1 mg/ml and increased to ca. 3 mg/ml in the presence of β-cyclodextrin 20 mM. Water solubility in 50 mM solutions of both trimethyl-β-cyclodextrin and dimethyl-β-cyclodextrin was ca. 3.5 mg/ml. Complexes (2:1 cyclodextrin-silymarin) prepared using the colyophylization method were investigated by in vitro tests (dissolution rate) using capsules containing 50 mg drug.The results showed a dramatic increase in the dissolution rate of the complex (> 85%) respect to the silymarin (< 35%).

Acknowledgments: This work was supported by MIUR (Ministero Istruzione, Università e Ricerca, Rome, PRIN2004).
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Spectroscopic investigation of the interactions between kavalactones and β-cyclodextrin

P 389

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Kavalactones represent the active constituents of kava-kava (*Piper methysticum* G. Forster), endowed with sedative effect (the use of which was discontinued after cases of hepatic failures) and anesthetic properties (1,2). In order to improve their solubility in water and eventaully their bioavailability if the extracts are readmitted for internal use, we evaluated the effect of β-cyclodextrin (β-CyD) on two main constituents of the extract, kavain (K) and 7,8-dihydrokavain (DHK). Both K and DHK are insoluble in water, and in the presence of β-CyD (10 mM) only DHK enhance its solubility. Thus, binary solution adducts of K/β-CyD and DHK/β-CyD were spectroscopically investigated through circular dichroism (CD) and NMR, and by means of computational tools. It is well known that the inclusion of organic chromophores into cyclodextrin cavities can induce CD bands allied to the electronic transitions of the chromophores. The coliophilized of (*S*)-DHK and β-CyD features a CD spectrum with three bands above 185 nm, which is different from the one of the free (*S*)-DHK, especially in the high energy region, where the transitions of the benzene chromophore are localized. Thus difference CD spectroscopy reveals the association between (*S*)-DHK and β-CyD, moreover it witnesses that the inclusion involves the phenyl ring rather the lactone moiety. The inclusion complexes were characterized by NMR, as well. Their occurrence was immediately revealed by the resonance shifts of both the kavapyrones and β-CyD. Moreover, ROESY features revealed several intermolecular cross peaks, the most intense involved H3 and H5 on β-CyD and the ortho and meta aromatic protons of the kavapyrones for both the substrates. This result confirms the structure of the inclusion complex, with the phenyl ring facing the secondary β-CyD hydroxyl groups. Molecular dynamics simulations (Amber force field), in a water bath confirmed the inclusion determined by CD and NMR. The binary system DHK/β-CyD at 300K relaxes toward a structure with the phenyl ring included in the β-CyD cavity and slightly tilted (15-20°) with respect to the main β-CyD axis.

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Compositions for health products obtained by treatment of tomato with beta-cyclodextrin

P 390

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Lycopene is a natural occurring carotenoid which is present in amounts higher then carotene in the human body. Several beneficial effect for human health have been attributed to diet supplementation with lycopene such as antioxidant effects, and protection against cancer. The main source of lycopene is tomato, which contains also a number of compounds with a recognized activity as radical scavengers. Powdered tomato extracts to use in nutraceutical products are obtained by extraction of fresh tomato with different organic solvents and added with inert excipients such as maltodextrins. The use of organic solvents is needed to increase the amount of extracted lycopene, which is high lipophilic. Our goal is to produce a powdered tomato extract that is stable, useful as bulk material to produce compositions for oral or topical administration, and allowing good bioavailability by using a solvent-free procedure. The approach employs treatment of tomato with beta-cyclodextrin (CD), which is regarded as a safe additive for pharmaceutical and food industry. The paste is dried by spray-drying to obtain a free-flowing powder. We demonstrate that CD-containing tomato powders have good flow properties, which allows their use in oral solid dosage forms, and show lycopene contents and chemical antioxidant activities higher than those of powders without CD.

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P 391 Oleo-gel-matrix. A possibility to reduce adverse effects caused by peppermint oil in the treatment of IBS

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The historical use of peppermint (*Mentha x piperita* L.) is not dramatically different to its use in modern herbal medicine. Classified as a carminative herb, peppermint has been used as a general digestive aid and in the treatment of intestinal spasms. Several studies demonstrated the safety and efficacy of peppermint oil in adults and children with irritable bowel syndrome (IBS)(1)**.** This in-vitro study evaluated the effect of different peppermint oil formulations which can cause side-effects like burning and cold sensation in the anal region. There were controversial reports on this subject. Several clinical trials showed that peppermint oil reduced the pain associated with intestinal spasms, commonly experienced in IBS. Some patients using peppermint oil capsules reported oesophageal reflux, burning and cold sensation in the rectum. Delayed release mechanisms have partially overcome these effects. The mucosa irritation properties of the different formulations were investigated by the HET-CAM assay (2). As an intermediate in vivo/in vitro system, it is not considered an animal experiment and can serve as a source of information to avoid side effects at the stage of formula development. The results showed that 100 % peppermint oil caused very strong irritation within the shortest time. These effects were also seen with formulations using only vegetable oils for dilution and peppermint oil concentrations higher than 30 %. With the oleo-gel-matrix formulations the same irritation effects started at peppermint oil concentrations higher than 60%. These data show that oleo-gel-matrix formulations are able to provide much higher levels of peppermint oil than standard capsule formulations to the bowel without provoking mucosal irritation. The results support observations from clinical studies , where products with oleo-gel-matrix formulations (Colpermin®, Medacalm®) have fewer reports of burning symptoms(3).

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P 392 Formulation Development of Cream containing *Artocarpus lakoocha* Extract and its Stability Evaluation

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Inhibition effect of the boiling water extract of heartwood of *Artocarpus lakoocha* on tyrosinase activity was examined. The extract (3 mg/ml) showed potent inhibition against tyrosinase which converts dopa to dopachrome in the melanin biosynthesis. The percent inhibition of in vitro assay showed to be 84.9%. The major component in the extract was identified as 2,4,3′,5′ tetrahydroxystilbene using chromatographic and NMR techniques. Since these results suggested that the water extract of *A. lakoocha* might be used as a whitening agent for skin, the extract was then formulated into creams and evaluated for both physical and chemical stabilities. A cream preparation of 2 % of *A. lakoocha* extract in cream base containing Sepigel, Lanol, Paraben concentrate, Sodium metabisulfite and Disodium EDTA appeared to be physically stable under a heating-cooling cycle test (12 cycles). In addition, the TLC-UV analysis of the preparation kept under accelerated conditions (45°C for 1 month) indicated no significant change in the amount of 2,4,3′,5′ tetrahydroxystilbene, suggesting a promising chemical stability of the formulation.

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Relationship between kavain solubility and structural parameters of different micellar systems

P 393

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Kavain is a representative molecule of the structurally related biologically active kavalactones of *Piper methysticum* G. Forster (kava-kava), whose extracts have been used largely in the last twenty years for their sedative properties. However, several cases of severe hepatic failures caused the withdrawal of kava-kava from the market (1). Pharmacological studies have pointed out the possibility to use kava-kava extracts for their local anesthetic properties by direct action on sodium ion channels (2). Kavalactones are lipophilic molecules scarcely soluble in water (<0.01 mg/ml). Surfactants can represent a very useful tool to increase the solubility of drugs forming micelles in water above critical micelle concentration (*CMC*) value (3). Solubilizing power of micelles strongly depends on many parameters: surfactant hydrophobic chain length, electrical nature (ionic or non-ionic) and length of polar head, presence of solute in liquid medium, and so on (4). In this work solubility of kavain in sodium dodecyl sulphate (SDS), sodium laurylethere sulphate (SLES) and octanoyl-6-0-ascorbic acid (ASC-8) micelles have been tested; kavain/micelles molar ratio (that is the average number of kavain molecules entrapped in one micelle) have also been investigated. Solubility power of surfactant toward kavain is SDS>SLES>ASC-8, i.e. 0.9, 0.8 and 0.1 mg/ml ca., respectively. These results are related to geometric properties of micellar complex, as hydrocarbon chain length, hydrocarbon chain volume and surface of polar head: all those properties influence the package of surfactant molecules reducing or increasing the available space between different chains in which kavain molecules are entrapped.

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Artemisinin nanosuspension for intramuscular administration

P 394

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Artemisinin, first isolated in 1972 by Chinese researchers from *Artemisia annua* L., is an antimalarial drug effective against drug-resistant *P. falciparum* strains (1,2). Due to the its low water and oils solubility, only tablets for oral administration and suppositories are marketed today. However, in patient with malaria and vomiting or patients with severe malaria artemisinin formulations for i.m. and i.v. administration are necessary. Nanosuspension are sub-micro colloidal dispersions of pure particles of drug, which are stabilized by surfactants. Different nanosuspension based formulations for oral administration of immunosuppresant and anti-emetic drugs are already marketed and different formulations for oral and i.v. administration are in I, II, III clinical phase studies. The aim of this work was to prepare a nanosuspension formulation of artemisinin, suitable for intramuscular administration. Different formulations were produced by high pressure homogenisation using 1% artemisin and different concentration of Poloxamer 188 or Phospholipon 90 as stabilisers and changing pressure and number of homogenisation cycles. The nanosuspension formulations were characterised in terms of particle size by photon correlation spectroscopy (PCS) and zeta potential. The surface morphology of the different formulations was studied using Scanning electron microscopy (SEM). The physical state of lyophilised or spray dried nanosuspension was characterised by X-ray diffraction and DSC. The in vitro dissolution study of lyophilised or spray dried formulations was compared with that of bulk artemisinin.

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P 395 Sedative norfriedelanes from genetic transformed cultures of the mexican species *Galphimia glauca*

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The Mexican plant *Galphimia glauca* is used locally to treat nervous system disorders. The nor-secotriterpenes galphimine B and its 6-acetoxy derivative are the two major principles with sedative action. We recently identified an additional series of related galphimines from the sedative plant crude extracts (1). The yield of the active compounds is low from wild specimens and varies seasonally, a situation that prompted us to initiate *in vitro* cultures that ensure sustainable and controlled production of these triterpenes. Callus and cell suspension cultures produced low amounts of galphimine B (2). In a recent work we established transformed root cultures of *G. glauca* that synthesized three major norfriedelanes (3). In this work we report the production of nor friedelanes in batch suspension cultures of a hairy root cell line(VYT) and a transformed cell suspension cell line (GgBa) that were grown for 45 and 32 days in B5 medium without hormones and under uniform conditions of agitation, light, and temperature. The title compounds were recovered from biomasses and nutrient media and quantified by HPLC.

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P 396 The Flavonoidal Constituents of *Herniaria nemistemon* and their Antioxidant Activity

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Investigation of *Herniaria* spp.(F.*Caryophyllaceae*) revealed the presence of saponins[(1)] ,coumarins, flavonoids and phenolic acid s[(2&3)] , and its mentioned in folk medicine to speed the healing of ulcers and to cure renal stones[(4)] . *Herniaria* species are represented in Egypt by five species[(5)] , and nothing was reported about the phytoconstituents of *H.nemistemon* , therefore the present work deals with the study of the flavonoidal constituents of the plant and also evaluation their antioxidant activity, using DPPH free radicals. About 2Kg of the aerial parts of *H.nemistemon* were dried, powdered and extracted with pet.ether and then with 70%methanol. The methanolic ext. after partitioner with chloroform, ethyl acetate and n-butanol yielded crude extracts containing flavonoids. The previous extracts were subjected separately to preparative PC.(3MM,15% acetic acid) and the main flavonoidal bands were cut and eluted separately with 90%methanol. The eluted fractions were further purified by using LH_{20} column. The isolated flavonoidal compounds were identified as kaempferol, quercetin-3-0-glucoside-7-0-rhamnoside, vitexin, kaempferol-7-0-rhamnoglucosyl and kaempferol /4 -methyl ether . Their identity were verified by TLC. PC. UV. ^{1}H-NMR and MS analysis. All the isolated flavonoidal compounds showed significant antioxidant activity compared to Trolox (standard antioxidant compound).

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Antimicrobial activities of fractions from aerial parts of *Francoeuria crispa* and *Globularia alypum*

P 397

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Francoeuria crispa Cass. (Compositae) and *Globularia alypum* L. (Globulariaceae) are perennial plants traditionally used in folk medicine of Libya for the treatment of various kinds of infections and inflammations (1).
The methanol extract obtained from aerial part of *G. alypum* by Soxhlet extraction was fractionated using a column chromatography on silica gel into six fractions (petroleum ether, toluene, dichloromethane, ethyl acetate, methanol and water), whereas the fractions of ethanol macerate of *F. crispa* aerial part were obtained using liquid-liquid extraction with chloroform, dichloromethane and water. *In vitro* antimicrobial activity was determined by the broth microdilution method using 96-well microtitre plates (2).
The dichloromethane fractions of *G. alypum* methanol extract showed the highest inhibitory activity against *Bacillus cereus* ATCC 11778 with minimum inhibitory concentrations (MICs) of $\geq 63\ \mu$g/ml. The dichloromethane fraction of ethanol extract from *F. crispa* inhibited *Staphylococcus epidermidis* ATCC 12228 and *Candida albicans* ATCC 10231 with MICs of 16 and 63 μg/ml, respectively.

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Growth Suppression of Hamster Flank Glands by Topical Application of an Extract from "Kwao Keur", *Pueraria mirifica*

P 398

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Hamster flank gland growth is androgenic dependent and a widely used model for the study of topically applied androgens and anti-androgens. A chloroform-methanol extract of "Kwao Keur" (*Pueraria mirifica*) was tested using this model. The extract was applied on one of the paired flank glands leaving other as a control. The results showed that the extract effectively suppressed flank gland growth. The local effect was also indicated by the different growth between treated site (-2.6 %) and untreated site (6.8 %). Acute dermal irritation test of the extract was also conducted according to the Test Guidelines (TG) No. 404 of OECD Guidelines for Testing of Chemicals (1993). Very slight degree of erythema formation was observed in one out of three treated sites on the skin of rabbits. This skin reaction was fully recovered within 24 hours. Since the effect of the extract was localized with low skin irritation, it may be potentially useful for treatment of androgen-dependent skin disorders.

P 399 Cytotoxic and antioxidant compounds from *Dioscorea membranacea*

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Commonly used among ingredients in Thai traditional anticancer preparations, the rhizomes of *Dioscorea membranacea* Pierre (Dioscoreaceae), was found potently cytotoxic and possibly contributed to such a therapeutic effect (1). Bioassay-guild isolation was used separation of its active ingredients and tested cytotoxic activity against three human cancer cell lines, i.e. large cell lung carcinoma (COR-L23), colon cell line (LS-174T) and breast cancer cell line (MCF-7), two normal human keratinocyte cell line (SVK-14) and normal human fibroblast (HF) using the SRB assay (2) and also tested for antioxidant activity with DPPH assay (3). Eight compounds were isolated , they are two novel naphthofuranoxepins (Dioscorealide A[**1**] and B [**2**]), one novel 1,4-phenanthraquinone (Dioscoreanone[**3**]), three steroids(β-sitosterol [**4**], stigmasterol [**5**]and β-D-sitosterol glucoside [**6**]) and two steroid saponins (3-0-α-L-rhamnopyranosyl (1→2)- β-D-glucopyranoside [**7**]and diosgenin 3-0-β-D-glucopyranosyl (1→3)-β-D-glucopyranoside [**8**]). **2**, **3** and **7** showed cytotoxic activity against three cancer cell lines, and **2** showed selective cytotoxic activity against lung and breast cancer but less active to two normal cells (see table). **3** showed the highest antioxidant activity. The structure activity relationships (SAR) were discussed for structure of **1,2** and **7,8.**

Table. IC_{50} values (μM) of cytotoxic compounds against cell lines

compounds	COR –L23	MCF-7	LS-174T	Keratinocytye	Fibroblast
2	5.3	1.7	17.5	145.0	26.3
3	10.2	13.2	35.1	58.2	23.5
7	5.5	4.6	4.0	3.0	4.0

Acknowledgements: Prince of Songkla University, Thailand

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P 400 Cytotoxic compounds of Thai medicinal plants for prostate cancer treatment

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Twelve Thai medicinal plants as the ingredients of a Southern Thai traditional formula for cancer treatment (1) were selected to test cytotoxic activity against prostate cancer cell line (PC3) and a normal human cell line, fibroblast cells (10FS) with SRB assay (2). Two extract (water and ethanolic extract) procedures used were similar to those practiced by Thai traditional doctors . The results found that the ethanolic extracts of five plants showed cytotoxic activity (IC_{50} < 30 μg/ml) against prostate cancer cell line (*Bridelia ovata, Curcuma zedoaria*, *Derris scandens*, *Dioscorea membranacea and Rhinacanthus nasutus*). *Dioscorea membranacea* roots showed cytotoxic activity against prostate cancer cell lines (IC_{50}= 17.55 μg/ml) and less cytotoxic activity against normal cell lines (IC_{50}= 66.05 μg/ml). *Rhinacanthus nasutus* root extract showed the highest cytotoxic activity against PC3 (IC_{50} = 2.1 μg/ml) and its extract also showed high activity against 10FS (IC_{50}=10.1μg/ml). The water extract of all plants exhibited no activity against all types of human cells. Cytotoxic assay guild isolation method was used for isolation eight compounds [dioscorealide A, dioscorealide B, dioscoreanone, stimasterol, β-sitosterol, diosgenin 3-0-α-L-rhamnopyranosyl (1→2)- β-D-glucopyranoside, diosgenin 3-0-β-D-glucopyranosyl (1→3)-β-D-glucopyranoside and β-sitosterol 3-0-β-D-glucopyranoside] from the ethanolic extract of *Dioscorea membranacea*. Among them, dioscoreanone and diosgenin 3-0-α-L-rhamnopyranosyl (1→2)- β-D-glucopyranoside showed high activity against prostate cancer cell line with IC_{50} of 8.14 and 5.88 μM respectively.

Acknowledgements: Prince of Songkhla University for the financial support

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Biological activities of Thai Medicinal Plants Preparation called Benjakul

P 401

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Benjakul[BEN], a Thai traditional medicine preparation,is composed of five plants, *Piper chaba* fruit [PC] , *Piper sarmentosum* root [PS] , *Piper interruptum* stem [PI] , *Plumbago indica* root [PL] *and Zingiber officinale* rhizome [ZO]. From selective interviews of folk doctors in Southern Thailand, it was found that Benjakul was used as the adaptogen drug for cancer patients. (1) These plants and the preparation have been selected to study cytotoxicity activity against three human cancer cell lines, large lung carcinoma (CORL23), two types of prostate cancer cell lines(PC3 and LnCaP) using by SRB assay(2). They were also tested the antioxidant activity by DPPH assay (3).The extract procedures were similar to practice by Thai traditional doctors (ethanolic and water extracts).The water extracts of these plants showed no cytotoxic activity and the ethanolic of plant extract showed cytotoxic against COR L23 and PC3 but no active against LnCaP(see in the table). These results can support using Benjakul to treat cancer patients.

Table. IC_{50} values (μg/ml) against cell lines and antioxidant activity [EC_{50} value (μg/ml)]

Plant extracts	PC	PS	PI	PL	ZO	BEN
CORL23	15.8	32.9	18.4	3.4	7.9	19.8
PC3	19.7	45.8	27.8	9.2	9.9	29.8
LnCaP	>50	>50	>50	>50	>50	>50
Antioxidant (ETOH)	91.6	64.0	>100	10.9	3.8	29.9
Antioxidant (water)	8.6	42.1	21.6	2.5	8.6	14.

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Anti-complement activity and the flavonoids of two cruciferous plants growing in Egypt

P 402

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The study of the flavonoids of the aerial parts of *Carrichtetra annua* (L.) DC and *Farstia aegyptia* Turra. family Cruciferae resulted in the isolation of quercetin, quercetin-3-O-arabinoside, quercetin-3-O-glucoside, the new acylated flavonol: quercetin-3-O-(6-feruloyl-β-glucopyransyl)(1 → 2)-β-arabinopyranoside)-7-O-β-glucopyranoside and quercetin-7-O-arabinosyl-3-O-glucoside from *Carrichtetra annua*. In addition to Isorhmentin, Isorhamnetin-3-O-rhamnosyl-7-O-glucoside and Isorhamnetin-3-O (feruoly1-sophroside)-7-O-rutinoside from *Farsetia aegyptia*. The aqueous alcoholic extracts of *Carrichtetra annua* and the chloroform, ethyl acetate, and butanol fractions obtained from the initial alcoholic extract as well as the isolated new compound (new acylated flavonol) were tested for their influence on the classical (CP) and alternative (AP) pathways of complement-mediated hemolysis. All the extracts showed anti-complement effect but the isolated compound has the strongest effect on both AP and CP pathways.

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P 403 HPLC quantification of columbin in *Sphenocentrum jollyanum* Pierre (Menispermaceae)

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Sphenocentrium jollyanum Pierre (Menispemaseae) is indigenous to Nigeria. The plant has been reported to possess antiviral antimicrobial anti-inflammatory antipyretic activities and of importance in the treatment of cough and constipation (1).Columbin, the major furano diterpene and predominant metabolite in *S. jollyanum* has been previously reported (2).In this study, we report the quantification of this metabolite in various morphological parts at different growing seasons using an HPLC method, with column:RP 18-3179,UV for detection. The highest value of columbin concentration (13.3 ppm) was obtained in the fruit collected in July while the least value was 1.4 pm from the leaf sample collected in December. Results obtained in this study have implications for the time of collection of the various morphological parts of *S. jollyanum* for optimal therapeutic activities.

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P 404 Evaluation of the antidiarrhoeal activity of *Byrsocarpus coccineus*

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Based on its use in traditional African medicine, the antidiarrhoeal activity of the aqueous leaf extract of *Byrsocarpus coccineus,* Connaraceae, (ABC) was evaluated on normal and castor oil-induced intestinal transit, castor oil-induced diarrhoea, enteropooling and gastric emptying. The extract (50,100,200 and 400mg/kg, p.o) produced a significant ($p<0.05$) dose dependent decrease in propulsion in the castor oil-induced intestinal transit in mice. The mean peristaltic index (%) for these doses of ABC, control (distilled water; 10ml/kg, p.o) and morphine (10mg/kg, s.c) were 55.27 ± 1.86; 53.12 ± 3.73; 38.60 ± 3.79; 30.25 ± 1.27; 89.33 ± 5.62 and 20.29 ± 3.38, respectively. The effect of ABC at the highest dose was significantly ($p<0.05$) lower than that of the standard drug. This effect was antagonised by yohimbine (1mg/kg, s.c) but not by isosorbide dinitrate (IDN, 150mg/kg, p.o). At 200mg/kg, ABC produced a significant decrease in propulsion in normal intestinal transit. ABC in a dose dependent manner delayed the onset of diarrhoea, produced a significant decrease in the frequency of defaecation, severity of diarrhoea and protected the mice treated with castor oil. Mean diarrhoea scores were 30.83 ± 1.72; 22.40 ± 1.71; 21.43 ± 1.32; 13.80 ± 0.33; 18.00 ± 3.94 and 7.67 ± 2.41 for control, ABC (50-400mg/kg) and morphine, respectively. This effect was not antagonized by IDN. ABC (400mg/kg) significantly decreased the volume (ml) of intestinal fluid secretion induced by castor oil (0.60 ± 0.23 vs 1.27 ± 0.12 for control). However, there was no significant effect on gastric emptying. The results obtained suggest that ABC possesses antidiarrhoeal activity due to its inhibitory effect on gastrointestinal propulsion, mediated through α_2 – adrenergic system, and also inhibited fluid secretion. Preliminary phytochemical analysis revealed the presence of alkaloids, tannins, saponins, reducing sugar, glycosides and anthraquinones.

Ethnopharmacological Survey of Plants used for Treatment of Schistosomiasis in the Niono District, Mali

P 405

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In Mali the use of traditional medicine is a wide spread phenomenon because of its cultural importance and also as the big majority of the people cannot afford the western drugs or medicines. In the district of Niono, particularly in the Office du Niger area, infections with *Schistosoma mansoni* (causative agent of intestinal schistosomiasis) and *S. haematobium* (causative agent of urinary schistosomiasis) have prevalences of 63% and 52% respectively (1). An ethnopharmacological survey, using a questionnaire, was conducted in the Office du Niger area of the Niono district to determine the knowledge about schistosomiasis and the plants used to treat the disease amongst traditional healers. 40 healers from 21 villages of six different health areas were interviewed. All interviewed healers knew about urinary schistosomiasis, while only six knew about the intestinal form. The majority of the healers reported haematuria as the main symptom of urinary schistosomiasis. 55 plants belonging to 30 families were reported to be used alone against urinary and intestinal schistosomiasis. Nine combinations of plants were reported to be used against urinary schistosomiasis, while three combinations were used against the intestinal form. Most of the remedies to treat both forms of disease were by decoction. *Cissus quadrangularis* L. (Vitaceae) and *Stylosanthes erecta* P. Beauv. (Fabaceae) were the plants most frequently used and were reported for the first time in Mali to be used against schistosomiasis.

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Senna podocarpa, roots – a west african medicinal plant with anti-*n. Gonorrhoeae* activity

P 406

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The leaves and roots of the *Leguminosae – Caesalpinioideae Senna podocarpa* (Guill. & Perr.) Lock, are traditionally used, in West Africa, to treat sexually transmitted diseases. In these countries the leaves of this medicinal plant are also considered a putative laxative that can replace official senna (1).
Previous studies on plants used as anti-infective agents by Guinea-Bissau's traditional medical practitioners showed that an 80% ethanol extract from the roots of the plant presented *in vitro* activity against nine *N. gonorrhoeae* strains with different susceptibilities to penicillin and tetracycline at minimum inhibitory concentration (MIC) of 100 - 200 µg/ml (2).
Hereby were present results of a bioguided study performed with this extract in order to localize the activity and identify the active compounds. Extract liquid-liquid partition showed that diethyl ether fraction was the most active one (MIC = 50 -100 µg/ml). Chrysophanol, physcion, emodin and rhein (major compound) were identified in this fraction, by means of LC-UV-DAD co-chromatography with standards.
Rhein (MIC $\leq$ 6.25 µg/ml) was identified as the compound responsible for the antibiotic activity, exhibited by the ethanol extract and diethyl ether fraction against *N. gonorrhoeae*.

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P 407 Isolation and identification of antimicrobial compounds from the Australian medicinal plant *Eremophila duttonii* (Schrophulariaceae)

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The arid adapted genus *Eremophila* features prominently in the Australian medical ethnobotanical literature with many citations describing traditional uses suggestive of antimicrobial activity, e.g. in the topical treatment of minor wounds, occular and otonasopharangeal complaints (1). In broad based screening programmes examining antibacterial activity of native indigenous medicinal plants, extracts of the species *Eremophila duttonii* have been shown to consistently produce the greatest levels of activity amongst plants studied, both by this group and elsewhere (2;3). The genus is characterised phytochemically by the acumulation of structurally and stereochemically unusual terpenoids, unique to the plant kingdom (1). Here we report on the isolation and identification of three compounds from a petroleum extract of *Eremophila duttonii* exhibiting antibacterial activity against *Staphylococcus aureus* and *Candida albicans*. Active compounds were detected and isolated by a combination of thin layer chromatography bioautography and flash column chromatography. Structural assignments for active compounds were performed using 2-dimensional ^{13}C and ^{1}H NMR spectroscopy. Major active compounds were identified as the serrulatane diterpenes, (serrulat-14-en-7,8,20-triol and serrulat-14-en-3,7,8,20-tetraol) and a novel furanosesquiterpene (11-hydroxy ngaione) exhibiting mild antibacterial activity.

Acknowledgements: Division of Chemistry, University of New England, Dr David Tucker
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P 408 The Relative Identification of *C. officinale* and *L. chuanxiong* by PCR-Mediated Fingerprinting

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The roots of *Cnidium officinale* and *Ligusticum chuanxiong* (Umbelliferae) have been used as herbal medicine known as 'Chuhn-Goong' in Korea. In the Oriental Medicine, it is considered to activate blood and circulate qi, alleviate pain, and to be effective on gynecological diseases.
This study was performed in order to establish the standard identification analysis on *L. chuanxiong* (Korea), *C. officinale*, and *L. chuanxiong* (China) by PCR-mediated fingerprinting. RFLP and RAPD method on nrDNA, ITS and rbcL regions were selected to compare and discriminate the three crude drugs. From the determined sequences of ITS, recognition sites of selected restriction enzymes were mapped. Among them, Alu I and Sac I provided useful molecular markers to distinguish the species of original plants.
In conclusion, especially, the molecular markers developed from PCR-mediated RFLP can be adopted as an identification tool to control the quality of 'Chuhn-Goong' in markets.

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Sedative and Hypnotic Effect of Korean *V. fauriei*

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Valerian has been used for more than 1000 years as an antianxiety agent and sleep aid especially in Europe and China. Also it is distributed in the wild life of entire nation. However, in Korea the employment of the herb is rare.
The essential oil of Korean *V. fauriei* was analyzed with GC-MS and TLC pattern was examined. And the biological activities were examined for sedative, hypnotic, anticonvulsive effects to assess the potential application in clinical treatment.
From the GC-MS analyses of essential oils and transferred essential oils from Korean *V. fauriei* root, approximately 25 and 42 compounds were detected respectively. The water and chloroform fraction of Korean *V. fauriei* significantly extended sleeping time of pentobarbital-induced mice. The convulsion of mice was induced with strychnine, picrotoxin and caffein. The water fraction of Korean *V. fauriei* inhibited strychnine- induced convulsion.
The biological activities as sedation, hypnosis, anticonvulsion of Korean *V. fauriei* extracts could be confirmed. Therefore, Korean *V. fauriei* root can be considered as a potential medicinal source for clinical application to alleviate or treat convulsion, insomnia, and other mental disturbances.

The African Plum: Larvicidal and Antibacterial investigations

P 410

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The plant, *Dacryodes edulis* (African Plum) is used in folkloric medicine in West Africa to treat earache, headache and fever. But its involvement in malaria control had neither been studied nor reported. However, this disease still affects huge number of the world population yearly. Routine preliminary assay to this effect in addition to its reported sensitivity to selected bacteria prompted the present research. Modifications of the methods of Ojewole *et al*., 2000 (1) and the agar dilution method as designed by the National Committee for Clinical Laboratory Standards 1990, were used for larvicidal and antibacterial assays respectively on the extracts and purified fractions. The ethanol extracts (leaf and root) of the plant at 5 and 10 % w/v were separately assayed against the fourth instar larvae of *Anopheles gambiae* incubated for 1, 6, 12 and 24 hours. At 5% w/v, the leaf and the root gave 20 and 10 % LA (Larvicidal activity) respectively, while at 10 % w/v, the leaf recorded 80 % and the root 25 % LA after 24 hours incubation albeit these activities (LA) were lower after 1,6 and 12 hours incubation period. Lethality recorded as percentage of deaths indicated a dose and incubation dependent trend. The purified N-Hexane fraction from the leaf was found to be the most active fraction with 55 and 100 % LA at 5 and 10 % respectively after 24 hours. The gram-positive bacteria, most especially *S. aureus,* were all sensitive to the leaf and root extracts (MIC: 375 µg/mL), while gram negative bacteria especially *k. pneumoniae* was sensitive only to the former (MIC:1.25µg/mL). On purification, the N-hexane (MIC: 187 µg/mL *Vs S. aureus*) against other fractions, concentrated most of the antibacterial activities. These results indicated activity enhancement on purification and a sort of correlation for the two assays used. In addition, the study has revealed the potential of the plant in mosquitoe control and also confirmed it's capability in treating ailments of bacteria origin and hence the justification of its folkloric uses.

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P 411 Ecological, Medicinal and Antibacterial Properties of *Ribes Khorasanicum* as an Endemic Plant Species in North-East of Iran

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Ribes Khorasanicum (Grossulariaceae) was first introduced and described by Assadi and Saghafi in North-East of Iran in1996. This species has longer calyx, pedicels and raceme comparing to *R. meyeri* that is the closest relative. *R. biebersteinii* is another relative of the species distinguished by leaf, sepal and style characters. *R. Khorasanicum* is a shrub with the height of 1.5-2.5 m. Flowering is in the second half of spring and fruit ripening is in the middle of summer. It is an endemic plant distributed locally in alpine area of Irano-Touranian regions in Hezar-Masjed chain mountains in Kalaate Naderi, Khorasan Province of Iran along the altitude of 2400-2700 m.a.s.l. During field work, we found that local people consume the dried fruits to treat blood pressure, cardiac problems and digestion poisoning. Alcoholic extract of the flowers, unripe and ripe fruits were done in the lab. Some pharmaceutical materials were recognized and evaluated in the different plant organs. Effects of alcoholic extract on certain intestinal bacteria viz. *Salmonella spp.*, *Shigella flexneri, Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were studied. The results showed existence of some pharmaceutical materials such as Alkaloids (A), Flavonoids (F), Saponins (S), Tanans (T). The grade of F, S and T were +2, -, +4 in flowers, +4, +1, +1 in unripe fruits and +1, - and +- in ripe fruits, respectively. The concentration of Alkaloid in flowers, unripe and ripe fruits was 5, 38 and 22 mg/lit, respectively. This study revealed that ripe fruit extract has an inhibitory effect on growth of the bacteria mentioned above. The extent of the inhibition was related to the concentration of the extract. More detail will be discussed in the paper.

P 412 Anticonvulsant effect of *Bunium persicum* fruit

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Bunium persicum (Boiss.) B. Fedtsch is a member of the Apiaceae family, which grows just in Iran. For thousands of years, *B. persicum* fruits have been used for edible and medicinal purposes. In Iranian traditional medicine, the fruits of the plant have been used as an antiepileptic remedy. However, there are no scientific reports concerning the central effects of the fruits. The aim of this study was to investigate the possible anticonvulsant activity of *B. persicum* against seizures induced by pentylene tetrazole (PTZ) and maximum electroshock (MES) in mice. In order to do the investigation, the essential oil and aqueous extract obtained form *B. persicum* fruit were tested in different doses and interval times. Besides, the effect of the oil and extract on motor coordination with the rotarod test was studied.
The essential oil protected mice against MES and PTZ induced seizures. The aqueous extract had no effect on the MES induced seizures but it showed an anticonvulsive activity in PTZ model. Some doses of the oil and extract produced sedation and motor impairment.
These results indicate that the oil and extract of *Bunium persicum* fruit may have some anticonvulsant activities.

Antimalarial Activity of *Cichorium intybus*

P 413

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Tropical infections are common place in some countries and most of their populations still rely on the use of plant extracts to combate the ravages of protozoa.
In Iranian traditional medicine, *Cichorium intybus* (chicory) fam. Compositae was used for releive of fever. In this study we evaluate antimalarial activity of chicory against Plasmodium berghei infections in mice (Peter's method). The test procedure was based on suppression of parasitemia compared with that in controls. The mices inoculated intraprituanally with Plasmodium berghei on day 0. Oral doses of plant extracts micronised in normal saline have given 3h later. Same oral dose have given every 24h for further days. Tests have used six concentrations of extracts each using 7 mices. Blood smears prepared and parasitemia determined. Doses 0.07 and 0.1 mg/kg were the most effective and provided 0 of parasitemia in 4th day. Chloroquine was used asa positive control.

In vitro screening of Australian traditional medicinal plants for activity against cancer cell lines

P 414

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We have studied the effects of extracts of various arid zone Eremophila species native to Australia as well as several other native plant species that have played an important role in the pharmacopoeia of Australian aboriginal people. Recent studies have identified Eremophila spp as potential antibacterial and antiviral agents but the current literature has little information concerning anticancer activity. Plant extacts were tested for activity against three common cancer cell lines: ovarian cancer cells C180-13S, prostate cancer cells DU 145 and human melanoma cells MM96L. Activity against these cancer cell lines was compared to cytotoxicity against primary untransformed human foreskin fibroblasts NFF. Ethanolic plant extracts were applied in serial dilution to microtitre 96 well plates seeded with 5000 cells/well. After six days of incubation the Sulfuorhodamine B cell growth assay was performed. Although all plant extracts exhibited substantial general cytotoxicity, an extract of *Eremophila duttonnii* exhibited 26 times higher activity against cancer cell lines as compared to normal cells

P 415 Characterisation and evaluation of antibacterial activity of a new type of essential oil from *Eremophila longifolia*

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Here we report on characterisation of a steam distilled essential oil of the traditional Australian medicinal plant *Eremophila longifolia* by GC-MS. During collection of this species variant populations of *E. longifolia* exibiting unusual morphological variations and an unusual essential oil fraction containing a large proportion of (+)-Menthone, α-terpineol and limonene was discovered growing in a region of Western NSW (Mutawintji National Park). The morphological variations and essential oil compositions reported here have not been previously described in any of the botanical or phytochemical literature pertaining to this species (1,2). Antibacterial MIC values of the oil were determined for *Escherichia coli* and *Staphylococcus aureus* using an agar dilution method (3). MIC values for these species were also obtained for various blends of *E. longifolia aeth.* and Lemon myrtle oil (*Backhousia citriodora aeth.*). The antifungal and antibacterial activity of citral rich lemon myrtle oil is well characterised, citral however is known to produce a sensitisation reaction when applied directly to the skin (3). This reaction has been found to be absent when citral or cirtral rich oils are combined with oils containing α-terpineol. Currently there are efforts to find α-terpineol containing oils that may be blended with lemon myrtle oil without significantly reducing activity (3). Growth inhibition equivalent to 100% lemon myrtle oil was obseved with 1:4 ratio blend of *E. longifolia* and lemon myrtle (MIC: 0.075% $^{v}/_{v}$ for *S. aureus* and 0.150% $^{v}/_{v}$ for *E. coli*).

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P 416 Hypoglycemic and hypolipidemic effects of dry extracts of *Teucrium polium* in normal rats

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Hypoglycemic and hypolipidemic activities of dry extracts of *Teucrium polium* were studied in normal rats. Dry extracts were obtained with spray-drying and liophilisation of 70% ethanolic extract of dried overground parts of *Teucrium polium*, administered in a single intraperitoneal and intragastral dose of 125 mg/kg. The serum glucose concentrations were measured within 24 hours. In the normal rats, both extracts exhibited hypoglycemic activity with significant reduction in the serum glucose concentrations with both intraperitoneal (24.5 %) and (35.5 %) single dose, within 24 hours period. These effects are comparable with the effect exhibited from a intragastral administration of reference antidiabetic drug (Glibeclamid) in a single dose of 2.5 mg/kg (38 % reduction in the serum glucose concentrations). The highest hypoglycemic effect (50 %) was exhibited 8 hours after single intragastral administration of 125 mg/kg spray-dried dry extract. In a oral glucose tolerance test, a single oral administration (intraperitoneal and intragastral) of both dry extracts at dose 125 mg/kg, lowered the serum glucose levels (30 % in average) in normal rats. Dry extracts exhibited no effect on normal serum concentrations of total cholesterol, triglycerides and HDL, both in the normal rats. These results demonstrate that dry extracts of overground parts of herba of *Teucrium polium* have hypoglycemic effect but did not affected lipid status in normal rats.

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Biological and phytochemical studies on *Cissus ibuensis*

P 417

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As parts of our studies on Nigerian plants used in the ethno-medicine, *Cissus ibuensis* Hook(F) (Ampelidaceae) was examined. *C. ibuensis* is a traditional remedy to relieve pain and inflammation, and for wound healing but no reports are present in the literature.
The *n*-BuOH soluble part of the EtOH extract was chromatographed over Si gel column. Fraction A-1 was characterized as rutin. A-2 was fractionated by RP-HPLC to give five main flavonols. Kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1->6)-β-L-rhamnopyranosyl-(1->2)]-β-D-galacto pyrano side (**1**), quercetin 3-*O*-[α-L-rhamnopyranosyl-(1->6)]- β-D-galactopyranoside (**2**), rutin (**3**), kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1->6)]-β–D-galactopyranoside(**4**), kaempferol 3-*O*-[α-L-rhamnopyranosyl-(1->6)]- β-D-glucopyranoside (**5**) were characterized by NMR and MS. The acute toxicity in mice (3.24 g/kg ip) was evaluated (1). The activity - analgesic by acetic acid-induced writhing in mice (2,3), and anti-inflammatory by egg albumin-induced edema in rats - were assayed (4). The extract show significant (5) ($P<0.001$) inhibition - of writhing in mice (45.0% at 50, 72.5% at 100 mg/kg), in comparison to piroxicam (66.7%) and - of edema in rats (52.1% at 50, 68.7% at 100 mg/kg). The observed activity supports the traditional uses.

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Antifungal effects of five herbal extracts compared with Nystatine .An in vitro study

P 418

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From many times ago herbal extracts have been used for curing diseases especially infectious ones.So many researches have been done on antimicrobial effects of Iran's herbs.The results have shown that many of these plants have great antibacterial and antifungal effects.In dental practice and especially periodontology,antimicrobials have so many applications and are used to decrease oral microflora before or after oral surgery.As mouthrinses are widely used by people,the need for better ones with less side effects ,better taste and more effective against resistant bacteria also increases. The aim of this study was to evaluate antifungal effects of 5 herbal extracts on candida albicans which is a well known pathogen of oral mucosa. In this quasi experimental study the antifungal effects of each extract were determined according to the diameter of no growth zone in the culture.The herbal extracts used were: Cinnamomum zeylanicum,Rheum rhaponticum,Althea officinalis,Cuminum cyminum and Querques infectoria.Disk plate technique was used to evaluate antifungal effects of the extracts.At first 10 grs of plant powder was solved in 100cc pure Mehanol and the solvant was placed in the shaker for 24 hrs.Disks containing 2mgs of each extract were prepared and placed in the culture.The diameter of no growth zone was compared with that of Nystatine. Cinnamomum zeylanicum,Rhem rhaponticum and Querques infectoria showed antifungal effects but other extracts did not have any effect.Mann-Whitney analysis was used to compare the effects of extracts with Nystatine.There was no significant difference between antifungal effects of Cinnamomum zeylanicum($P>.05$) ,Rhem rhaponticum($P>.05$) and Querques infectoria($P>.05$) and Nystatine.Control disks were used but none of them showed any effect. As a conclusion,evaluating antimicrobial effects of herbal extracts can lead us to find some new products with great effects and probebly better taste or even cheaper than commercial ones.

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P 419 Ameliorative effects of *Nyctanthus arbortristis* on pyrogallol induced hepatotoxicity and immunosuppression

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Reactive oxygen species are involved in the pathogenesis of several degenerative diseases such as atherosclerosis, cancer and play a vital role in inflammatory processes. Pyrogallol, a strong generator of free radicals induces immuno-suppression besides causing oxidative stress. Leaves of *Nyctanthus arbortristis* Linn. *(*Family: Oleaceae) traditionally used in treatment of hepatic derangements showed anti oxidant activity in preliminary studies. Hence present work investigated its protective effects in hepatotoxicity and immuno-suppression induced by pyrogallol. Wistar rats (170-200 g) were treated with methanolic extract of *Nyctanthus arbortristis* (MNA) at doses of 100, 250 and 500 mg/kg, p.o. and were concomitantly administered pyrogallol 50 mg/kg i.p for 14 days. To assess the cell-mediated response, rats pre-immunized on 7th day with sheep red blood cells (SRBC), were challenged by injecting SRBCs in subplantar region of hind paw on 15th day and an increase in paw volume was recorded after 24, 48 and 72 h. Haemaglutination antibody titer as well as total leucocyte and differential leucocyte count were determined. Pyrogallol also induced hepatotoxicity as evident from the elevated levels of liver marker enzymes in plasma such as ASAT, ALAT, ALP and LDH while liver showed decreased levels of reduced glutathione (GSH) and catalase with an increased lipid peroxidation. The treatment with MNA significantly ($p<0.01$) inhibited suppression of both humoral and cell mediated immunity. It also significantly ($p<0.001$) prevented the alterations in the biochemical levels in pyrogallol-induced hepatotoxicity. The histopathological studies of livers revealed that MNA reduced the severity of the lesions induced by pyrogallol. In conclusion, methanolic extract of *Nyctanthus arbortristis* ameliorated the detrimental effects of pyrogallol induced hepatotoxicity and immunosuppression in a dose dependent manner.

P 420 Oenothein B from *Epilobium angustifolium* L. induced neutral endopeptidase activity in prostate cancer PC-3 cells

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Willow herb (*Epilobium angustifolium* L., *Oenotheraceae*) is used in folk medicine for benign prostate hyperplasia (PBH) and associated problems of micturition. Its use to treat prostatic adenoma also has been mentioned. In the prostate gland neutral endopeptidase (NEP; EC 3.4.24.11) deactivates bombesin, endothelin-1 and calcitonin-gene-related peptide. The lost of NEP activity or expression presumably promotes peptide-mediated cell proliferation and progression of prostate cancer (1). We investigated the influence of Epilobium extract and its main constituent oenothein B on NEP activity in androgen-independent prostate cancer cell (PC-3). An aqueous extract of *Epilobium angustifolium* and oenothein B, a dimeric macrocyclic ellagitannin, were specifically able to induce the neutral endopeptidase (NEP) in prostate cancer cells. Additionally, a weak but statistically significant inhibition of cell proliferation was observed. Simultaneous treatment of the cells with arabinosylcytosine and the extract as well as the oenothein B resulted in an additional enhancement of NEP activity. Taking into account the role of this peptidase in prostate cancer progression, our results might offer a pharmacological explanation for the use of *Epilobium* in folk medicine.

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Tissue culture and Pharmacognostic studies of *Sphenocentrum jollyanum P*ierre (Menispermaceae)

P 421

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Sphenocentrium jollyanum Pierre (Menispemaceae) is indigenous to Nigeria. The plant has been reported to possess antiviral antimicrobial, anti-inflammatory antipyretic activities and of importance in the treatment of cough and constipation (1). Pharmacognostic and microscopic characters revealed the anisocytic and anomocytic type of stomata, non- glandular hair and uniseriate trichome, lifeform type of fibre,prism crystals.Stomata frequency at upper and lower epidermis were 2.29 per mm^2 and 1.56 per mm^2 respectively while stomata index at upper and lower epidermis were 28% and 23 % respectively. An evaluation of the seasonal variation of yield to solvent for various morphological parts revealed that the fruit gave the highest yield [3.42 $\pm$ 0.07] for MeoH extracts for samples collected in July while the lowest yield to solvent is [0.18 $\pm$ 0.01] hexane extracts of the root collected in September. A callus culture for *S.jollyanum* was also established for the first time using modified MS media although attempts to mature to field and green house grown failed. This study is very important in future preparation of pharmacopoeia, monographs as well as for the identification, classification and other taxonomic considerations.

Reference: Burkill,H. M[1995] The useful plants of West Tropical Africa Vol.1 families A-D Royal Botanical Gardens,Kew

Assessment of the essential oil composition of *Tornabenea insularis* Parl. ex Webb grown in Santo Antão, Cape Verde Islands

P 422

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Tornabenea insularis Parl. ex Webb is an endemic *Apiaceae/Umbelliferae* from Cape Verde archipelago. This annual or short perennial shrub grows up to 120cm high and it is found in almost all islands in humid and sub-humid hills, from sea level up to 1000m and also occurring on cultivated areas (1). *T. insularis* is commonly known as "aipo" in São Nicolau and Santo Antão Islands and as "funtcho" in Fogo Island, being used in popular medicine against cough (1). The essential oils from the aerial flowering parts and from the seeds of *T. insularis*, collected in Santo Antão Island, were isolated by hydrodistillation and distillation-extraction and analysed by GC and GC-MS. The yellowish oil was obtained in a yield of 0.67% (v/w) for the aerial parts and of 0,05 % (v/w) for the seeds. Major differences were seen in essential oil composition between the two plant parts. Limonene (12%) and two unidentified compounds, A (b/p 41, *m/z* 220, 10%) and B (b/p 159, *m/z* 220, 28%) were the main components of the essential oil from the aerial flowering parts. These compounds were either not detected (A) or present in a relative amounts $\leq$0.2% (limonene and B) in the seed oil, which was dominated by myristicine (87%).

References: 1. Martins E.S. (1996) Flora de Cabo Verde: Plantas vasculares. 67. *Apiaceae.* Lisboa-Praia, IICT-INIDA.

P 423 Chemical Composition. Antiviral and Antimicrobial Activities of the essential oils of Aster *novi-belgii*, *Solidago canadensis* and *Myoporum laetum* growing in Egypt

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Aster genus has been used for the relief of coughs and as an expectorant and it possesses diuretic, antitumor, antibacterial, antiviral and anti-ulcer activities[1]. The essential oils of several *Solidago* species have been investigated before (2). A number of furanoid sesquiterpenes ketone have been isolated from the essential oils of dfferent *Moporum* species (3). Essential oils of the fresh leaves of *Aster novi-belgii, Solidago canadensis and Myopoum laetum.*were prepared by hydrodistillation methods using Clevenger apparatus. The three essential oils were analyzed by GC/MS. The major components of *Aster* essential oil were β-pinene, (31.52%), β- myrecene (21.16%) and limonene (18.74%), whereas the essential oil of *Solidago* was characterized by β-myrcene (25.59%), limonene (16.48%) and β-phellandrene (11.51%) as major components. GC/MS of the essential oil of *Myporum* revealed the presence of 16 components ten of them were identified by GC/MS and four compounds include the major one could not be identified by their mass spectra and retention times therefore they were isolated and identified by spectroscopic analysis, the major and three minor furanoid sesquiterpene ketone representing the majority of the essential oil (82.30%). The isolated major compound ngaione (79.63%), The antiviral activities of the essential oils were investigated against *Herpes simplex* virus type I (HSV-I). Essential oil of *Myopoum laetum* showed the highest inhibitory effect (80.0 %) than the other two oils. The essential oils of the three plants possessed antimicrobial and antifungal activities. The aim of the present investigation was conducted to study the chemical composition of the essential oils isolated from *Aster novi-belgii*, *Solidago canadensis* aerial parts and *Myoporum laetum* leaves growing in Egypt. Moreover, the examination of antiviral activity *in vitro* against herpes simplex type I (HSV-I) and the antimicrobial activity of the oils.

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P 424 Flavonoids and anti-diabetic activity of *Grewia asiatica* L. leaves

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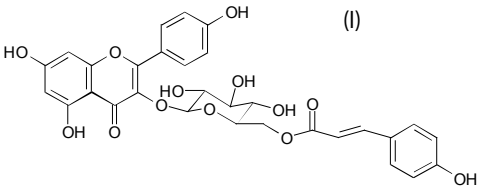

(I)

Grewia asiatica Linn (family Tiliaceae) is known as Phalsa in Hindi. The plant is widely used in traditional medicine for the treatment of various ailments (1, 2). The present work deals with successive extraction of dried powdered leaves of the plant with solvents of increasing polarities. The different extracts were investigated for their anti-diabetic activity in alloxan-induced diabetic rats (3). The obtained data were statistically analyzed using Students't' test. Significant reduction in blood glucose level was observed especially with the ethyl acetate and methanol extracts. Bioactivity guided fractionation led to the isolation and purification of six major flavonoids from the ethyl acetate extract.. They were identified as, isorhamnetin, quercetin, kampferol, isorhamnetin 3-*O*-rhamnoside, kampferol 7- *O*-glucoside and kampferol 3-*O*-(6''-*O*-E-*p*-coumaroyl)-glucoside (I) by determination of their hydrolytic and spectral data including UV, ^{1}H NMR and ^{13}C NMR (4,5).

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Biflavones from *Rhus* species with affinity to the $GABA_A$-benzodiazepine receptor

P 425

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Plant material from three *Rhus* species were collected in South Africa, one of these species, *Rhus pyroides*, is traditionally used in the treatment of epilepsy. From the leaves of *Rhus pyroides* Burch., *Rhus dentata* Thunb. and *Rhus pentheri* A. Zahlbr., six different extracts were made which were tested for activity in the [^{3}H]-flumazenil binding assay. The ethanol extract of *R. pyroides* and the ethyl acetate extracts of *R. dentata* and *R. pentheri* had the highest activity. From the extract of *R. pyroides* two active compounds were isolated by HPLC, they were by structure elucidation (^{1}H-NMR and ^{13}C-NMR) determined to be agathisflavone and amentoflavone. The K_i values were calculated to be 28 nM and 37 nM respectively. The active fraction collected from *R. dentata* contained agathisflavone, apigenin, a triterpene and at least one other unknown compound. The fraction collected from *R. pentheri* contained agathisflavone, apigenin and more than one unknown compound. Apigenin, agathisflavone and amentoflavone were fitted into the pharmacophore model of ligands binding to the $GABA_A$ receptor benzodiazepine site. The fitting into the pharmacophore model reflected the affinity of the compounds in the [^{3}H]-flumazenil binding assay. Apigenin, agathisflavone and amentoflavone were evaluated to be unfit as CNS active drug candidates due to their unspecific pharmacological profiles and their low penetration of the blood-brain barrier.

Stimulation of Insulin Release from Isolated Rat Pancreatic Islets by *Panax ginseng* C.A. Meyer and Its Ginsenosides

P 426

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We determined the ability of aqueous ethanolic extract (AEE-PG), crude saponin fraction (CSF-PG) and several ginsenosides of Panax ginseng (PG) roots, to stimulate insulin release from isolated rat pancreatic islets. In acute 60-min tests, AEE-PG (0.05-0.2 mg/mL), CSF-PG (0.05-0.2 mg/mL) and the ginsenosides (0.002-0.05 mg/mL) significantly ($P<0.01$) evoked a 2- to 20-fold stimulation of insulin release in a dose-dependent manner at 3.3 mM glucose compared to the control. Experiments at different glucose concentrations showed that AEE-PG, CSF-PG and the ginsenosides significantly ($P<0.01$) stimulated on their own whereas they did not potentiate insulin secretion induced by glucose. The extracellular Ca^{2+}-free condition, a L-type Ca^{2+} channel blocker and an ATP-sensitive K^+ channel opener significantly ($P<0.01$) inhibited insulin secretion evoked by PG. Incubations with inhibitors of ATP synthesis showed that the secretagogue action of PG seemed to be independent on an intracellular content of ATP. In conclusion, PG stimulated insulin release from the islets by inhibiting ATP-sensitive K^+ channel activity but not through ATP synthesis.

P 427 Antifungal activity of alkaloid extract of *Erythrina americana* Miller

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The genus *Erythrina* (Leguminoseae) comprises more than 100 species distributed in tropical and subtropical regions of the world (1). These plants produce flavonoids, isoflavonoids and alkaloids with high pharmacological, anti-microbial and anti-inflammatory activities (2). In another hand the fungal diseases are one of the major problems on the production and storage of meals. As part of our investigation of the *Erythrina* alkaloids, we describe the antifungical activity of one alkaloid extract of *E. americana*. The alkaloid extract was obtained from one month age seedlings grew in an controlled environment chamber at 25ºC, relative humidity of 80% and 12 h of light. The seedlings were lyophilized, 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 was done on *Botrytis cinerea*, *Fusarium oxysporum*, *Monilia fructicola*, *Penicillium sp*., *Rhizopus stolonifer* and *Trichoderma sp*., using the disc diffusion method (4). The data showed that *B. cinerea* in dose of 1000 µg L^{-1} the alkaloid extract diminished significantly its growing. At 2000 µg L^{-1} *F. oxysporum*, *M. fructicola* and *Trichoderma sp*. were affected too. Finally the extract at 4000 µg L^{-1} exhibited moderate activity against *Penicillium sp*. and *R. stolonifer*.

Acknowledgements: To the mushroom laboratory of the Phytopathology Academy of the Colegio de Postgraduados by the donation of the fungi.
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P 428 Wound healing activity of *Alocasia odora* (Roxb.) Koch

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Stems of *Alocasia odora* and *A. macrorrhizos* have been used in Vietnamese traditional medicine for the treatment of wounds.(1) While some experimental studies and clinical observations have shown positive effects of *Alocasia macrorrhizos* on wound healing (2), there is still no literature about this activity of *Alocasia odora*. Proliferation and antioxidant assays were used to test the ability of extracts to stimulate growth of human skin fibroblast cells *in vitro,* as well as the ability to protect the same cells from oxidative damage. Neutral Red assay was used to assess cell growth and death. Bioassay results of an active fraction from methanol extract and its four compounds (P3-P6) isolated by HPLC are dose dependent and shown in Table 1.

	Cell proliferation		Antioxidant activity	
Sample	*Concentration, µg/mL**	*% Growth vs Neg.*	*Concentration, µg/mL**	*% Protection vs Neg.*
Total Fraction	50; 100	160;207	12.5; 100	43;100
P3	25;; 100	119;157	100	20
P4	25;; 100	126;358	3.13; 100	53;85
P5	50; 100	129;254	3.13; 100	59;72
P6	100	114	50; 100	38;45

*: Concentration at which the effect of the fraction/compounds was significantly stronger than that of the negative control ($p<0.05$); Neg: Negative control group with the identical conditions as those of the test groups but without test substances
Table 1: Effect on fibroblast growth and antioxidant effect of an active fraction and its compounds from *A. odora*.

Acknowledgements: Vietnamese Ministry of Education and Training; Hanoi Institute of Materia Medica. P K Man, Dr P V Hien,
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Alkaloids from unripe rind of *Citrus reticulata*

P 429

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Besides essential oils and flavonoids, alkaloids are minor components of *Citrus reticulata* peel. Among alkaloids, synephrine (**1**), an indirect beta-sympathicomimetic agent, has been used to lose weight in obese individuals (**2, 3**).The purpose of this study was to extract and isolate major alkaloids from unripe rind of *Citrus reticulata*. Crude alkaloids were extracted with ethyl acetate after alkalizing the powder of *Citrus reticulata* peel with 25% ammonia solution. Crude alkaloids were washed with ethyl acetate many times and crystallized in solvent system methanol: water (1: 3). By comparing spectrum data (MS, NMR) of white crystals obtained with those of reference substances, they were determined to be synephrine, a main alkaloid of *Citrus reticulata* peel. From the mother solution after filtering crude alkaloids above, the second alkaloid was isolated by column chromatography. Elucidation of its structure is in progress.

Acknowledgements: Prof. Peter J. Houghton for checking the abstract.
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Investigation of some Chinese traditional medicines used to treat cancer

P 430

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Five traditional Chinese medicines (TCM) *Illicium verum* Hook f fruits (IV), *Lonicera japonica* Thunb flowers (LJ), *Aristolochia manshuriensis* Kom stem (AM), *Dolichos lablab* L seeds (DL), *Gekko swinhoana* Gunther entire animal (GG)were selected for their reputed anticancer effects (1). In vitro cytotoxicity screening of extracts made with hexane, chloroform, methanol and water was carried out by using the Sulphorhodamine B (SRB) assay (2). Three cancer cell lines, COR-L 23 (Human non-small cell lung cancer, C32 (Human amelanotic melanoma) and HepG2 (Human Caucasian hepatocyte carcinoma,) were used for the primary screening, employing previously-determined optimal plating densities. The greatest cytotoxic activity was shown by the chloroform extracts of IV, LJ and AM with IC_{50} values of 52µg/ml, 24µg/ml and 14µg/ml respectively against COR-L 23 (Table 1). Interestingly, the chloroform extract of IV was selectively cytotoxic for lung cancer with IC_{50} values of 66µg/ml after 72 hours treatment, and has IC_{50} >100µg/ml for MRC-5, a non-cancer human fetal lung fibroblast cell line.

Cell lines	IV	LJ	AM	DL	GG
COR-L23	52±2.74	24±0.63	14±2.28	>100	>100
C32	72±1.99	>100	28±1.28	>100	>100
HepG2	>100	92±1.04	>100	>100	>100

Table 1. IC_{50} values (µg/ml) for chloroform extracts in 72h treatment.

Acknowledgements: Royal Botanic Gardens, Monique Simmonds, Christine Leon for plant identification
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P 431 Bioassay-guided fractionation of Thai medicinal plants used to treat cancer

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Seven Thai medicinal plants traditionally used to treat cancer were selected and extracted, according to traditional methods, to obtain water and alcoholic extracts. The extracts were tested *in vitro* for cytotoxicity using the Sulforhodamine B (SRB) assay to assess cell growth inhibition (**1**) against normal cells (SVK-14, human keratinocyte) and 4 cancer cell lines (COR-L23, large cell lung carcinoma; MCF7, human Caucasian breast adenocarcinoma; C32, human amelanotic melanoma and CACO-2, human colon carcinoma). Of the 7 Thai medicinal plants, an ethanol extract of *Ammannia baccifera* (Family Lythraceae) showed the strongest cytotoxicity against both cancer cell lines and normal cells. It exhibited the lowest IC_{50} against COR-L23 (27.12 ± 1.40 μgmL^{-1} for 48h exposure period and 19.95 ± 0.87 μgmL^{-1} for recovery period). The ethanol extract of *A. baccifera* (30g) was subjected to vacuum liquid column chromatography over silica gel eluted with *n*-hexane: dichloromethane: methanol (step gradient) to obtain 15 fractions. Active fractions were subjected to column chromatography (silica gel eluted with dichloromethane: methanol) followed by multi-preparative thin-layer chromatography. 4-Hydroxy-1-tetralone, 4-acetoxy-1-tetralone and β-sitosterol have been purified and identified using NMR spectroscopy and Mass spectrometry. Work to isolate more pure compounds is in progress.

Acknowledgements: I. Techatanawat thanks the Thai Government Pharmaceutical Organisation for financial support
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P 432 *In-vitro* cytotoxicity of selected Nigerian medicinal plants

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There is only scant literature on the anticancer components of Nigerian medicinal plants.(**1**) This necessitated an ethnobotanical survey of plants commonly used by traditional healers in South-Western Nigeria. 8 of the species identified were extracted with methanol and tested for cytotoxicity using the SRB assay.(**2**) Three cancer cell lines (human breast adenocarcinoma cell line MCF-7, human large cell lung carcinoma cell line CORL-23 and human amelanotic melanoma C32) and one normal cell line (normal human keratinocytes SVK-14) were used. Cytotoxicity was observed in 5 species (*Acanthospermum hispidum, Cajanus cajan, Morinda lucida, Nymphaea lotus* and *Pycnanthus angolensis)* with IC_{50} values shown in the Table. The extract of *C. cajan* was further partitioned with hexane, CH_2Cl_2, $CHCl_3$, ethyl acetate and acetone. The CH_2Cl_2 fraction was found to be most active with IC_{50} values of 10, 10, 12 and 7µg/ml respectively. Work is in progress to identify the active compounds .

Plants	MCF7 $_{(n=2)}$	CORL23 $_{(n=2)}$	C32 $_{(n=2)}$	SVK14 $_{(n=2)}$
A. hispidum	13.50±1.27	8.99±1.05	13.54±0.81	10.25±3.18
C. cajan	14.55±1.20	11.60±2.52	33.07±0.69	25.00±8.48
M. lucida	41.00±4.38	30.10±3.37	43.82±0.52	37.75±10.96
N .lotus	30.10±0.71	47.31±1.88	36.26±2.98	28.50±2.12
P. angolensis	48.45±2.05	28.64±0.57	66.88±0.55	66.00±9.90

Acknowledgement: Commonwealth Scholarship Commission*for financial support for JA*
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Antibacterial and cytotoxic activities of two medicinal plants from Botswana

P 433

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There is an increasing interest in plants as sources of agents to fight microbial diseases and cancer (1) and there is a constant need for new and effective therapeutic agents. Botswana herbal medicine has not been studied extensively (2). Chloroform and water extracts from *Commiphora glandulosa* (Burseraceae) and *Clerodendrum uncinatum* (Verbenaceae) were evaluated for therapeutic potential as antimicrobial and anticancer agents using microdilution assay. Chloroform and water extracts of *C.glandulosa* showed activity against (*Bacillus subtilis* (NCTC 10073), *Staphylococcus aureus (NCIMB 9518)*, *Clostridium perfringens* (NCTC8237) and exhibited cytotoxicity against normal cell line RAW 264.7 macrophages and leukaemia cell line U937 monocytes. Water extract of *C.uncinatum* showed neither antibacterial nor cytotoxic activity. None of the plant extracts showed activity against *Escherichia coli* (NCTC 9002) and *Pseudomonas aeruginosa* (NCIMB 10421), *Klebsiella aerogenes* (NCTC 5055) and *Candida albicans* (NCPF 3179), *Aspergillus fumigatus* (NCPF 7097). Active crude extracts exhibited minimum inhibitory concentrations of 7.8 - 500µg/ml. Active crude extracts yielded IC_{50} values of 15- 47µg/ml in the cytotoxicity assay.

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Two new cassane diterpenenoids with antituberculous activity from *Calliandra californica* Benth. (Fabaceae)

P 434

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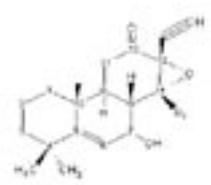
The ethyl acetate extract of *C. californica* was subjected to a bioassay-guided fractionation by different chromatographic columns to give compounds **1** and **2**. Their chemical structure was elucidated by MS, IR, $^{+}$H and ^{13}C NMR. The Minimum Inhibitory Concentration (MIC) of **1** and **2** against *Mycobacterium tuberculosis* H37Rv strain and a clinical isolate that is resistant to Streptomycin, Isoniazid, Rifampin, Ethambutol, and Pyrazinamide was determined by the Alamar Blue Microplate Method. The MIC values for **1** were 25 µg/mL for the H37 Rv strain and 12.5 µg/mL for the clinical isolate and for **2** were 50 µg/mL for the H37Rv strain and 100µg/mL for the clinical isolate.



1: R^1= CH=O **2**: R^1 = CH2-OH

Acknowledgements: 1. Zaida Piñuelas Cota. 2. CONACyT-SIMAC 2000, 3. CYTED. Project X.11:PIBATUB.

Reference: 1. Encarnación-Dimayuga. R.(1992) Medicina Tradicional y Popular de B.C.S. 1ª Ed. Artes Graficas. UABCS. México p.75

P 435 Antibacterial and resistant modifying activity of *Paullinia pinnata*

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In traditional medicine in Ghana, *Paullinia pinnata* (SAPINDACEAE) is used for the treatment of wounds and other microbial infections. Bioassay-guided fractionation of the methanol extract of the roots using an antibacterial assay led to the isolation of five compounds, K1, K2, K3, K9 and K11 which were assessed for their antibacterial activities against different strains of *Staphylococcus aureus* (XU212 (TetK), RN4220 (MsrA) and SA1199B (NorA)) possessing efflux mechanism of resistance (**1**). All had antibacterial effects. Incorporation of K11 in the growth medium at 0.1µg/ml caused an 8-fold (norfloxacin), 256-fold (tetracycline) and 712-fold (erythromycin) potentiation of activities against SA1199B, XU212 and RN4220 respectively (Table 1). Identification of the compounds is underway in our laboratory.

Table1. Antimicrobial susceptibility of test strains in the absence and presence of 0.1µg/ml of K11 and 10 µg/ml of Reserpine (a standard resistance modulator).

Strains	MICs (µg/ml) of antibiotics				
	Tetracycline	Erythromycin	Norfloxacin	+Reserpine	+K11
XU212 (Tet K)	128	-	-	32	0.5
RN4220 (MsrA)	-	256	-	256	0.5
SA-1199B (NorA)	-	-	32	8	4

All MICs were determined in triplicate.

Acknowledgement: Kofi Annan is supported by the Ghana Government.
Reference: 1. Oluwatuyi, M. et. al (2004) Phytochem. **65,** 3249-3254

P 436 *Microsorum scolopendria*, a traditional herbal medicinal Polynesian fern, a rich source of ecdysteroids

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Microsorum scolopendria is a fern belonging to the Polypodiaceae family. Largely widespread in French Polynesia, this plant is one of the most widely used medicinal ferns there. Many traditional remedies include it in their recipes (1, 2). While many medicinal plants have been abandoned to cure people, *M.scolopendria* is still used today, mainly as a vermifuge or purgative for children[1]. As far as we are concerned, no phytochemical investigation of this plant has been reported so far.
Following a bibliographical synthesis (1, 2) as well as a survey among traditional practitioners, we studied the chemical composition of an extract of *M.scolopendria* leaves (extraction, purification by chromatographic methods, identification by N.M.R., M.S., I.R., U.V.). The two main components that we managed to isolate belong to the ecdysteroid family: the ecdysone and the 20-hydroxyecdysone. Ecdysteroids are known to be active agents in the stimulation of protein synthesis and the reduction of blood glucose and cholesterol levels (3).
This result provides us with a good idea of the bioactive molecules contained in this medicinal fern.

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Study of three plants traditionally used in Mali in the treatment of dysmenorrhoea

P 437

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The use of traditional herbal remedies is becoming increasingly popular all over the world. Traditional medicine is a very old practice, commonly encountered not only in the rural area in Africa but also in the towns. Traditional medicine has been described by the World Health Organisation (WHO) as one of the surest means to achieve total health care coverage of the Africa's population. In Mali where more than 80 percent of the population depends upon traditional medicine and medicinal plants for primary health care.The 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.The three plants were selected according to results ethnobotanical literature survey of traditional herbal remedies used to treat dysmenorrhoea in Mali. Bibliography results were validating by traditional healers and herbalist of Bamako. In the present poster is about ethnobotanical information on the three plants: *Maytenus senegalensis* Lam. (Celastraceae), *Stereospermum kunthianum* Cham. (Bignoniaceae) and *Trichilia emetica* Vahl. (Meliaceae).

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Isolation and partial characterization of biological active pectic olysaccharides from Combretum glutinosum Perr. ex DC.

P 438

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In Malian traditional medicine, a water extract of the leaves from *Combretum glutinosum* Perr. Ex DC. (Combretaceae) is used, among other ailments, in the treatment of wounds. The extract is used externally on cuts as disinfectant, and to promote healing. It was therefore of relevance to investigate whether bioactive high molecular weight components could be present in the hot water extract. The complement system plays an important role in the immune system and can thus be an important part of the wound healing process. Polysaccharide polymers in *C. glutinosum* were extracted with water at 50°C, and separated into one neutral and three acidic polymer fractions by anion exchange chromatography. One of the acidic fractions, Cg50S2, was further separated into three fractions by gel filtration, and all were shown to exhibit potent dose-dependent complement fixating activity, when compared to the positive control PMII from *Plantago major*. The monosaccharide composition showed a typical pattern for pectic substances, and linkage analysis confirmed this. To further elucidate the structure of the isolated pectic polymers they were enzymatically degraded using α-L-arabinofuranosidase and β-D-galactosidase from *Aspergillus niger*.
Wound healing is a multifactor process where microbial infections and the formation of free radicals may contribute to retard or inhibit the resolution. The crude water extract were shown to exhibit mild antibacterial activity against Gram-positive bacteria, and a MeOH-extract, DPPH-scavenging activity.

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P 439 Comparative Chemical Composition of *Agastache mex*icana subsp. *mexicana* and *A. mexicana* subsp. *xolocotziana*

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In Central México a medicinal plant complex formed by red, white and blue hyssops is known as "los tres toronjiles" (the three hyssops). This complex is widely used in folk medicine for alleviate nervous disorders (1). Despite the fact that, the red and white hyssops showed important morphologic differences, both were classified as *A. mexicana.* However, it has been proposed that *A. mexicana*, actually is formed by two subspecies: *A. mexicana* (H. B. K.) Lint & Epling subsp. *mexicana* Bye, Linares & Ramamoorthy and *A. mexicana* (H. B. K.) Lint & Epling subsp. *xolocotziana* Bye, Linares & Ramamoorthy (2). In order to support this proposal, we decide to carry out comparative chemical analyses of both subspecies. The results showed that the acacetin and (2-acetyl)-7-0-glucosyl acacetin are the main constituents in both methanol extracts. However, GS-MS analyses showed that methyl chavicol, limonene and linalool were the main constituents of *A. mexicana* subs. *mexicana* essential oils, while pulegone, menthone and isopulegone were the major constituents in those of the subspecies *xolocotziana*. Additionally different chemical compositions were found in their hexane extracts. These chemical dissimilarities between the two taxa support their recognition as distinct subspecies.

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P 440 Antiprotozoal activities of lanaroflavone isolated from *Campnosperma panamense* (Anacardiaceae)

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Activity-guided fractionation of the leaves from *Campnosperma panamense* Standl. (Anacardiaceae), collected near Buenaventura, on the Occidental coast of Colombia, was undertaken as a part of a screening program to evaluate the antiprotozoal activities of tropical underexploited plants. The bioactive biflavonoid lanaroflavone was isolated and identified using spectroscopic and spectrometric methods. Lanaroflavone showed high *in vitro* antiplasmodial activity against K1 resistant strain of *Plasmodium falciparum* (IC_{50} = 0.48 μM) and low cytotoxicity towards L6 cells (1). Complementary studies showed that the mechanism of action underlying the antiplasmodial activity involved strong inhibition of ferriprotoporphyrin IX biomineralization. Furthermore, lanaroflavone showed mild *in vitro* leishmanicidal activity.

Aknowledgements: Agence Universitaire de la Francophonie, Vanessa Douville
References : 1. Weniger et al. (2004) Fitoterapia 75:764

Elaeodendron schlechteranum: biological and phytochemical investigations

P 441

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Elaeodendron schlechteranum (Loes) Loes (Celasteraceae) is widely used in Tanzania by traditional healers. The root decoction is taken orally for treatment of anaemia, female infertility, and cardiovascular problems. A paste from the root is applied on abcesses and carbuncles and the stem/root powder is applied on foul smelling septic wounds. Based on these claims, leaves, stem and root barks were separately collected for investigations. Crude extracts (*n*-hexane, 80% MeOH and H_2O) were prepared separately by maceration / percolation, and screened for antibacterial, antifungal and anti-HIV activities. Root bark extracts and their fractions were further evaluated for antioxidant activity using the DPPH method. Significant antibacterial activity was observed in the *n*-hexane extract against *B.cereus* and *B.subtilis* (MIC 1 – 3 μg/ml), and *S.aureus* (MIC 4 – 20 μg/ml). Crude MeOH and water extracts, EtOAc, *n*-BuOH and H_2O fractions from root bark gave IC_{50} values of 5 - 17 μg/ml against HIV-1 (strain III_B) and 8 - 68 μg/ml against HIV-2 (strain ROD). IC_{50} values ranging from 9 to 47 μg/ml were obtained for the antioxidant activity of the crude MeOH extract, $CHCl_3$ EtOAc, *n*-BuOH and water fractions. The ongoing bioassay-guided isolation from the *n*-hexane extract has led to the isolation of three triterpenoids, viz., 5-glutinene-3,29-diol, tingenin B and cangoronine. Of these, tingenin B exhibited antibacterial activity against *S.aureus* (MIC 0.5 μg/ml). The EtOAc fraction yielded three proanthocyanidins, viz., a mixture of 4'-O-methyl-epigallocatechin (major) and 4'-O-methyl-gallocatechin (minor) possessing a stronger antioxidant activity (IC_{50} = 8.0 μg/ml) compared to Trolox used as a standard (IC_{50} = 12.2 μg/ml), and a dimer identified as gallocatechin-(4α-8)-epigallocatechin-4″,4‴-dimethylether, or 4″,4‴-di-O-methyl-prodelphinidin B_4. This prodelphinidin derivative has not been reported before. The isolation and structure elucidation of further active compounds is in progress.

Neuroprotective coumarins from the roots of *Angelica gigas*: Structure-Activity Relationships

P 442

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We previously reported that coumarin constituents isolated from methylene chloride fraction of roots of *Angelica gigas* Nakai (Umbelliferae) had neuroprotective, acetylcholinesterase inhibitory *in vitro* and anti-amnesic activities *in vivo* (1, 2, 3). In our continuation on the study of neuroprotective compounds from *A. gigas* roots, neuroprotective activity-guided isolation on its *n*-butanol fraction, yielded nine coumarins, nodakenin, marmesinin, columbianetin-O-β-*D*-glucopyranoside, (*S*)-peucedanol-7-O-β-*D*-glucopyranoside, (*S*)-peucedanol-3'-O-β-*D*-glucopyranoside, skimmin, apiosylskimmin, isoapiosylskimmin and magnolioside. Among nine coumarins, three dihydrofuranocoumarins, nodakenin, marmesinin and columbianetin-O-β-*D*-glucopyranoside exhibited significant neuroprotective activities against glutamate-induced toxicity exhibiting cell viability of about 50 % at concentrations ranging from 0.1μM to 10μM. To evaluate and compare neuroprotective activities of coumarins from *A. gigas* roots, activities of sixteen coumarins isolated from methylene chloride fraction we previously reported[1, 2] were also screened simultaneously in the same system. As a result, it was revealed that the formation of cyclized isoprenyl units such as dihydropyran or dihydrofuran or furan ring at C-6 of coumarin and the addition of lipophilicity-enhancing functional groups are important in the neuroprotective activity of coumarins.

Acknowledgements: This work was supported by the Korea Reaserch Foundation Grant (KRF-2003-105-E00216).

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P 443 Bioactive polyphenols from *Euphorbia stenoclada* as inhibitors of human airway smooth muscle proliferation

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In the search for bioactive principles from Malagasy species (1,2), we examined aerial parts of *Euphorbia stenoclada* Baill. (Euphorbiaceae) (ES), a traditional remedy against respiratory diseases. Since an excessive proliferation of the airway smooth muscle (ASM) is reported in asthma, we tested if a crude extract of ES aerial parts had anti-proliferative properties in human airway smooth muscle cells in culture (HASMC). Then, we conducted a bioassay-guided fractionation on ASM to identify their bioactive constituents. HASMC were pre-treated for 1 hour with a hydro-alcoholic (80% EtOH) crude extract or with five fractions (A-E) obtained by RP-flash chromatography. Cell proliferation was induced by IL-1β (10U/ml), a well known mitogen for ASM. Cell proliferation was assessed by the XTT technique after 4 days treatment. The ES crude extract inhibited the IL-1β-induced proliferation of HASMC *(CI_{50} = 0.78 μM)*. The most active fraction (E) containing exclusively flavonoids, totally inhibited HASMC proliferation at 5μg/ml *(CI_{50} = 0.86 μM)*. The later fraction was purified by preparative HPLC to give 6 flavonols. Only one of them was active, inducing a total inhibition of HASMC at 0.5 mg/ml.

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P 444 Phenolic Constituents and Antioxidant Capacity of *Alternanthera tenella* Colla

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The family of Amaranthaceae includes many species, which are used in traditional medicine for the treatment of several diseases (1-3). *Alternanthera tenella* Colla (Gomphreneae tribe, Amaranthaceae) is frequently found in Brazil and has been used for the treatment of fever, infections and inflammation (3, 4). In an initial evaluation the ethanolic extract of *A. tenella* revealed high levels of phenolic compounds as well as high antioxidant activity (Oxygen Radical Absorbance Capacity (ORAC) assay) (5). Subsequent phytochemical investigation of the ethanolic extract resulted in the isolation of a new flavone C-glycoside, acacetin 8-*C*-[α-L-rhamnopyranoyl-(1→2)-β-D-glucopyranoside] (**I**) as well as know flavonoids, including 2″-*O*-α-L-rhamnopyranosyl-vitexin (**II**), 2″-*O*-β-D-glucopyranosyl-vitexin (**III**), vitexin (**IV**), quercetin (**V**) and kaempferol (**VI**). All structures were identified by spectroscopic methods (UV, IR, 1D and 2D NMR and ESI-MS). In addition, total phenolic content of the extract and fractions were measured using the Folin-Ciocalteu reagent (6) and the quantitative analysis of the flavone C-glycosides major constituents has been performed by an HPLC method. Findings from this study demonstrated that the ethanolic extract of *A. tenella* possess antioxidant/free-radical scavenging activity, which seems to be correlated to its total phenolic content. The extract appears to contain, a series of C-glycosyl flavones and apigenin derivatives as major constituents. ORAC assays were performed for flavones **I-IV** and for quercetin, isoquercitrin, chlorogenic acid and caffeic acid as reference compounds. All examined compounds showed relative radical scavenging activity in the range of 0.72 - 5.62 Trolox equivalents. By means of HPLC analysis the concentrations of each compound in the ethanolic extract were calculated as 0.24% w/w for compound **I**, 3.31% w/w for compound **II**, 0.10% w/w for compound **III** and 0.16 w/w for compound **IV**. Antioxidant and quantitative analytical experiments were performed in triplicates (%RSD 0.29 - 6.65%).

Acknowledgements: to FAPESP and to CNPq for financial support and to Professor J. C. Siqueira for identifying the plant material.
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Essential oil of wild *Ocotea quixos* (Lam.) Kosterm. (*Lauraceae*) leaves from Amazonian Ecuador: chemical characterization and biological properties

P 445

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Ocotea quixos is a medium sized tree native to Amazonian Ecuador and neighbouring countries, which is reputed to have known aromatic properties since the period of the Incas but is not well known outside Ecuador (1). Flower calyces and leaves are used by the Amazonian indigenous people as appetizer spices appreciated for their cinnamon-like perfume, but also for eupeptic, disinfectant and local anesthetic activities (2). The essential oil extracted by steam distillation from the leaves of wild *Ocotea quixos*, *Lauraceae* was analyzed by GC and GC-MS. Sixty-one compounds were identified, representing 93.6% of the total detected. The main components were *β*-caryophyllene (15.1%), cinnamylacetate (11.4%), sabinene (7.6%), geranial (5.6%) and *trans*-cinnamaldehyde (5.1%), *α*-pinene (4.4%). Remarkable differences were noted with respect to the flower calyces essential oil of the same plant (3) which reflect a weaker cinnamon-like smell and a pungent woody tone of leaves essential oil. *In vitro* antioxidant properties of the essential oil, obtained by DPPH (1,1-diphenyl-2-picrylhydrazyl) and *β*-carotene bleaching assays, were also evaluated with respect to synthetic antioxidants (trolox® and BHA: buthylhydroxyanisole) and *Thymus vulgaris* essential oil taken as natural reference. The results evidenced a weak activity of the *O. quixos* leaves essential oil (13.6% DPPH inhibition) both in comparison to the synthetic compounds - 28.2% and 86.9% for trolox® and BHA respectively - and thyme essential oil (75.6%). Antimicrobial activity tests are in progress, but preliminary data pointed out weak activity with respect to the flower calyces essential oil both against gram negative and positive bacteria, and yeasts. Finally, the *O. quixos* leaves essential oil showed potential applicative perspectives mainly linked to its delicate scent, and composition, moreover evidenced by the absence of potentially toxic compounds as safrole. Therefore, these data taken as a whole could suggest that the essential oil could find possible practical employ in flavour and cosmetic industry.

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The Effect of GMPE(a Mixture of Glycyrrhizae Radix, Gingered Magnoliae Cortex, Parched Puerariae Radix, and Euphorbiae Radix) on the Suppression of the Formation of AGEs and Sorbitol

P 446

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AGEs and sorbitol accumulation play an important role in the progression of diabetic complications(1). To find the candidates from the herbal medicines, the effectiveness of 200 medicinal herbs and prescriptions was examined *in vitro*. Among them, GMPE had a stronger AGEs formation-inhibiting activity (17.89 μg/ml) than that of aminoguanidine (63.41 μg/ml). In the STZ-induced Diabetes rats, when 500 mg/kg of GMPE was administered, the formation of the AGEs was significantly suppressed in the lens, the sciatic nerve, and the kidney, and the concentration of sorbitol was also significantly decreased in kidney and sciatic nerve ($P<0.05$). This result suggests that GMPE is a good candidate herbal medicine for the prevention of diabetic complications such as diabetic retinopathy, and diabetic nephropathy.

Acknowledgements: The Ministry of Science and Technology, the Korean government, [M 10413010001].

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P 447 Antioxidants from *Lannea velutina,* a medicinal plant from Mali

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In Mali *Lannea velutina* A.Rich (Anacardiaceae) is used against chest pain, gastric ulcer, wound, skin diseases, respiratory tract diseases and fever. In our earlier studies (1) on the antioxidant activities of some medicinal plants of Mali the ethanol and methanol extracts of bark of *L. velutina* showed the highest activities for radical scavenging and 15-lipoxygenase inhibition (IC50<20 µg/ml). In the present work we have evaluated the radical scavenging and 15-LO inhibition of the constituents of the alcohol extracts of the root bark of *L. velutina*. The root bark was collected in Mali in February 2002. Dried pulverized material was extracted successively with petroleum ether, dichloromethane, chloroform; MeOH and EtOH 80 %. The alcohol extracts were purified by column chromatography, and substances were identified by NMR and by degradation with phloroglucinol/HCl. The major components are procyanidins with an average chain length of ca. 10 and epicatechin (major part) and catechin (minor) as monomeric units. Minor amounts of monomers and dimers were isolated, as well. The radical scavenging activity (measured by the reaction with diphenylpicrylhydrazyl, DPPH) and inhibition of 15-LO from soybeans was determined as described previously (1). The procyanidins isolated showed high activities as radical scavengers (IC50<10 µg/ml) and 15-LO inhibitors (IC50<20 µmg/ml).

Acknowledgements: The authors thanks NUFU for financial support.
References: 1. Maiga A et al (2005) *Submitted*.

P 448 *Lithospermum erythrorhizon*, an anti-inflammatory and anti-oxidant Chinese medicinal plant

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Radix Lithospermi (RI), the dry root of *Lithospermum erythrorhizon* (Boraginaceae), is officially listed in the Chinese Pharmacopoeia and is used in traditional Chinese medicine as an anti-inflammatory and antipyretic agent (1). The root's principal components, shikonin and its derivatives, have previously been described as the main antimicrobial principles and the agents responsible for the plant's wound healing and anti-inflammatory effects (2); thus, the aim of our work with this medicinal plant has been to study its anti-oxidant and anti-inflammatory activity. First, the dry roots were exhaustively extracted with MeOH at room temperature. The MeOH extract (M) was successively extracted with *n*-hexane (H), CH_2Cl_2 (D), EtOAc (E), and BuOH (B), and all the extracts were then assayed for antioxidant activity (3). Extracts M, H, D, and E, but not B, exhibited significant antioxidant activity in the DPPH, ABTS and Fe^{2+}/ascorbate/brain homogenate tests, giving similar effects in all the tests with IC_{50} ranges between 113-90 µg/ml in the DPPH test, 24-11 µg/ml in the ABTS test, and 1.2-13 µg/ml in the lipid peroxidation assay. In contrast, none of the extracts showed scavenger activity on the superoxide radical nor did they inhibit xanthine oxidase activity. Fraction D was tested for anti-inflammatory activity (4) and was found to reduce carrageenan-induced paw oedema by 40% (*P< 0.05) and 51% (**P< 0.01), at 1 and 3 h, respectively, but the effect disappeared after 5 h. In the TPA-induced acute ear oedema, extract D (1 mg/ear) reduced the swelling by 82% (**P< 0.01), whereas indomethacin reduced the oedema by 92% at 0.5 mg/ear. These experimental data support the clinical use of RI to treat inflammatory processes.

Acknowledgements: Z.Y. is recipient of a grant from the A.E.C.I. (Spanish Foreign Ministry). This work was supported by the Spanish Government (SAF2002-00723)
References: 1. Tang, W., Eisenbrand, G. (1992) Chinese Drugs of Plant Origin. Springer-Verlag. Berlin. **2.** Papageorgiou, V.P. et al. (1999) Angew. Chem. Int. Ed. 38: 270. **3.** Schinella, G.R. et al. (2002) Life Sci. 70: 1023. **5.** Giner, R.M. et al. (2000) Eur. J. Pharmacol. 389: 243.

Phytochemical Investigation and Estrogenic Activity of *Vitex agnus-castus* Fruits growing in Egypt

P 449

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Chaste tree (*Vitex agnus-castus* Linn.), *Verbenaceae,* is an ornamental shrub or small tree widely distributed in Mediterranean costal region and central Asia, Brummitt(1) It is traditionally used for various medicinal purposes. Halaska, et al.,(2) and Wuttke, et al.,(3). Casticin, vitexilactone, pinnatasterone and 17-OH-progesterone were separated from the chloroformic extract of (chaste tree) fruits their structures were identified on the basis of spectroscopic data. In addition, the hormonal effects of the chloroform extract was investigated in normal and ovariectomized female rats. Significant increase in the score numbers of cornified cells, which were Significant detected by vaginal smear test. Significant change in the body and uterine weight were also observed. The endocrinological activities of the extract were carried out. Significant increase in plasma levels of estrogen and progesterone hormones, while significant reduction in prolactin levels in comparison to normal control and standard estradiol hormone.

References: 1. Brummitt, R.K.(1992). Vascular Plants Families and Genera. Royal Botanic Gadens, Kew. P. 690. **2.** Halaska, H.; Beles, P.; Gorkow, C. and Sieder, C. (1999). Treatment of cyclical mastalgia with a solution containing a Vitex agnus- castus extract: results of a placebo-controlled double-blind study. The Breast, 8: 175-181. **3.**Wuttke, W.; Jarry, H.; Christoffel, V.; Splenger, B. and Seidlova-Wuttke, D. (2003). Chaste tree-pharmacology and clinical indication. Phytomed., 10: 348-357

Screening for Antimicrobial Properties of 147 Extracts of Chinese Medicinal Plants

P 450

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147 *n*-hexane, methanol and water extracts of traditional Chinese herbal drugs, which are used against infectious diseases, were screened for antimicrobial activities. The *in vitro* antibacterial activity of the extracts was tested against representative Gram-negative (*Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 11778, *Bacillus licheniformis* laboratory strain), and against the pathogenic yeast *Candida albicans* ATCC 10231. The *in vitro* activities are reported as zones of inhibition determined by agar diffusion test with a disc potency of 100 µg of tested extract (1). *Staphyloccus aureus* was affected by the methanol extract of Cortex Moutan and the *n*-hexane extract of Radix Salviae miltiorrhizae. *Bacillus cereus* and *Bacillus licheniformis* were affected by the *n*-hexane extracts of Radix Salviae miltiorrhizae and Radix Saussureae lappae. The methanol extract of Semen Phaseoli had a weak effect on *Bacillus licheniformis*. The methanol extracts of Rhizoma Coptidis chinensis, Cortex Moutan, all extracts of Cortex Phellodendri, methanol and water extracts of Radix Sanguisorbae and the n-hexane extracts of Radix Saussureae lappae and Herba Taraxaci showed significant activities against the yeast Candida albicans. No effects were observed against Gram-negative bacteria. Although all this plants are used in TCM for the treatment of infectious diseases, only very few of them showed a direct effect on bacteria. So immunomodulatory effects have to be considered.

References: 1. Größnitzer, E., Feierl,G. et al. (2002) Eur J Pharm Sci 15: 49-61

P 451 Sesquiterpene lactones from *Centipeda minima* with iNOS inhibitory activity

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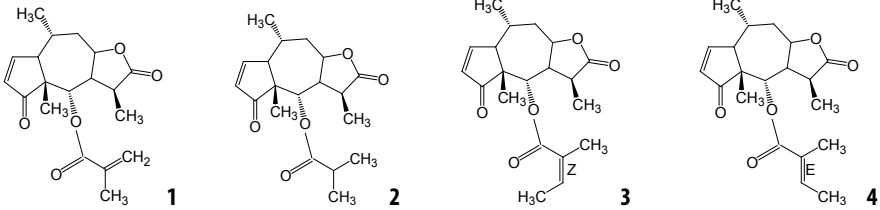
In traditional Chinese medicine *Centipeda minima* (Asteraceae) is used for the treatment of sinus infections. In a screening we tested extracts of the aerial parts of *Centipeda minima* for their effects on inducible nitric oxide synthase (iNOS) in the macrophage cell line RAW 264.7 after induction by interferon-gamma and bacterial lipopolysaccharide. The effect was determined by measuring nitrite release into culture supernatants. The *n*-hexane extract showed an iNOS inhibitory effect of 41.1 % at a concentration of 10 µg/ml. By activity guided fractionation four sesquiterpene lactones, ester derivatives of plenolin (11,13-dihydrohelenalin), were isolated from this extract. They were identified by nmr and ms as 6-O-methylacrylplenolin **1**, 6-O-isobutyroylplenolin **2**, 6-O-angeloylplenolin **3** and 6-O-tigloylplenolin **4**. The latter substance has been isolated from *C. minima* for the first time. Besides 6-O-isobutyroylplenolin, all compounds exhibited strong iNOS inhibitory properties in the low µM range. 6-O-angeloylplenolin was the most active compound. This effect may explain at least part of the anti-inflammatory effect of *Centipeda minima.*



1 2 3 4

References: 1. Baer, H. P., Schmidt, K. et al. (1995) Life Sci 57: 1973-1980

P 452 Acetylcholinesterase Inhibitors from *Stephania venosa* Spreng

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Acetylcholinesterase (AChE) inhibitors have lately gained interest as potential drugs in the treatment of Alzheimer's diseases. From the screening of more than thirty Thai medicinal plants used in Thai traditional rejuvenating and neurotonic remedies, methanolic extract of *Stephania venosa* Spreng. showed high AChE inhibitory activity. We, therefore, aimed at investigation of AChE inhibitors in this plant.
The methanolic extract of the plant was fractionated using column chromatography. The inhibitory activities of the fractions on AChE were determined using Ellman assay on a 96-microplate. The chemical structures of the compounds isolated were elucidated using spectroscopic techniques. The quaternary protoberberine alkaloids i.e. stepharanine, cyclanoline and N-methyl stepholidine expressed inhibitory activity on AChE with the IC_{50} values of 14.10 ± 0.81, 9.29 ± 3.47 and 31.30 ± 3.67 µM respectively while the tertiary protoberberines, stepholidine and coryldamine showed significantly less activity. These data suggest that protoberberine alkaloids from *S. venosa* could be a lead for AChE inhibitors.

Boswellic acid with acetylcholinesterase inhibitory properties from frankincense

P 453

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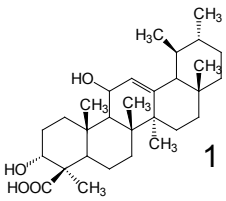

1

Frankincense, the oleo-gum-resin from *Boswellia carterii* and related species, has a reputation as a memory enhancer amongst its many traditional uses. Extract of frankincense displayed inhibition of acetylcholinesterase (AChE) using the Ellman method (1). The methanol extract from frankincense and ethyl acetate-soluble fraction both showed significant AChE inhibitory effects (0.9mg/ml gave 64.1% and 60.5% inhibition respectively). Bioassay-guided fractionation of the methanol extract resulted in the isolation of an active compound, 11-hydroxy-β-boswellic acid **1,** whose structure was elucidated by mass and NMR spectroscopy. Related boswellic acids were also tested but did not have an inhibitory effect. AChE inhibitory effects appeared to be associated with the presence of free hydroxyl group in this structural type.

References: **1**. Ellman, G.L. et al (1961) Biochem.Pharmacol. 7:88-95.

Antioxidant Activity and Stability of Roselle (*Hibiscus sabdariffa* L.) Dried Extracts

P 454

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Dried calyxes of Roselle were extracted in boiling water and then dried using 2 different techniques: spray dry and vacuum dry. Total anthocynins and total phenolic compounds in both extracts as well as their DPPH radical scavenging activities were determined. The amount of monomeric anthocyanins was found to be superior in the spray-dried extract, **SR**, (33.4±0.5 mg/g) than that in the vacuum-dried extract, **VR**, (2.5±0.1 mg/g). However, the phenolic contents and antioxidant activities were comparable in both extracts. The phenolic contents, in gallic acid equivalents, in **SR** and **VR** were 300.7±4.4 and 281.7±6.2 mg/g and the EC_{50} values of radical scavenging activities were 15.1±0.5 and 11.3±0.1 µg/ml, respectively. The stability studies of both Roselle extract were carried out under accelerated conditions (45ºC, 75%RH) for 4 months. In both samples, the polymerization of anthocyanins was observed, showing that monomeric anthocyanins decreased while polymeric anthocyanins increased. Then again, their DPPH scavenging effects and total phenolic contents maintained nearly the same values during testing period. These results suggested that there was no significant influence of polymerization of anthocyanins on the antioxidant activities of Reselle extracts. Its antioxidant effect seemed to be related to total phenolic contents.

Acknowledgements: 1. National Research Council of Thailand 2. Prince of Songkla University, Thailand

References: **1**. Tsai, P.J. and Huang, H.P. (2004) *Food Research International*. 37: 313-318, **2**. Gradinaru, G., et al. (2003) *Food Chemistry.* 83: 423-436. **3**. Tsai, P.J., et al (2002) *Food Research International*. 35: 351-356.

P 455 Blood-sugar-reducing effects of bitter melon (*Momordica charantia* L.) leaves

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In order to find the possibility of practical use of bitter melon (*Momordica charantia* L.) leaves, which are most of the times discarded, the present study examined the blood-sugar-reducing effects of leaves and isolated several compounds contained in them.

The blood-sugar levels, in the intact rats was measured with time by cannlation of the jugular vein, after oral administration of some organic solvent extracts of bitter melon leaves following α-starchy. The MeOH, chloroform, and *n*-BuOH extracts of bitter melons leaves reduced blood-sugar levels in rats. We further isolated 12 compounds, and among them there were dehydrodiconyferyl alcohol derivatives and Momoridicoside L that have blood-sugar-reducing effects.

Refrences: Clara L. et al. (1998) J. Pharm.Pharmacol., 50, 84.

P 456 Chemopreventive Effect of Brazilian Traditional Medicine, *Tabebuia avellanedae*

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Tabebuia avellanedae Lorentz ex GRISEB (Bignoniaceae), which is native to South America from Brazil to northern Argentina, is well known in traditional folk medicine used for the treatment of various diseases. Previously we reported that 5-hydroxy-2-(1-hydroxyethyl)-naphtho[2,3-*b*]-furan-4,9-dione, one of the antitumor constituents in this plant inhibited TPA-induced EVB-EA activation in Raji cells and thereby acted as a chemopreventer *in vitro* (1). This fact promoted us to examine chemopreventive effects of *T. avellanedae* since the inner bark of this plant produced in Brazil is distributed in Asia as a herb tea. Oral administration of the aqueous extract of the powdered inner bark (provided by Taheebo Japan Co,Ltd.) inhibited the promotion stage of carcinogenesis in mouse skin (carcinogen/promoter: DMBA/TPA) and in mouse lung (4NQO/ 8% glycerol), suggesting that the extract might be a functional material for cancer prevention as well as fruits and vegetables. In this presentation, we will describe the *in vivo* chemopreventive activity of *T. avellanedae* together with the *in vitro* activity of several constituents including a novel cumarin derivative.

References: 1. Ueda S. et al. (1994) *Phytochemistry* 36:323-325.

Cytotoxicity and Inhibition of Lymphocyte Proliferation of Xanthones and Cinnamate Esters from *Hypericum hookerianum*

P 457

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Hypericum hookerianum Wight et Arn., a traditional tribal wound-healing agent used in India (1) is the only species occurring in Thailand. Though its extract was claimed to possess the antibacterial activity (2), it has not been previously investigated chemically. The chloroform extract of its stem wood furnished 5-hydroxy-2-methoxyxanthone (**1**), 2-hydroxy-3-methoxyxanthone (**2**), *trans*-kielcorin (**3**), betulinic acid 3β-yl caffeate (**4**) and 4-hydroxy-3-methoxyphenyl ferulate (**5**). Compounds **1-5** were evaluated for their effect on the *in vitro* growth of three human cancer cell lines: MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS). The results showed that cinnamate esters **4** and **5** exhibited strong inhibitory effect against all three cell lines; that of *trans*-kielcorin (**3**) was moderate while the inhibitory effect of xanthones **1** and **2** were only weak. The effect of compounds **1-5** on the mitogenic response of human lymphocytes to PHA was also evaluated. Again, xanthones **1** and **2** exhibited weaker antiproliferative effects than cinnamate esters **4** and **5** while *trans*-kielcorin (**3**) was devoid of activity.

Acknowledgements: FCT (Unidade de I&D nº 226/94), FEDER, POCTI (QCA III), NCI (USA), Thailand Research Fund for the RGJ PhD Program.
Reference: 1. Mukherjee, P. et al. (2000) J. Altern. Complement. Med. 6, 61. **2.** Mukherjee, P. et al. (2001) Fitoterapia 72, 558.

Investigation and Analysis of *Polygonum chinense* L. in Taiwan

P 458

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Some of Taiwanese folks have been using the leaves and stems of *Polygonum chinense* L. (Figure 1), prepared in hot vinegar solution, to treat their seriously damaged skins of hands as well as feet. The folks there claim their skins got renewed beautifully after two weeks' treatment.
The ways of *Polygonum chinense* L. that is grown (1) and used by folks in Taiwan as well as the above-mentioned facts-finding, are further investigated. For scientifically analyzing this natural herb and traditional medicinal solution as well as various extracts, series of laboratory work and analytical method development using chromatographic techniques (such as HPTLC and HPLC primary) and other instrumentations are carried out.

References: 1. W. S. Kan., (1969), Medicinal Plants, National Research Center of Chinese Medicines, Published in Taiwan

P 459 Effect of livomyn tablet and livomyn syrup in acute hepatic injury induced by carbon tetrachloride

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Herbal drugs are playing an important role in health care programmes worldwide with a resurgence of interest in herbal medicines for the treatment of various ailments including hepatopathy. Livomyn tablets and syrup are the herbal formulations containing extracts of various medicinal plants mentioned in Ayurveda for hepatic disorders. Hence, the present study was aimed at evaluating their hepatoprotective activity in carbon tetrachloride (CCl_4) induced acute hepatic damage, as the changes associated with this are similar to that of acute viral hepatitis. Wistar rats (170-200 g) were used for the study and treatment was given for 14 days with Livomyn tablet: 350 mg/ kg body weight and Livomyn syrup: 2.7 ml/ kg body weight p.o. respectively. Silymarin (100mg/kg) was used as a positive control. On 14th day CCl_4 (1.25 ml/kg, p.o.) was administered to induce acute hepatic injury, manifested by significant ($p<0.01$) rise in plasma ASAT, ALAT, ALP and LDH levels compared to respective control values. Administration of CCl_4 also caused alterations in plasma total protein, albumin, cholesterol and total bilirubin levels. Pre-treatment of rats with Livomyn tablets and syrup significantly ($p<0.01$) inhibited the alterations in these biochemical levels. The formulations also countered the increase in liver weights and reduced the severity of histological lesions in liver caused by CCl_4 with aversion of variations in glutathione levels, TBARS levels and DNA content of liver. These results indicate that Livomyn tablets and syrup exhibited significant hepatoprotective activity against CCl_4 induced acute hepatotoxicity.

P 460 Hepatoprotection by herbal formulations: livomyn tablet and syrup in chronic hepatic injury induced by carbon tetrachloride

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Despite of the tremendous strides of modern medicine, there is hardly any drug that offers protection to the liver damage. Alternatively, herbal drugs can play an important role in the treatment of various ailments including hepatopathy. Livomyn tablets and syrup are herbal formulations containing extracts of various medicinal plants mentioned in Ayurveda for hepatic disorders. The present study aimed at evaluating their hepatoprotective activity in chronic hepatic injury induced by carbon tetrachloride (CCl_4) as the changes associated with this are similar to that of viral hepatitis. Wistar rats (170-200 g) were treated with Livomyn tablet: 350 mg/ kg body weight and Livomyn syrup: 2.7 ml/ kg body weight p.o. respectively for 21 days. Silymarin (100mg/kg) was used as a positive control. Hepatic injury was induced by concomitant administration of CCl_4 (1 ml/kg, p.o.) twice a week for three weeks, manifested by significant ($p<0.01$) rise in plasma ASAT, ALAT, ALP and LDH levels compared to respective control values. Administration of CCl_4 also caused increase in cholesterol and bilirubin levels along with decrease in total protein and albumin levels. Treatment of rats with Livomyn tablets and syrup significantly ($p<0.01$) inhibited the rise in the enzyme levels showing reduced leakage of enzymes from the hepatocytes and countered variations in cholesterol, bilirubin, total protein and albumin levels. It also significantly ($p<0.01$) restored reduced glutathione and DNA content of liver, which were depleted in CCl_4 induced hepatotoxicity. The lipid peroxidation was lowered by treatment with the test formulations as evident from reduced TBARS levels. Treatment with formulations inhibited the increase in liver weights caused due to inflammation. It also reduced the severity of hepatic lesions including necrosis with leucocyte infiltration, vacuolar degeneration and showed regeneration of hepatocytes in necrotic areas. These results indicate that the herbal formulations Livomyn tablets and Livomyn syrup exhibited significant protective activity at therapeutic doses against chronic CCl_4 induced hepatotoxicity *in vivo*.

Situation of Herbal Medicine in Iran: Training, Research and Products

P 461

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Traditional medicine is gained increasing importance in the National Health Systems of the nations. Integration of traditional medicine into the modern medicine has been growing up in many worldwide countries. Medicinal plants, with their diversity have been one of the most valuable sources in both traditional and modern systems of medical practices. Islamic Republic of Iran has a long and rich history of the use of herbal medicines in its traditional and old systems of medicine, the activities and authorships of the famous old Persian physicians like Avecinna, Rhazis (Razi), Jorjani and others are well-known in the world. There are various geographical climates in Iran which made it unique in the region and even in the world. More than 7500 plant species is recorded in this country due to such specific situation. Among these species, there are some endemic plants which grow only in Iran and they aren't found in any other place in the world. According to this abundance of plant covering, medicinal herbs has been attracted many scholars to search upon it.

The government of Islamic Republic of Iran and the Ministry of health are strongly committed to promoting the use of traditional medicine in the health sector. Some other ministries like Ministry of Agriculture are also involved implementing good agriculture practice for herbal medicine. Iranians are still interested in using herbal drugs and many of the allopathic physicians use herbal drugs to cure their patients.

In this article situation of education, industrial activity, research and published articles of Iranian researchers are discussed.

Anti-HIV-1 protease- and HIV-1 integrase activities of Thai medicinal plants called Hua-Khao-Yen

P 462

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Ethanolic- and water extracts from five species of Thai medicinal plants called Hua-Khao-Yen were tested against HIV-1 protease (HIV-PR) and HIV-1 integrase (HIV-1 IN) activities (1, 2). The result revealed that *Smilax corbularia* (EtOH extract) exhibited the most potent against HIV-1 IN with an IC_{50} value of 1.9 μg/ml, followed by *Dioscorea burmanica* (water extract, $IC_{50} = 4.5$ μg/ml), *Dioscorea burmanica* (EtOH extract, $IC_{50} = 4.7$ μg/ml), *Smilax corbularia* (water extract, $IC_{50} = 5.4$ μg/ml), *Smilax glabra* (EtOH extract, $IC_{50} = 6.7$ μg/ml) and *Smilax glabra* (water extract, $IC_{50} = 8.5$ μg/ml), whereas *Pygmaeopremna herbacea* and *Dioscorea membranacea* were inactive ($IC_{50} >$ 100 μg/ml). Regarding HIV-1 PR inhibitory activity, only *Dioscorea membranacea* (EtOH extract) showed appreciable activity ($IC_{50} = 48$ μg/ml), while others possessed mild activity. This result may support the traditional use of *Smilax corbularia* and *Dioscorea membranacea* for AIDS treatment.

Acknowledgements : The Thailand Research Fund and National Institute of Health, Bethesda, USA.

References: 1. Min, B.S. et al. (1999) Phytother. Res. 13 : 680-682, **2**. Tewtrakul, S. et al. (2003) Phytother. Res. 17 : 232-239

P 463 *Athrixia phylicoides* (bush tea): possibilities for a new health-promoting beverage

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Athrixia phylicoides DC. (Asteraceae), commonly known as Zulu or bush tea, is widely used as a beverage in South Africa. The commercialization of this tea in a similar vein to popular herbal teas such as rooibos (*Aspalathus linearis*) and honeybush tea (*Cyclopia intermedia*) is being pursued. Decoctions and infusions of *A. phylicoides* and a related species, *A. elata* Sond. (daisy tea) were prepared following the traditional approach. The cytotoxicity of the extracts was investigated using the brine shrimp lethality and MTT cytotoxicity assays but showed negligible activity. Ethanol extracts prepared in the laboratory, however, showed noteworthy cytotoxic activity in both assays.
The Trolox equivalent antioxidant capacity (TEAC) of the extracts was found to be comparable to that of rooibos (0.257). The highest TEAC value was calculated to be 0.269 for the *A. phylicoides* decoction, and the other extracts displayed slightly lower values. The total phenolic content in each sample was determined spectrophotometrically according to the Folin-Ciocalteu method and calculated as gallic acid equivalents. The *A. phylicoides* decoction exhibited a value of 45.18 compared with a value of 35.64 for rooibos. The presence of antioxidant compounds in beverages may contribute to a decreased incidence of certain cancers and other ailments. There was no detectable caffeine in the *Athrixia* infusions and decoctions following analysis using TLC and I/HCl spray reagent. The lack of caffeine and the presence of antioxidant activity, as well as the pleasant taste, support the development and commercialization of bush tea as a healthy alternative to caffeine-containing beverages.

P 464 Anti-inflammatory and Antinociceptive Activitiy of *Eryngium* species

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The genus *Eryngium*, belonging to the Umbelliferae subfamily Saniculoideas, is known to contain acetylenes, flavonoids, coumarins and triterpene saponins (1). *Eryngium* species are represented by 317 accepted species, subspecies and varieties (2). *Eryngium campestre* grows in most parts of Europe and northern Africa and has been introduced into North America. It is reported that the root is unprovenly used as an in the treatment of bladder and kidney stones, renal colic, kidney and urinary tract inflammation, urinary retention and oedema, coughs, bronchitis, skin disorders and respiratory disorders (3)**.** The genus *Eryngium* is represented by 23 species (24 taxa) in the Flora of Turkey and East Aegean Islands. Ten species of these are endemic (4-6).
The root and aerial parts of various *Eryngium* species are used as folk remedy worldwide for the treatment of bladder and kidney stones, renal colic, kidney and urinary tract inflammation, urinary retention and oedema, coughs, bronchitis, skin disorders and respiratory disorders. Ethanolic and aqueous extracts obtained from eight *Eryngium* species were evaluated for their *in vivo* anti-inflammatory and antinociceptive activities. Among the plant extracts screened, the ethanolic extract from aerial part of *E. isairicum* and water extract from aerial part of *E. kotschyi* showed remarkable anti-inflammatory activities; ethanolic extracts from aerial part of *E. isairicum* and *E. maritimum* possess *significant in vivo* antinociceptive activity.

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Guggal (*Commiphora mukul*): A Cholesterol Reducing Herb

P 465

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Guggal is a spiny shrub with many branches, usually grows upto 2-3 meters high and found in the arid, rocky tracts in India. The extracted 'gum resin' of the plant is used. In Ayurvedic it is used for multiple treatments from healing bone fractures to cardiovascular disease, obesity. It has antispasmodic, diaphoretic, antisupperative, emmenagogue and aphrodisiac qualities. It has been found that extract 'guglipid' from the Guggal plant can help in reducing cholestoral. It is found that the extract blocks the body's Farnesoid X Receptor (F X R). This receptor is important for Cholesterol level because it triggers the conversion of cholesterol to bile acid. Another positive aspect of guglpid is that it does not create any harmful side effects associated with drugs commonly used for cholesterol disorders. Guglpid received regulatory approval in India in 1987.

Acknowledgements: BBC Health News, 2 May 2002; Dr. Virendra Sodhi: Herbal Spotlight:Guggal;

Studies on the nutritional value and antitumour property of the bark of *Spondias mombin* L.

P 466

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This study was conducted to investigate the anti-tumour property of the bark of *Spondias mombin*, commonly used traditionally for the treatment of certain malignancies. Forty wister rats of about seven weeks old were randomly sampled and grouped into three. Group A, the main control, was fed with 50g marsh only over a definite period, while the test control received 50g marsh and 5g *Cycas revoluta* , a carcinogenic plant. Three subgroups from the B groups were fed with carcinogencontaining diet and *S. mombin* simultaneously at different concentrations. Group C rats had their diet changed to marsh and *S. mombin* at different concentration for each subgroup after initial exposure to carcinogencontaining diet.
Group B rats fed with carcinogenic feed alongside treatment with *Spondias mombin* bark for induced cancer showed some recovery, as the treatment suppressed some effects of the carcinogen. By contrast, group C rats, which were similarly induced with cancer and later treated at 50% level showed significant improvement compared to the test control. Most of the symptoms observed in the latter such as hair loss, reduced agility, low food intake and hyperplastic nodules were reduced. Ultrasound findings showed significant tachycardia in group B rats with increased dosage of the treatment plant while the test control rats showed relative bradycardia, indicating that tachycardia is a possible side effect of the treatment plant. Histopathology of the tissues showed significant pathological differences especially in the liver, small intestine and kidney ($p<0.05$) as observed in SGOT, SGPT, ALP, cholesterol and bilirubin levels. Proximate analysis carried out on *S. mombin* bark showed a high concentration of crude fibre and calcium ion, which have been identified to have anti-tumour properties. The results obtained suggest a role for *S. mombin* in the treatment of certain malignancies.

P 467 Antiulcer activity of Calmogastryl® used in malian traditional medicine

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Calmogastryl®, a Traditional Improved Drug obtained from the stem bark decoction of *Pteleopsis suberosa* Engl. et Diels (Combretaceae), a tree distributed from Senegal to Mali, is orally administered in Mali folk medicine for the treatment of gastric ulcers (1). Previous pharmacological studies on the plant extracts showed antiulcer activity (2,3). In this bioassay-guided isolation we evaluated the antiulcer activity of chloroform-methanol (9:1), methanol, and aqueous extracts of the dried powdered stem bark of the plant in the acute model of ethanol-induced gastric ulcers in rats, orally administered at a dose corresponding to 1000 mg/kg. Results showed that the aqueous soluble fraction from methanol extract exhibited a significant protection reducing the incidence of gastric lesions induced by ethanol administration. The fractionation of the extracts on Sephadex LH-20 afforded some fractions that were tested for antiulcer activity. The levels of total thiols (T-SH) and non-protein thiols (Np-SH) as markers of oxidative stress and thiobarbituric acid-reactive substances (TBARS) as an index of lipid peroxidation were evaluated in the stomach homogenate, showing the fraction that significantly lowered the elevated lipid peroxide levels and restored the decreased Np-SH products in ethanol-induced gastric ulceration.

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P 468 Extracts of 30 South African *Combretum* and *Terminalia* species have antifungal activities with MIC's as low as 20 µg/ml

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Combretaceae species are used for many medicinal purposes by traditional healers. There have been reports of antifungal activity in members of the Combretaceae, but there have been no follow up studies. As a first step in the isolation of antifungal compounds from *Combretum* and *Terminalia* species 24 *Combretum* species and 6 *Terminalia* species were screened for antifungal activity. Methanol extracted the highest quantity, but the acetone extracts had the highest antifungal activity. Most of the extracts had MIC values of c. 0.08 mg/ml; some with MIC values as low as 0.02 mg/ml. *Microsporum canis* was the most susceptible fungus and *T. sericea* extracts was the most active against nearly fungi tested with *Terminalia* extracts. Acetone and DCM extracts of *Terminalia* species had the best average MIC values on the tested organisms, (0.146 and 0.147 mg/ml after 24 hours). Acetone extracts of *C. molle* and *C. celastroides* ssp. *orientale* were the most active against all the fungi tested (average MIC values, of 0.19 and 0.13 mg/ml). The methanolic extracts of *C. moggii* and *C. petrophilum* were very active against all the fungi. All extracts of *C. nelsonii* were also very effective against all the pathogens. Hexane and dichloromethane extracts of *Terminalia* species contained more antifungal compounds than the other extracts. Based on Rf values the active compounds were clearly not tannins. All *Terminalia* species contained the same compound ($R_f = 0.46$ in BEA) active against all tested pathogens.

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Phytochemical Studies on *Thymus sipyleus* Boiss. subsp. *sipyleus* var. *sipyleus*

P 469

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Thymus sipyleus subsp. *sipyleus* var. *sipyleus* (Lamiaceae) is an endemic species and widely grows in Turkey (1). *Thymus* species known as "Kekik, nemamulotu and sater" are used by the public for its antibacterial, secretolytic and broncospasmolytic effects in Turkey (2). *T. sipyleus* subsp. *sipyleus* var. *sipyleus* is used as spice Erzurum Province (Turkey) (3). In this study, phytochemical studies were performed on aerial parts of plant. The isolation of the compounds was carried out using several and repeated chromatographic techniques from n-hexane, chloroform, ethyl acetate, n-butanol and water phases that partitioned from methanolic extract obtained from plant. Ursolic acid was isolated from chloroform phase, rosmarinic acid, luteolin and luteolin 7-O-(6"-feruloyl) β-glucopyranoside from ethyl acetate phase, luteolin 5-O-β-glucopyranoside from n-butanol phase and luteolin 7-O-β-glucuronide from water phase. The structures of the compounds were elucidated by means of spectral analysis (^{1}H-NMR, ^{13}C-NMR, 2D-NMR (COSY, HETCOR and HMBC) and EI-MS) .

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Antioxidant properties of two Chilean medicinal *Haplopappus* species

P 470

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"Bailahuén" is the common name used for several medicinal Chilean plants belonging to the genus Haplopappus (Asteraceae). The nine species in this "bailahuén complex" are used along the country in relief of liver ailments (1). A chromatographic analysis of extracts from *H. multifolius* (2) and *H. taeda* (3), the most consummed species in Central Chile, showed striking differences in chemical composition. Thus, while *H. multifolius* accumulate coumarins, *H. taeda* mainly produces dihydroflavonoids and terpenes. Different types of extracts (infusions, methanolic extract and resins) obtained from both species, showed high free radical scavenging properties when tested with DPPH. On the other hand, resins and the main pure compounds isolated from them (7-methylaromadendrin from H. taeda and prenyletin from *H. multifolius*), strongly inhibited microsomal lipid peroxidation induced by Fe^{+3}/ascorbate system. In this assay the flavanone sakuranetin from *H. taeda* was pro-oxidant.
Considering the relationship established between the antioxidant activity and hepatoprotection, we can now attribute these properties mainly to prenyletin (and related coumarins) in *H. multifolius* and to dihydroflavonols in *H. taeda*, in a similar fashion as silymarin from *Silybum marianum* seeds.

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P 471 Antioxidants from *Prunus myrtifolia* (Rosaceae): detection of flavonol glycosides and caffeic acid esters by LC-UV-MS

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Prunus myrtifolia belongs to Rosaceae, a plant family known for its edible fruits: quince (*Cydonia*), pear (*Pyrus*), strawberry (*Fragaria*), apple (*Malus*), plum, peach and cherry (*Prunus*), most of which contain almond smelling seeds, associated with their benzaldehyde contents. *Prunus* species contain flavonoids, arylpropanoids, terpenoids, in addition to cyanogenic glycosides [1,2]. As part of our studies on species from Tropical Rain Forest (Mata Atlântica), leaves and stems of *Prunus myrtifolia* were collected and their ethanolic extracts showed positive results when screened for antitumoral (assay based on growth inhibition of genetically modified strains of *Saccharomyces cerevisae*) and antioxidant (bleaching of β-carotene TLC autographic assay [3]) activities. Phytochemical work on the EtOH fraction of the leaves extract led to the isolation of kaempferol and quercetin glycosides, catechin, benzoic acid, mandelonitrile and *p*-hydroxymandelonitrile glycosides, in addition to two new phenethyl glycosides. The EtOH extract from stems of *P. myrtifolia* gave five caffeic acid esters, which showed strong free radical scavenging activity towards DPPH. These results support the use of extracts of *P. myrtifolia* in traditional medicine and suggest their actions as possible chemopreventive agents or phytoceuticals.

Acknowledgements: This work was sponsored by the program BIOTA-FAPESP, CAPES and CNPq.
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P 472 The African Medicinal Plant Standards [AMPS] project has identified the most important African medicinal plant species and developed an open access database

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Africa contains 25% of the world's species diversity, but African species contribute only a low percentage of plants commercialized and used as herbal medicines in Europe compared to species from China and India. This may be because insufficient information is available on the efficacy, safety and quality control of African medicinal plants. The ACP-European Union Centre for the Development of Enterprise funds a project to identify the 21 (and later another 29) African medicinal plant species with the highest potential of commercialization and to write trading standards/monographs for these species. This is a pan African project involving a variety of role players in the medicinal plant industry. The criteria used for the selection of the species, the aspects to be covered in the profiles and the profile content have been validated by a workshop involving key role players from Africa and Europe. Safety of all products were evaluated in *in vitro* studies and identification criteria were developed to ensure adequate quality control. The results of this project will be made available on the Internet with open access before the end of 2005. This project should lead to a greater demand for African medicinal plants, to the production of these species in Africa thereby creating jobs and increasing the quality of life of Africans while delivering products that may be useful to inhabitants of Europe. This project would also identify areas requiring research and increase research cooperation within Africa. The database will be continually updated with latest information. This project may be a modest start to the eventual development of an African Herbal Pharmacopoeia

Acknowledgements: ACP-EU provided funds for this project and CTA-EU provided funds for an international workshop to evaluate the results of the AMPS project.

Ethnobotany of the chittagong hill tracts' tribes- traditional medicinal uses compare with modern biological science

P 473

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The indigenous communities living within the forests of Chittagong Hill Tracts (CHT) have long been a source of admiration as they possess a unique understanding of, and ability to utilise, the plants around them. The present work presents a comparative ethnobotanical study of the tribal communities of CHT of Bangladesh. The five largest tribal communities (Chakma, Marma, Murong, Tanchangya and Tripura) inhabiting the three districts of CHT (Bandarban, Khagrachari and Rangamati) were investigated and data on their use of plants was obtained by discussions with tribal informants. The discussions were tape recorded, data documentation sheets prepared with the help of interpreter and a set of photographs were taken during the visits.
A total of 330 species have been documented for the treatment of 114 illnesses by the CHT tribes. A literature survey using Dictionary of natural products CD ROM, BIOSIS and SCI was undertaken for all reported species (or genera if no records of the species were obtained) to discover whether any ethnopharmacological evidence was available to support the use report to make a comparative study of traditional medicinal uses with modern biological sciences. A total of 115 species used by the tribes have been found potential pharmacological activity from the literature. Currently the tribal communities are being exposed to outside influences and risk losing their local knowledge as they increasingly import into their community industrial products and modern medicinal treatments.

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Investigation of In-vitro Antibacterial Activity of Three Medicinal Plants

P 474

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In this study, the aqueous extract of *Fluerya aestuans, Ocimum bacilicum* and *Acalypha wilkesiana* were investigated for in-vitro antimicrobial activities by agar diffusion and tube dilution techniques. The extract except *Fluerya aestuens* inhibited the growth of standard and local strains of bacteria including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*.
The aqueous extracts of these plants did not exert any inhibitory action on *Candida albicans*. The minimum inhibitory concentrations of the extracts ranged between 8mg/ml to 64mg/ml, while the minimum bacteriocidal concentration were between 32mg/ml and 64mg/ml.

P 475 Anticancer Triterpenoid Saponin from *Lecaniodiscus cupanoides*

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The plant *Lecaniodiscus cupanoides* Planch is an evergreen shrub or small tree in dry forest environments[1]. Ethnobotanical information revealed that the plant is used as an aphrodisiac, a galactogen, a laxative and in cases of sexual asthenia[2].
The powdered stem (1kg, dry weight) was extracted with ethanol. The filtrate was concentrated under reduced pressure to give dried extract. Bioassay guided fractionation of this extract using VLC followed by column chromatography and HPLC as well as preparativeTLC afforded compounds 1 and 2. The compounds were identified as triterpenoid saponins 3-O-{α-L-arabinofuranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-}-hederagenin and 3-O- {α-L-arabinopyranosyl- (1→3)-α-L-rhamnopyranosyl- (1→2)-α-L-arabinopyranosyl-}-hederagenin based on chemical investigations and spectroscopic data (^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, ^{1}H-^{1}H COSY and MS.). The compound 1 exhibited anticancer activity against human colon carcinoma H-116, human lung carcinoma A-549 and human lung carcinoma HT-29 with IC_{50} of 5.0µg/ml, 2.5µg/ml and 2.5µg/ml respectively and compound 2 exhibited similar activites with IC_{50} of 5.0µg/ml, 5.0µg/ml and 2.5µg/ml respectively.

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P 476 Anti-stress activity of herbal formulations, Amala Mus and Amala Plus by modulation of cold stress induced perturbations in rats

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Stress is known to alter the physiological homeostasis of the organism with complex mechanisms leading to etiopathogenesis of a variety of diseases like depression, anxiety and cognitive dysfunctions. Using agents, which could induce a state of non-specific increase of resistance to diverse aversive assaults can provide the answer to this problem. Amala Mus and Amala Plus are the herbal formulations containing various constituents from the traditionally used medicinal plants. Hence, the present work was undertaken to evaluate the anti-stress activity of Amala Mus and Amala plus. Wistar rats (150-180g) used for the study were pre-treated with Amala Mus: 2.7 g/kg body weight and Amala Plus: 105 mg/kg body weight p.o for 21 days. Revital syrup containing ginseng: 110 mg/ kg body weight served as the positive control. After pre-treatment, all animals except for vehicle control group were concomitantly exposed to cold restraint stress (4^{0} C for 1 h) for 7 days. The test formulations significantly ($p<0.01$) countered the increase in adrenal gland weights and atrophy of spleen caused by cold restraint stress. Increased corticosterone levels due to stimulation of hypothalamus pituitary adrenal (HPA) axis in stress, altered plasma glucose, cholesterol, total protein and triglycerides levels. The treatment with test formulations significantly ($p<0.01$) ameliorated the stress-induced variations in these biochemical levels. Cold restraint stress significantly increased nor-epinephrine, 5-Hydroxy Tryptamine and dopamine levels in brain and treatment with test formulations significantly ($p<0.001$) reverted the increase in these catecholamines levels. Histopathological studies of the adrenal glands of stress group revealed moderate diffuse vacuolar degeneration, moderate congestion and minimal diffuse vacuolar degeneration of zona glomerulosa and zona fasiculata. The Amala Mus group showed mild vacuolar degeneration of zona glomerulosa and zona fasiculata where as Amala Plus and normal control groups did not show any abnormalities. The above findings suggest that Amala Mus and Amala Plus demonstrated non-specific anti-stress activity by modulation of stress-induced perturbations.

Protective effects of *Nyctanthus arbortristis* L. Leaves against chronic carbon tetrachloride induced hepatotoxicity in vivo

P 477

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In practice of traditional ayurvedic medicine a number of herbs have been recognized for their potential benefits in the treatment of liver disorders. A decoction of fresh leaves of *Nyctanthus arbortristis* L. *(*Family: Oleaceae) is used traditionally in viral hepatitis induced hepatic derangements, hence present work focused on investigating its protective effects against chronic carbon tetrachloride induced hepatotoxicity *in vivo*. The methanolic extract of *Nyctanthus arbortristis* (MNA) was used for the study as it exhibited potential *in vitro* anti oxidant activity during preliminary screening. Wistar rats (150-200 g) used for the study were treated with MNA extracts 100, 250 and 500 mg/kg body weight p.o. respectively for 21 days. Silymarin (100mg/kg) was used as a positive control. Hepatic damage was induced by concomitant administration of CCl_4 (1 ml/kg, p.o.) twice a week for three weeks, manifested by significant ($p<0.01$) rise in plasma ASAT, ALAT, ALP and LDH levels compared to respective control values. Administration of CCl_4 also caused increase in cholesterol and bilirubin levels along with decrease in total protein and albumin levels. Treatment of rats with MNA extracts significantly ($p<0.01$) inhibited the rise in the enzyme levels showing reduced leakage of enzymes from the hepatocytes and countered variations in cholesterol, bilirubin, total protein and albumin levels in a dose dependent manner. It also significantly ($p<0.01$) restored the depleted levels of catalase, reduced glutathione and DNA content of liver in CCl_4 induced hepatotoxicity. The lipid peroxidation was lowered by MNA treatment as evident from reduced TBARS levels. Treatment with MNA extracts inhibited the increase in liver weights due to inflammation and reduced the severity of hepatic lesions including necrosis with leucocyte infiltration and vacuolar degeneration and showed regeneration of hepatocytes in necrotic areas. These results indicate that methanolic extract of *Nyctanthus arbortristis* leaves exhibited significant protective activity against chronic CCl_4 induced hepatotoxicity *in vivo*, comparable with the standard silymarin (100 mg/kg).

Effects of dihydrocucurbitacin B on cell-mediated allergy

P 478

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Cayaponia tayuya (Cucurbitaceae) is a South American climbing plant. Its roots are used in folk medicine as an anti-inflammatory and anti-allergic crude drug (1). From the $DHCl_3$ extract of the dry roots, Recio et al. (2) have isolated 23,24-dihydrocucurbitacin B (DHCB) and demonstrated its anti-inflammatory activity in different experimental models of acute and subchronic inflammation. Thus, while oxazolone and dinitrofluorobenzene (DNFB) are the most common agents used to induce contact dermatitis, sheep red blood cells (SRBC) were used to induce systemic delayed-type hypersensitivity (DTH). One common factor involved in all of these reactions, however, is that they are mediated by specific T cells. In our work with these 3 experimental models (3), we have thus assayed the anti-allergic effect of DHCB, as well as its effect on lymphocyte proliferation and lymphocyte cell cycle (4) and have found that DHCB showed anti-allergic effects in all three models assayed, with an inhibition of 46% (**$P < 0.01$, Dunnett´s *t*-test) in oxazolone-induced DTH and 62 % at 72 h (**$P < 0.01$, Dunnet´s *t*-test) in DNFB-induced DTH. In the SRBC model, although the inhibition reached 54% (**$P < 0.01$, Dunnett´s *t*-test) at 18 h, the effect disappeared after 48 h. In the histological studies of the contact dermatitis models, the DHCB-treated group exhibited only a mild inflammatory lesion along with a reduction of both the oedema and the inflammatory cell infiltration; a reduction of the epithelium thickness with respect to the control group was also observed. Other parameters of the inflammatory process such as papillomatosis, acanthosis, hyperkeratosis, and spongiosis were also attenuated in the DHCB group. In addition, DHCB inhibited the lymphocyte proliferation with a CI_{50} of 1.46 µM, stopping the cell cycle in G_0 phase. These results demonstrate the anti-allergic effect of DHCB, which exhibited an immunosuppressive effect in the experimental models and lymphocytes.

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P 479 Anti-inflammatory Properties and Phytochemical Characteristics of *Crassocephalum rabens*

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Crassocephalum rabens (Compositae) is a popularly used folk herbal medicine and food supplement in Taiwan. *C. rabens* that has been used anecdotally for preventing various inflammation related syndromes. We observed in the present study that the BuOH and EA fractions derived from total *C. rabens* extracts exhibited significant dose-dependent inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages. The active sub-fractions were further purified on the basis of bioactivity-guided fractionation. Twenty-five pure phytocompounds including phenolic compounds and glycolipid derivatives were identified. An enriched galactolipid fraction (EA8) was observed to possess inhibitory effects on nitric oxide (NO) and PGE_2 productions induced by LPS in macrophages. A specific galactolipid, designated as CRE-7, was isolated from the EA8 fraction which displayed an effective inhibitory effect on NO production with no detectable cytotoxicity. Immunohistochemical studies in mouse skin demonstrated that TPA (a tumor promoter)-induced COX-2 protein and protein nitrotyrosine over-expressions can be effectively suppressed by the CRE-7 treatment, with a comparable effect to that of celecoxib, a well-known non-steroidal anti-inflammatory drug. Since NO, PGE_2, and COX-2 are important mediators for inflammation and tumor development, the results obtained from this study thus suggest the potential therapeutic benefits of *C. rabens* for inflammatory disorders or cancers.

P 480 Cytotoxic Activity and Nitric Oxide Production Enhance of Tirucallane-type Triterpenes

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Continuing with our systematic study of Mexican medicinal plants (1), now we wish to report the cytotoxic activity and nitric oxide (NO) production enhance of the triterpenes present in *Amphipterygium adstringens* (cuachalalate). The results showed that, the hexane extract from the bark of *A. adstringens*, as well as its principal constituents, masticadienonic (**1**) and 3α-hydroxymasticadienolic (**2**) acids, inhibited the growth of HCT-15 (colon), MCF-7 (breast), U251 (CNS), PC-3 (prostate) and K562 (leukaemia) human cancer cell lines. Furthermore, derivatives 24,25S-dihydromasticadienonic (**3**) and masticadienolic (**4**) acids both obtained from **1** were evaluated. The results showed that both **3** and **4** presented a higher activity than **1** on colon cancer cell lines. On the other hand, the effects of **1**-**4** in production of nitric oxide (NO), both from resting macrophages as well as from those LPS-stimulated, were determined. It was founded that **1**, and **2** elicited an increment in NO release from resting cells, while in LPS-activated macrophages only **2** and **4** caused the increment in the NO production.

Acknowledgements: Partial financial support from UNAM (DGAPA, IN-203198) is acknowledged.

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Chemical and microscopic analysis of the aphrodisiac Bois Bandé

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The popular Caribbean aphrodisiac Bois Bandé gains increasing significance in Europe due to the growing number of respective remedies available as "Bois Bandé" on the Internet and due to tourism. In the different West Indian islands different species are collected and offered for sale at the local markets. The Caribbean island of Grenada furnishes the Bois Bandé which consists of the stem bark and the roots of the native tree *Chione venosa* (Sw.) Urban var. *venosa,* Rubiaceae (1), growing in the island's rain forest. Market samples and authentic stem bark and root samples acquired in different years were compared by microscopy and TLC. The fingerprints of the methanol extracts showed homogeneity for all samples. Furthermore HPLC-UV-DAD and HPLC-ESI-MS methods were developed in order to identify the acetophenone derivatives, iridoids and neolignans whose structures were recently published by our research group (2, 3). The GC analysis of the dichloromethane extracts indicated a high concentration of acetophenone in the root in contrast to the stem bark. There are warnings that too extensive consumption or high dosages of the drug may cause unwanted side-effects in the urogenital tract. Thus methanolic-aqueous extracts were checked for their cytotoxicity.

Acknowledgements: We thank Telfor Bedeau, nature guide in Grenada, for guiding us to the drug-furnishing trees.
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Studies on the immunomodulatory activity of the active fraction of *Elephantopus scaber* L. in mice

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The influence of the administration of the active fraction of *Elephantopus scaber* (100 mg/kg) on the humoral and cellular immune response in mice was investigated. The data revealed that the active fraction of *E. scaber* significantly enhances the antibody response to sheep red blood cells (SRBC) with elevation of titre values of specific antibodies in the sera of treated animals. It also induces an increase in the number of splenic plaque forming cells (PFC). The antibody complement mediated cytotoxicity (ACC) showed detectable activity from 5th day after EAC tumor transplantation, reaching a peak on the 10thday and thereafter showed a gradual decline. Elevated delayed type hypersensitivity reaction (DTH), observed in this study suggested the activation of the cellular immune response. Treatment with the active fraction of *E. scaber* also resulted in an enhancement of peritoneal exudates cells and macrophage count. The serum enzyme activities, SAKP, SAST, SALT and serum γ-GT, serum urea, serum creatinine and serum calcium levels of rats indicated that there was no toxicity even at higher concentrations. Thus, the present study has shown the immunostimulatory activity of active fraction of *E. scaber*.

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P 483 Hepatoprotective activity of *Spilanthes ciliata* on ethanol – intoxicated liver in Wistar rats

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Spilanthes ciliata H. B. K. (Asteraceae), commonly known as 'Akravu' or 'Kuppamanjal' is used in tribal medicine for liver ailments. To scientifically validate this claim, the hepatoprotective effect of the ethanolic extract of the whole plant of *S. ciliata* was studied on rat liver damage induced by ethyl alcohol, by monitoring serum enzymes, serum cholesterol, serum total lipids and studying the histopathological changes of control and treated rats. The ethanolic extract of whole plant of *S. ciliata* produced significant hepatoprotection against ethyl alcohol at a dose of 200 mg/kg. Thus our investigation justified its use in tribal medicine for liver diseases.

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P 484 Pharmacognostic Standardization of *Andrographis paniculata*: an antiviral & immune boosting herbal medicine

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Andrographis paniculata Nees, is an annual herb that is used as an antiviral, hepatoprotective, antimalarial and immune boosting herbal medicine (1,2). The need for the establishment of pharmacognostic standards for the plant to authenticate it, guide against adulteration and the preparation of a monograph necessitated this research work. Macroscopic, microscopic, organoleptic and chemomicroscopic investigations on powdered and anatomical sections of the leaves and floral parts were done according to official books. The result of these investigations indicated a very bitter leaf taste, characteristic odour, 50–100 cm plant height, angular stem shape, opposite/decussate leaf shape, simple leaf arrangement and reticulate venation. A characteristic distribution of cystoliths (specialized cells containing chemical constituents) on both epidermises; a layer of palisade below upper epidermis containing oil globules, non-granular pollen grains, paracytic stomata, peculiar uniseriate and glandular trichomes were obtained from the microscopic investigation. Chemomicroscopy on the plant revealed presence of ligin, oil globules, calcium oxalate crystals, cutin with absence of tannin and aleurone grains. The result indicated the pharmacognostic profile of *Andrographis paniculata* which are necessary towards its standardization and quality control especially since the plant is being cultivated in different regions of the world. The determination of these diagnostic features amongst other parameters, are of importance in the identification, determining the purity and quality of the plant. These investigations are also required towards the preparation of a monograph on the plant due to its relevance in plant drug research.

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Hodia gordonii: preliminary phytochemical and clinical studies

P 485

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Hodia gordonii (Asclepiadaceae) is a succulent plant of Kalahari desert which has been used for thousands of years by Xhomani Bushmen as anorexant during hunting trips (1). Recently it was proposed to reduce the food intake, but no phytochemical data neither clinical studies have been published about its effectiveness and safety.
In this work we have investigated the phytochemical composition of *H. gordonii* extract. Our investigations lead to the isolation of two new pregnene glycoside derivatives, 12-*O*-tigloyl-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4) β-D-oleandropyranosyl-3β, 12β, 14β, 20-tetrahydroxypregn-5-ene (**1**) and 12-*O*-tigloyl-3-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranosyl-3β, 12β, 14β, 20-tetrahydroxypregn-5-ene (**2**). The structures of compounds were elucidated by HR mass spectrometry, 1D and 2D NMR experiments.
To evaluate the anorexant effects of plant extracts 40 mildly overweight women (BMI>25 Kg/m^2), were treated for 90 days with hypo caloric diet and with 400 mg of *H. gordonii* dried extract (HG) or with hypo caloric diet and placebo in a double blind trial. No significant variations were observed in haematological parameters, thyroid and liver functions. The treated group showed a significantly higher body weight reduction vs placebo. This study confirm our previous data on HG effectiveness in caloric intake reduction (2).

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Antihyperglycemic and hypolipidemic activities of *Helicteres isora* root extracts

P 486

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Helicteres isora Linn (Sterculiaceae) is a sub deciduous tree bearing the fruits in a peculiar twisted form. The roots and fruits possess expectorant, demulcent, astringent, antigalactagogue and other medicinal properties. Traditionally the juice of roots is claimed to be useful in cough, asthma, intestinal infections and diabetes(1). The antidiabetic and hypolipidemic activities of *H. isora* root extracts were evaluated in alloxan-induced diabetic rats by a prolonged treatment according to established method(2). The possible mechanism of blood glucose lowering action was investigated. It was found that the butanol and aqueous ethanol extracts (250 mg/kg; 10 days) has shown significant reduction in blood glucose, total cholesterol, triglycerides and urea levels. The beneficial effects of these extracts were supported with histopathological examinations of liver, pancreas and kidney. The treatment with *H. isora* root extracts in experimental animals showed comparable repair and regeneration of the cell structure compared to the necrosis in the alloxan induced diabetic control animals. These studies proved the antidiabetic activity of *H. isora* root beyond doubt. Further investigations are needed to isolate the active compounds.

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P 487 Evaluation of medicinal plants from Mali for their *in vitro* and *in vivo* trypanocidal activity

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Water, methanol and dichlormethane extracts prepared from various parts of 40 medicinal plant species from Mali were investigated for their trypanocidal activity against *Trypanosoma brucei brucei*. Of a total of 165 extracts tested *in vitro* in the Low Inoculation Long Incubation Test (LILIT), 24 extracts showed a high trypanocidal activity. Using the Long term Viability Assay (LtVA) for corroboration of the results of the 24 extracts, it was found that 15 samples had minimum inhibitory concentration (MIC) values > 100 µg/ml, 8 MIC values of 100 µg/ml and one MIC values of 50 to 100 µg/ml. So far, 4 extracts with MIC values ≤ 100 µg/ml were tested for antitrypanosomal activity in mice, experimentally infected with *T. brucei brucei.* Only the aqueous extracts of the leaves of *Terminalia avicennioides* Guill. & Perr. (Combretaceae) and of the stem bark of *Ceiba pentandra* (L.) Gaertn. (Bombacaceae) were able to reduce the parasitaemia in animals treated at the dose of 100 mg/kg b.w. (intraperitoneally, twice daily for 3 days) and of 150 mg/kg b.w. (orally, twice daily for 3 days), respectively. The reduction of parasitaemia was, however, statistically significant ($p = 0{,}002$) only in the case of treatment with *T. avicennioides*.

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P 488 A cytotoxic steroid saponin from *Dioscorea burmanica*

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Plants named 'Hua-Khao-Yen' are common ingredients in traditional cancer remedies in Thailand (1) and are best-selling medicinal plants in traditional drugstores. Hua-Khao-Yen was found to comprise at least five species, the rhizomes of *Dioscorea burmanica* Prain et Burkill (Dioscoreaceae) being the most common. The ethanolic rhizome extract showed high and selective cytotoxic activity against human large cell lung carcinoma (COR-L23), colon cell line (LS-174T) and breast cancer cell line (MCF-7) (IC_{50}= 7.04, 22.6 and 16.3 µg/ml respectively) using the SRB assay (2,3). Bioassay guild isolation used to isolate a cytotoxic compound. A known steroid saponin glycosides, gracillin (Diosgenin-3-O--β-D-glucopyranoside –(1→3) –[α-L-rhamnosyl (1→2) -β-D-glucopyranoside) was isolated from the ethanolic extract of *D. burmanica*. This compound showed specific cytotoxicity against colon and lung cancer cell lines (IC_{50}=4.34 and 6.07 µg/ml respectively) but less active for breast cancer cell lines (IC_{50}= 30.64µg/ml). This result could support using for cancer treatment.

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Cytotoxic effects of extracts of *Combretum*, *Terminalia* and *Pteleopsis* against some selected human cancer cell lines and BBCE endothelial cells

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The pantropical genera *Combretum* and *Terminalia* comprise some species with powerful anticancer properties. At least seven species of *Terminalia* have traditionally been used for the treatment of cancer (1). While 24 species of *Combretum* are well known in African traditional medicine, *C. zeyheri* Sond. is to our knowledge the only species used for the treatment of cancer (2). Combretastatin-A-4, a powerful cytotoxic stilbene, has been isolated from the bark of the South African bushwillow, *Combretum caffrum* (3). Combretastatin-A-4 inhibits cell division by inhibiting the polymerization of tubulin. Combretastatin-A-4 is also able to elicit selective and irreversible vascular shutdown within solid tumors, leaving normal vasculature intact (4). In this work we have screened 30 extracts of nine species of *Combretum*, five species of *Terminalia* and *Pteleopsis myrtifolia* collected from Tanzania for their antiproliferative and cytotoxic effects in search for new active species by using the ethnomedical and chemotaxonomic approach. The antiproliferative and cytotoxic effects of the extracts were evaluated by counting the cells with a cell counter (Coulter counter, Beckman) and by using the Alamar Blue assay (Promega) which measures the mitochondrial activity of the treated cells. The most outstanding cytotoxic effects were obtained with a leaf extract of *Combretum fragrans* F. Hoffm. which inhibited the proliferation of the cellines used in this investigation with 85.9- 99.9 % when 50 µg/ml of the extract was used. The other extracts of *Combretum* and *Terminalia* (50 µg/ml) showed moderate or no cytotoxic effects. The mechanisms by which the strongest cytotoxic extract arrests the cell proliferation are going to be investigated with Hoechst staining and with Western blot gel electrophoresis in order to see eventual changes in the levels of enzymes promoting and blocking apoptosis.

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P 490 Vasorelaxant Effect of Mexican Medicinal Plants on Isolated Rat Aorta

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The vasorelaxant effect of methanol extracts (0.86-50 µg/mL) of *Iresine calea*, *Psyttacanthus calyculathus, Laelia autumnalis, Brickellia cavanillesii* and *Lepechinia caulescens*, plant species used in Mexican folk medicine for the treatment of hypertension and related diseases (1), were evaluated in isolated rat aortic rings (2). The extracts of *I. calea* and *P. calyculathus* did not show a vasorelaxant activity on norepinephrine-evoked contraction (NE, 1 x $10^{-7.5}$ M) in endothelium-intact (+E) and endothelium-denuded (-E) rat aorta rings. On the other hand, *L. autumnalis* and *B. cavanillesii* induced a concentration-dependent and endothelium-independent relaxation on rat aorta. However, the methanolic extract of *L. caulescens* produced a significant vasodilator effect in a concentration-dependent and endothelium-dependent manner. In order to determine the mode of the vasorelaxant action evoked by *L. caulescens*, the extract was evaluated in the presence of $_{L}$-NAME (inhibitor of nitric oxide synthase at 1 x 10^{-4} M) and indomethacin (inhibitor of cyclooxygenases at 1 x 10^{-5} M). Relaxation was blocked by $_{L}$-NAME, indicating the extract vasodilating properties are endothelium mediated due to liberation of nitric oxide.

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P 491 Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay

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Numerous plants are used traditionally to treat mental diseases in South Africa. Fifty six ethanol extracts from 39 plants, both indigenous and exotic, that are traditionally used predominantly as sedatives or to treat various CNS-related ailments, were tested in the $GABA_A$–benzodiazepine receptor binding assay, where the binding of ^{3}H-Ro 15-1788 (flumazenil) to the benzodiazepine site is measured. The $GABA_A$–benzodiazepine receptor complex is involved in sedation, epilepsy and convulsions. Of the 56 extracts tested, 11 extracts showed activity. The most active extracts were the ethanolic leaf extracts of *Arctopus echinatus* (Apiaceae), *Helichrysum ruderale* (Asteraceae) and *H. umbraculigerum* (Asteraceae) which all showed good dose-dependent activity. None of the active compounds have been isolated.

Confirmation of Biological Activities of Prasaplai, a Thai Traditional Medicine

P 492

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Prasaplai is a Thai traditional medicine which is composed of ten medicinal plants, *Acorus calamus* L., *Allium sativum* L., *Citrus hystrix* DC., *Curcuma zedoaria* Roscoe, *Eleutherine palmifolia* (L.) Merr, *Nigella sativa* Linn., *Piper chaba* Hunt, *Piper nigrum* L., *Zingiber cassumunar* Roxb., *Zingiber officinale* Roscoe and two chemical compounds, sodium chloride and camphor (1). Prasaplai is widely used by Thai traditional doctors as a remedy for relieving dysmenorrhea and adjusting the cycle of menstruation, but it has not been proven scientifically (2). The purpose of this research was to confirm the efficacy of the Prasaplai by testing inhibition of rat uterine contraction, anti-inflamatory and estrogenic activities. Inhibition of uterine contraction of ethanol and aqueous extracts of Prasaplai was tested on rat uteri which were induced by acetylcholine ($2.04x10^{-4}$mg/ml), oxytocin ($1.54x10^{-4}$ mg/ml) and PGE_2 ($6.00x10^{-4}$ mg/ml). IC_{50} values of the aqueous extract of Prasaplai against acytylcholine, oxytocin and PGE_2 were 11.70, 10.04 and 5.75 mg/ml, respectively while of the ethanol extract were 2.09, 1.74 and 2.95 mg/ml, respectively. Prasaplai also promoted anti-inflammatory activity by inhibition of COX-1 and COX-2. Plasaplai extracted with oil, dichlorometane and hexane were tested. The hexane extract (25 μg/ml) could inhibit both COX-1 and COX-2 up to 64.43 and 84.50 %, respectively. Estrogenic activity was determined by comparing between rat uterine increasing weight of the control and subject groups. The results showed that Prasaplai did not have estrogenic activity.

Acknowledgements: The Royal Golden Jubilee Ph.D. Program, the Thailand Research Fund (TRF), the German Academic Exchange (DAAD).

References: 1. Poomchusri NT.(1973) Ayurvedic study 2 nd ed. Bangkok: Promjakkanpim, p194. **2.** Nation list of essential drugs committee. (1999) National list of essential drug A.D. 1999 (List of herbal medicine products), p.2.

Flavonoids of *Ailanthus excelsa* (Roxb) and biological evaluation

P 493

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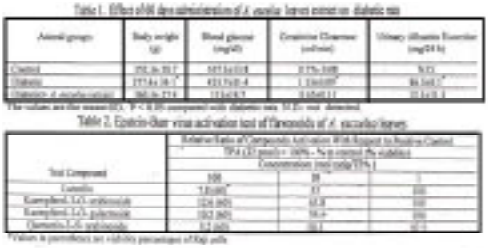
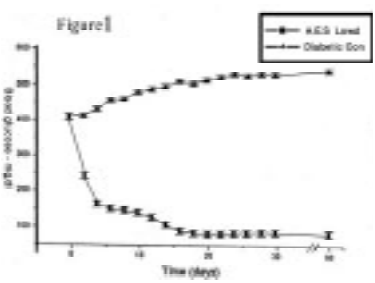
Flavonoids Isolated from *Ailanthus excelsa* leaves (70%) methanol show hypoglycemic effect Table (1) Fig. (1).using Manuel et al. [(1)] method . Six flavonoids were isolated for the first time, vitexin was isolated previously[(2)]. Significant decrease in blood glucose level, creatinine and urinary albumin in diabetic rats, no effect in normal rats. Four flavonoids were tested against Epstein Barr-virus early antigen activation Table (2), quercetin-3-O-arabinoside was the most active one.





(1) Manuel, J.A. et al. , (2001), J. Ethnopharmacology, 74, 125.

(2) Kapoor, S. K., et al., (1971), Phytochemistry, 10, 3333.

P 494 Theoretical fundamentals of Islamic Medicine

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Islamic medicine is a holistic and regular system which primarily comprises two main parts: theoretical and practical. This article presents general principles of theoretical part of this system which itself includes theoretical and practical aspects. The first aspect consists of four important points dealing with natural affairs, states of the body, causes and symptoms. Natural affairs have been abstracted in below diagram.
States of human body includes health, disease and the intermediate states. Causes contain the obligatory causes which are weather, food and drinks, physical and psychic movements and conditions of rest, depletion and retention, sleep and wakefulness. Symptoms indicate one of the three states of the body already mentioned i.e. health, disease and the intermediate states. The practical part of medicine is of two kinds:
a) Knowledge of regulating the body so as to maintain its health. It is called hygiene.
b) Knowledge of managing the diseased body and the methods of restoring it to health. It is called the Knowledge of treatment and comprises three parts: administrations, drugs and manual operations.

Reference: 1. Avicenna (1993) Al-Qanun fi'l-tibb. Jamia Hamdard. New Delhi. **2.** Ibn Nafees (2001) Al-Mujaz fi'l-tibb. Al-ahram. Cairo.

HPLC-DAD-MS analysis of phenolic constituents from several extracts of verbena and lemon verbena

P 495

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Verbena (Verbena officinalis L) and lemon verbena *(Lippia citriodora* Humb. B. et K.) are species which contain several flavonoids and phenolic acids (1,2). In view of the pharmacological interest in natural phenolic compounds as antioxidants, this study examined the chemical composition of a commercial aqueous extract (1:4 D/E) of verbena and two extracts obtained from the herbal drugs, the EtOH one and the lyophilized decoction. Chromatographic HPLC 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^{-1}. 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 5 extracts. The lyophilized decoction of verbena was the richest extracts and 15 constituents were identified, mostly of them represented byn verbascoside and its analogues, besides luteolin derivatives and a few iridoids. The commercial extract of verbena presented a very similar HPLC profile with a total of 11 constituents. Verbena EtOH extract showed the presence of elenoic acid, verbascoside, isoverbascoside and two derivatives. The HPLC profiles of EtOH and the lyophilized decoction of lemon verbena were very similar to the EtOH extract of verbena. All the extracts showed as principal components verbascoside and its analogues. It was very interesting to note that the commercial aqueous extract of verbena contained less than 0.5% total verbascoside and its analogues, while a similar content (about 3%) of verbascoside and its analogues was found in both the lyophilized decoctions. The EtOH extracts showed the highest percentages of verbascoside and its analogues: ca. 10% in verbena and ca. 21% in lemon verbena.

Acknowledgments The financial support of MIUR (PRIN 2004) is gratefully acknowledged.
References: **1.** Deepak, M., Handa, S.S. (2000) Phytother. Res., 14: 463-465. **2**. Valentao P. et al. (2002) Biol. Pharm. Bull., 2: 1324-27.

Preliminary investigation on monoterpene and phenolic profile in leaves of different Italian populations of *Rosmarinus officinalis* L.

P 496

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The objective of this preliminary study was to investigate the terpene and phenolic composition of *Rosmarinus officinalis* L. Leaf samples of were collected from different populations in Tuscany. Chiral and non chiral monoterpenes were determined by means of GC analysis and the identification of the constituents was obtained by comparison with pure standards. The major components of the essential oils were a-pinene, camphfene, b-pinene, myrcene, limonene, 1,8-cineole, camphor, linalool, and borneol. Analysis of terpene profiles showed variation in the monoterpene composition between the different provenances .
The HPLC/DAD/MS analysis of the phenolic fraction was applied, after extraction with ethanol and ethanol/water, with the help of ultrasounds. A rapid method was optimized to detect in the same extract more then 20 different components, among them rosmarinic acid, rosmanol and analogous compounds, and flavonoids.
These data warrant further studies on geographic variability of the terpene and phenol composition of essential oils as well as of the more polar phenolic compounds of *R. officinalis* in order to select superior chemotypes that can be used for commercial applications in pharmacy, food industry and flavoring industries.

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P 497 Assessment of genetic diversity and estimation of caffeoyl quinic acids and flavonoids in *Cynara cardunculus* L.

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The species *Cynara cardunculus* L. is native to the Mediterranean basin and belongs to the *Asteraceae* family (*Compositae*). It includes three taxa: globe artichoke (*C. cardunculus* L. var. *scolymus* L.), cultivated cardoon [*C. cardunculus*. L. var. *altilis* (DC)] and wild cardoon [*C. cardunculus* L. var. *sylvestris* (Lamk) Fiori]. Recent molecular studies (**1**) demonstrate that wild cardoon is the ancestor of both cultivated forms, which presumably evolved separately as a result of different selection criteria. Globe artichoke was selected for the production of heads or capitula (immature inflorescences), while cultivated cardoon for the production of fleshy leaf-stalks.
By means of amplified fragment length polymorphism (AFLP) markers (**2**) we evaluated genetic relatedness among 124 *Cynara cardunculus* accessions. Genetic similarities were calculated according to Jaccard's Similarity Index and used to construct a dendrogram based on the unweighted pair group method using arithmetic averages.
The lowest JSI value of 0.238 was detected between an accession of wild cardoon and one of artichoke (variety 'Terom'). Wild and cultivated cardoon accessions were also highly genetically differentiated, and the lowest JSI value detected was 0.327. Within artichoke, the smallest JSI value (0.373) was found between the varieties 'Terom' and 'Spinoso di Palermo'.
On the basis of molecular data a limited number of accessions (nearly 30 samples, analysed in triplicate) representative of the genetic variation were assayed in order to estimate leaf polyphenolic composition by HPLC/DAD and HPLC/MS. Mono and di-caffeoyl quinic derivatives, apigenin and luteolin glycosides were characterised and quantified by HPLC (**3**). Three compounds whith the quinic acid moiety esterified with succinic acid were firstly identified in the leaves of *C. cardunculus* L..

References: 1. Lanteri, S. et al. (2004) Theor. Appl. Genet. 108: 1534-1544. **2.** Vos, P. et al. (1995) Nucleic acids Res. 23: 4407-4414. **3.** Mulinacci N. Et al. (2004) J. Pharm. Bio. 34: 349-357.

P 498 New insights and a new HPLC method for the determination of anthraquinones in Radix Rhei

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Since the current photometric method used by the European Pharmacopoeia for the determination of anthraquinones in Radix Rhei is time consuming and hard to automatize, we have developed an HPLC method, as it was also suggested for senna leaves and fruits (1,2). Using an optimized reversed phase system, the individual hydroxyanthranoid glycosides and aglykones can be separated within 35 min. Since the ratio of glycosides and aglykones is relevant from an activity and pharmakokinetic point of view (3), it is suggested to calculate the sum of glycosides and the sum of aglykones. From the analyses of ca. 90 batches it was apparent that glycosides are dominant in regular rhubarb roots. Comparing the results of the classical photometric method with the HPLC method, no clear correlation could be found, indicating that the HPLC method leads to more relevant results.
During the validation of the extraction method we found out, that the yield of glycosides is highly dependant on the water content of the solvent. From the results it was clear that enzymatic hydrolysis takes place within minutes, when using aqueous solvents. Considering this intensive enzymatic activity in rhubarb roots, determination of glycosides is highly needed in order to get information on the hydrolysis status of the raw material.

References: 1. Kabelitz, L., Reif, K. (1994) Dtsch. Apoth. Ztg. 134, 37-40. **2.** Metzger, W., Reif, K. (1996) J. Chromatogr. A, 740, 133-138. **3.** Lee, J.H., Kim, J.M., et al. (2003) J. Ethnopharmacol. 84: 5-9.

On-line HPLC detection of inhibitors of the human cytochrome P450 1A2 enzyme

P 499

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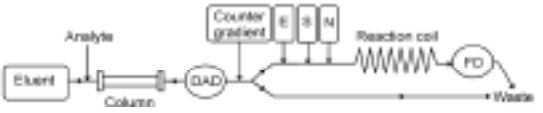
DAD (diode array det.); FD (fluorescence det.); E (enzyme); S (substrate); N (NADPH)



An on-line HPLC screening method for the detection of inhibitors of the enzyme cytochrome P450 1A2 (CYP1A2) in plant extracts was developed. An HPLC column is coupled with a diode array detector and a continuous bioassay, that is based on the methoxyresorufin O-dealkylation assay. It uses recombinant human CYP1A2 enzymes (Supersomes™) to convert methoxyresorufin to its fluorescent metabolite resorufin in the presence of NADPH. Analytes that inhibit CYP1A2 and thus decrease resorufin production are detected by fluorescence detection as negative peaks. The detection limits for the CYP1A2 inhibitors and/or substrates furafylline, tacrine, fluvoxamine and propranolol were ≈ 30 ng. This method provides rapid information about the inhibitory activity of individual constituents in complex mix-tures. Its application to vege-tables or herbs used in our diet will detect naturally occurring 1A2 inhibitors which could modulate the formation of carcinogens or are involved in drug interactions.

Dereplication of Natural Products using HPLC-DAD-SPE-NMR

P 500

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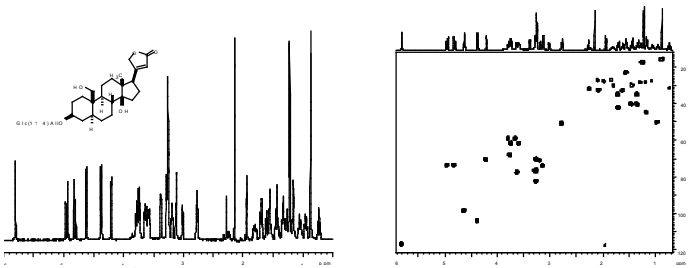

Figure 1. 600 MHz ^{1}H NMR and HSQC spectrum obtained from a K. laniflora extract

Extracts of natural origin usually contain a range of chemically diverse constituents occurring in varying concentrations. This poses a number of analytical challenges for the rapid identification of constituents of these inherently complex mixtures. The development of hyphenated techniques, particularly HPLC-NMR, has greatly increased the analytical capabilities in natural product research. The recent introduction of on-line solid phase extraction (SPE) in HPLC-NMR has further enhanced the sensitivity of this technique by enabling multiple peak trapping and concentration of the eluted analytes in a highly sensitive, small-volume NMR flow-cell. This improves the S/N ratios in the NMR spectra and facilitates the acquisition of ID and 2D NMR data, necessary for structure elucidation. The potential of HPLC-DAD-SPE-NMR hyphenation is demonstrated by structure determination of a range of complex constituents from various plant extracts. The technique is shown to allow acquisition of high-quality 1D and 2D NMR data (Figure 1) following analytical-scale HPLC separation of crude plant extracts, thereby enabling rigorous structure determination of known as well as previously unknown constituents. Examples include dereplication of extracts of *Perovskia atriplicifolia*, *Kanahia laniflora*, *Smirnowia iranica*, *Hubertia ambavilla* etc. It is believed that the HPLC-DAD-SPE-NMR technique will be an increasingly important analytical platform in natural product research and in other areas where rapid structural analysis of complex mixture constituents is required.

P 501 LC-NMR/LC-MS analysis of 2,3,10,11-oxygenated protoberberine alkaloid metabolites in *Corydalis* cell cultures

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Protoberberine alkaloids differ from each other in the number and placement of various oxygen functions on the aromatic rings. The two oxygenation patterns most frequently are oxygen atoms at carbons 2,3,9,10 and 2,3,10,11. The former is the most commonly occurring type, while the latter has been labeled "pseudoprotoberberine", and is not as widespread in its occurrence(1). Some representatives of the 2,3,10,11-oxygenated alkaloids display higher activity in some biological testa than the corresponding 2,3,9,10-substituted alkaloids(2). While the biosynthetic conversion of the 2,3,9,10-oxygenated protpberberines into other alkaloidal types, such as the protopines, benzophenanthridines, rhoeadines, benzindanoazepines, and spirobenzylisoqyinolines has been demonstrated(3,4), no studies on the biosynthesis of 2,3,10,11-oxygenated protoberberines have been presented, in spite of their occurrence in some plant species including *Corydalis* species(1). Now the metabolism of 2,3,10,11-oxygenated protoberberine alkaloids was studied in cell cultures of Corydalis species. Without prior isolation, the structures of the matabolites were determined by LC-MS and LC-NMR snalysis(5). Tetrahydropseudocoptisine a-N-metho salt, pseudoprotopine, and pseudomuramine were identified for the first time, and preliminary evidence for matabolic pathways to the formation of these alkaloids were obtained.

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P 502 Quantitative HPLC analysis of indigotin and indirubin in *Baphicacanthus cusia* root

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A method for the quantification of indigotin and indirubin in *Baphicacanthus cusia* was developed and validated through HPLC. Standardized solutions of indigotin and indirubin were used, from which the parameters of linearity, precision, accuracy, specificity, and robustness of the method were evaluated (1). A reversed-phase high-performance liquid chromatographic separation and quantitative method using methanol and water (73:27) isocratic elution was developed to analyze the isomeric active compounds, indigotin and indirubin, present in the ethyl acetate extract of *Baphicacanthus cusia* root. The results obtained showed that this approach present a good linearity ($r^2 > 0.999$) of between 0.02 and 0.14 μg/ml, precision (R.S.D. values lower than 5% for both compounds) and accuracy (96 and 98 %recovery for indigotin and indirubin, respectively). In addition, the method possessed specificity and robustness.

Acknowledgements: Faculty of Pharmaceutical Sciences, Prince of Songkla University
Reference: 1. Snyder, L.R., Kirkland, J.J. (1997) Practical HPLC Method Development. John Wiley & Sons, Inc. Canada.

Comparative TLC and HPLC analysis of phenolic compounds in E*quisetum palustre* extracts

P 503

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Flavonoids are considered as the most pharmacologically active compounds in *Equisetum* species. In the frame of our study of chemical and pharmacological properties of *Equisetum* species in Serbia, here the results of comparative HPLC-DAD and TLC analysis of phenolic compounds of *E. palustre* are presented. For the analysis, crude MeOH extract was separated into the three following fractions - EtOAc, n-BuOH and H_2O. HPLC was carried out using previously described method (1). All examined extracts show considerable qualitative and quantitative differences.
In the EtOAc extract two kaempferol glycosides assumed as kaempferol 7-O glucoside and kaempferol 3-O rhamnoside as well as one phenolic acid, most probably di-*E*-caffeoyl-*meso*-tartaric acid have been detected. Few more polar glycosides were detected by the TLC. In the n-BuOH extract kaempferol mono- and di- glycosides were present in considerable amounts. By the TLC some kaempferol tri- glycosides and phenolic acids were also detected. In the H_2O extract, phenolic acids were abundant compounds. Three phenolic acids (probably monocaffeoyl-*meso*-tartaric and one hydroxycinnamic acid) were present in this extract. The dominant flavonoids were kaempferol di-glycosides.

References : 1. Viet, M. et al. (1995) Phytochem. 38 : 881-891

Determination of phenolic glycosides and saponins in *Primula elatior* and *P. veris* roots by HPLC using ELSD and MS-detection

P 504

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Preparations of *P. elatior* L. (oxlip) and *P. veris* L. (cowslip) roots are common remedies for the treatment of respiratory tract problems [1]. Triterpene saponins (e.g. primulasaponin I and II) are considered to be responsible for the secretolytic and secretomotoric effects of the plants, which also contain characteristic phenolic glycosides (primulaverin, primeverin) and flavonoids [1, 2].
The first liquid chromatographic method suitable for the simultaneous analysis of all quality or activity related compounds in primula root is described. Optimum separations were obtained with a Synergi 4 µm Fusion RP 80 Å column, using 0.025 % TFA and 5% acetonitrile in methanol as mobile phase. Saponins were detected by evaporative light scattering detection (ELSD), whereas the phenolic glycosides were monitored by UV at 210 nm. The method was validated for repeatability ($\sigma_{rel} \leq 4.5$ %), precision (intra- and inter-day variation ≤ 5.0 %), accuracy (recovery ≥ 97.1 %) and sensitivity (LOD ≤ 22 ng (UV) and 53 ng (ELSD) on-column, respectively). LC-MS experiments in negative APCI mode allowed a final peak assignment. Both *Primula* species could easily be differentiated by their saponin pattern. The total saponin content was highest in *P. veris* roots (max 14.8 %), the aerial parts or *P. elatior* contained significantly less; primeverin (0.64 - 1.42 %) showed to be the most dominant phenolic glycoside.

Acknowledgements: Bionorica AG, 92318 Neumarkt, Germany for financial support
References: 1. Hänsel R. et al. (1994) Hagers Handbuch der Pharmazeutischen Praxis – Vol. 6, Springer, Berlin. **2.** Karl C. et al. (1981) Planta Med. 41 (1): 96-99

P 505 A combined HPLC/ESI-MSMS and HPLC/UV-DAD method for the identification of lignans

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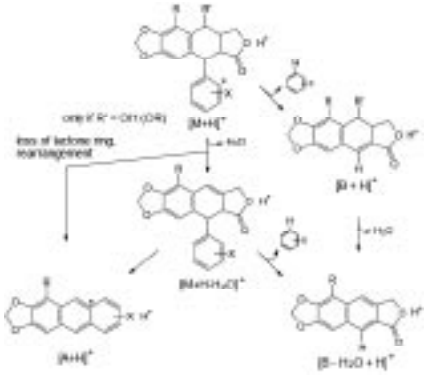

In the course of our chemosystematic study (1) of Lignans in *Linum* species, an analytical method for the fast and unambiguous identification of Lignans was developed. The methodology is based on analysis of the crude DCM extracts by means of HPLC/UV-DAD and HPLC/ESI-MSMS. Analysis of 25 reference lignans of the aryltetralin-, arylnaphthalene-, dibenzylbutyrolactone and diphenylfurofuran types led to the establishment of common fragmentation schemes for the different lignan groups (e.g. figure: aryltetralins of diff. substitution), allowing for structural identification of unknown constituents in plant extracts.

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P 506 Phytochemical investigation of lignans from *Linum tauricum* ssp. *bulgaricum*

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Naturally occurring lignans or neolignans have been used as a template for the development of many potential new therapeutic agents (1). In continuation of our research on lignans in *Linum* species, we have investigated *Linum tauricum* Willd. ssp. *bulgaricum* (Podp.) Petrova (Linaceae), which is an endemic subspecies to Bulgaria The phytochemical investigation of the aerial parts of *Linum tauricum* ssp. *bulgaricum* led to the isolation of five aryltetralin lignans by means of semi-preparative HPLC: podophyllotoxin, 6-methoxypodophyllotoxin, 4′-demethylpodophyllotoxin, 4′-demethyl-6-methoxypodophyllotoxin and isolariciresinol. LC-MS and NMR studies were used to characterize the structures of the isolated compounds. 6-Methoxypodophyllotoxin is reported as a common component of many *Linum* species (2) while podophyllotoxin, 4′-demethyl-6-methoxypodophyllotoxin, 4′-demethylpodophyllotoxin and isolariciresinol are not previously isolated from *L. tauricum* (3). The results from our phytochemical study approve the assumption that aryltetralin lignans are characteristic for the section *Syllinum* of the genus *Linum* (4). Our phytochemical findings in *Linum tauricum* ssp. *bulgaricum* suggest that lignans with a 3,4,5-trimethoxy substitution in the pendent aryl ring and those with a 4-hydroxy-3,5-dimethoxy-substituted pendent ring may undergo analogous reactions of oxygenation at C-6 and C-7. In addition, phytochemical results can cast light on the systematic classification of *L. tauricum* species.

Acknowledgments: FEBS Short-Term Fellowship is gratefully acknowledged for the financial support to N.P.Vasilev.

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A flavonoids from *Cirsium oleraceum* (L.) Scop.

P 507

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Cirsium oleraceum (L.) Scop. belongs to the family Asteraceae, since long ago the folk medicine uses the plant for treating a great variety diseases. Compounds from plant extract show an antimicrobial activity on the microorganizms. (1).
Several studies of the flavonoid chemistry of large genus *Cirsium* have appeared. (2). The herbs were extracted subsequently with MeOH and 50%MeOH. The combined extracts were concentrated under reduced pressure, treated with hot water and the resulted precipitate was filtred off. The filtrate was extracted with petroleum ether, ethyl acetate, ethyl acetate + 5% methanol and n- buthanol.
In this work we show chromatographic separation of the methanolic extract of herbs followed by isolation and identyfication flawonoids by TLC, employing diferent adsorbents (cellulose plates, polyamid and silics gel) and diferent mobile phases. Spots were observed in UV light (λ =366nm) before and after spraying with 1% solution Naturstoffreagenz A in MeOH.
The HPLC on A Hewlett– Packard, model 1100 liquid chromatograph equipped with a 20 µl sample injector (Rheodyne) and a variable UV –VIS wavelength DAD detector was also used. Flavonoids were identified by TLC and HPLC by comparison with standards spectrum.

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Quantitative determination of oenothein B in *Epilobium* extracts

P 508

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Willow herb (*Epilobium angustifolium* L., Oenotheraceae) is used in folk medicine for benign prostate hyperplasia (PBH) and associated problems of micturition. Oenothein B, dimeric macrocyclic ellagitannin, appears to be responsible for the pharmacological activity of *Epilobium* [1, 2]. Additionally, oenothein B has shown to have antitumor activity [3].
The aim of our work was to quantify oenothein B in aqueous and methanolic extracts prepared from Epilobii herba. The HPLC method (Cf [1]) and HPTLC-densitometry (a method developed by the authors) have been used. The results obtained by the both methods have been compared. The content of oenothein B determined by densitometry corresponded well to the one obtained by HPLC method.

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P 509 Metabolite profiling of Chinese liquorice root (*Glycyrrhiza glabra*) by ESI-MS/MS and LC-ESI-MS/MS

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Liquorice root (*G. glabra*) is used worldwide as a natural sweetener, as well as a flavouring additive for the preparation of sweet foods. On the other hand it is one of the most commonly used herbal drugs in traditional Chinese prescription. Anti-inflammatory, anti-viral, anti-allergic, anti-ulcer and anti-oxidative activities have been reported for liquorice extracts and preparations [1-2]. These activities have been attributed to the two classes of main constituents, saponins and flavonoids. The intake of high levels of liquorice extract is known to increase blood pressure, effect due mainly to glycyrrhizic acid [3], the main constituent of this root. Glycyrrhizic acid is also supposed to have chemo preventive activity and it is used clinically in patients with AIDS [4]. In literature there are several methods for the determination and quantification of glycyrrhizic acid in fresh, dried roots, root extracts, formulations of *Glycyrrhiza* species, and biological samples [5-6]. These methods are all related to the analysis of isolated glycyrrhizic acid, or of the corresponding aglycon after hydrolysis. Several saponins related to glycyrrhizic acid and different phenolic compounds occur in liquorice roots [7], and it is not to be excluded that some of them are involved in the liquorice roots activities. We have undertaken chemical evaluation of liquorice root considering the large number of secondary metabolites by using liquid chromatography hyphenated with mass spectrometry to obtain a full metabolite profiling of this drug. By using tandem in time mass spectrometry it was possible to characterize the compounds on the basis of their specific fragmentation. In preliminary studies *G. glabra* from China showed the most complex profile both in saponins and phenolic constituents. The differences observed in the metabolite profiles allowed us to recognize readily the origin of the plant, by using a fast analysis performed by direct introduction ESI-MS/MS; more in detail the different qualitative contents in metabolites among various samples were evaluated by using the LC-ESI-MS/MS method realized by a polarity switch.

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P 510 Determination of Amentoflavone derivatives by negative ion LC-MS

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Eight biflavonoid aglycones –amentoflavone and amentoflavone derivatives- from *Selaginella bryopteris* and *Juniperus communis* were analyzed with high performance liquid chromatography negative ion electrospray ionization mass spectrometry (HPLC-ESI-MS^n) with the aim to characterize their mass spectrometric behaviours in more detail.
Each compound was injected directly in to the ESI source by continuous perfusion. The $[M-H]^-$ ions were selected for fragmentation to produce MS/MS spectra. The prominent MS/MS ions were then selected for further MS^n analysis (n=3 up to 5). Collisions energy ranged from 50 to 55%.
The MS^n spectra obtained allowed us to propose plausible schemes for their fragmentation and to compare them with the known fragmentations of monoflavonoid aglycones (1). 375, 443, 417, 399 and 493 are prominent ions of amentoflavone obtained by the electrospray ionization. CO, CO_2, C_2H_2O, C_3O_2 and ring B are typical losses of biflavonoid and monoflavonoid fragments monitored in the negative ion mode (1). The fragments allowed us to distinguish the different amentoflavone derivatives.
Electrospray ionisation (negative ion mode) is powerful tool for the structural characterization of biflavonoid aglycones by LC-MS.

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LC-MS analysis of the Methanol-soluble Fraction of *Pelargonium sidoides* extract Eps® 7630

P 511

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Pelargonium sidoides DC (Geraniaceae) is a medicinally used plant indigenous to South Africa. A standardized ethanolic root extract, Eps® 7630 contained in the pharmaceutical product Umckaloabo® (licencee ISO, Ettlingen, Germany) is therapeutically used for the treatment of bronchitis, sinusitis and rhinopharyngitis (1-2). In order to gain insight into its composition, we have analysed a part of the phytochemical profile of Eps® 7630 by LC-MS. For this, Eps® 7630 was treated with MeOH to obtain a MeOH-soluble and -insoluble fraction containing, among other, polymeric polyphenolic compounds and polysaccharides, respectively. From the methanol-soluble residue a stock solution (5 mg/mL) was prepared. Analytical studies were performed by LC-MS using a Superspher RP-18 column (25 cm; 4.6mm i.d.) and a gradient of (A) H_2O and (B) acetonitrile, both with 0.1%formic acid, at a flow rate of 1ml/min (5 min. under isocratic conditions at 3% B, followed by a linear gradient from 3 to 50% B over 20 min., and finally to 90% B within 5min.). At the selected wavelength of 345 nm twelve major peaks were detected representing 90 % of the total area under the curve (AUC). Peak identifications were based on mass spectral data in combination with R_t values of our in-house reference library. The results showed that Eps® 7630 contains a series of highly oxygenated coumarins, gallic acid methyl ester, shikimic acid, its 3-O-gallate, and four individual proanthocyanidins. Among the coumarins scopoletin and umckalin recorded at R_t 19.51 min and 20.51 min, respectively, were found. Four known tri- and tetra-oxygenated coumarins were detected between R_t 14.5 min and 19.0 min including the unique umckalin-7-sulfate and umckaloside (umckalin-7-glucoside). The proanthocyanidins appearing between R_t 10.76 and 12.36 min displayed an $[M]^+$ at *m/z* 610, suggesting to represent dimeric prodelphinidins. Shikimic acid, gallic acid methylester and shikimic acid-3-gallate were recorded at R_t 2.45, 2.46, and 2.48 min, respectively. It should be noted, that all aforementioned components represent minor components of the standardized extract Eps®7630.

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Phenolic constituents from Aloe vera (*Aloe barbadensis* Mill.) flowers

P 512

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Aloe vera has become very popular in cosmetics and nutraceutical formulations due to the ascribed beneficial properties of the inner gel from the fleshy leaves (Aloe vera gel). The big yellow flowers of *A. barbadensis* are not of commercial interest yet although other *Aloe* flowers were shown to contain various biologically active substances (1, 2). The aim of this study was to identify the phenolic constituents of the flowers with regard to a possible antioxidative effect as described for the flowers of *Aloe vera* L. var. *chinensis* (2). The overall polyphenol content was determined as 1.11 % by the method of Folin-Ciocalteu, the flavonoid content amounted to 0.28 % (oxalic/boric acid). The methanolic extract of the dried and ground Aloe vera flowers (origin Andalusia, Spain) was investigated after a clean-up by liquid-liquid-partitioning.The identification of the phenolcarboxylic acids was accomplished by means of HPTLC, HPLC-DAD and LC-MS/MS. Chlorogenic acid, caffeic acid, *p*-coumarylquinic acid, caffeoylshikimic acid, feruloylquinic acid, *p*-coumaric acid and ferulic acid could be verified. Searching for anthranoids in the flowers, the methanolic flower extract was compared with a methanolic solution of the Curaçao-Aloe (Ph. Eur.), the laxative dried sap of the leaves. Aloin A and B, the main constituents and pharmacologically active components of the Curaçao-Aloe, could not be detected (HPTLC, HPLC, detection limit 1 ppm) and hence are not accumulated in the flowers. However, the main 2-alkylchromone of Curaçao-Aloe, Aloeresin B (=Aloesin), could be identified in the flower extract by HPTLC, HPLC-DAD and LC-MS.

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P 513 The LCMSMS analysis of guttation fluids for the presence of fungal alkaloids

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Perennial Ryegrass (*Lolium perenne)* is the most important pasture grass species in New Zealand. This plant grows in symbiosis with a fungus (endophye) that lives in the intercellular spaces of the grass. The endophyte lives from the grass and produces a range of alkaloids that protect the grass against grazing animals and insects. These fungi also produce ergot alkaloids and indole diterpenes that are toxic to livestock and also the unusual pyrrolopyrazine alkaloid peramine, which is not toxic to animals or insects, but deters insects from feeding on the plant. Peramine appears to be continuously produced by the endophyte, but does not accumulate. No mechanism for the removal of peramine by its further metabolism or any other process has been reported. We have developed a highly sensitive method for the analysis of peramine, using a linear ion trap mass spectrometer. We studied the fragmentation pathway of peramine using ESI MS^n, and developed a single reaction monitoring method using the fragmentation of the guanidinium moiety. This detection method was coupled to reversed phase (C18) liquid chromatography with H_2O/MeCN gradient, with 0.1 % formic acid. Ryegrass plants (Grasslands Nui) infected with a strain of the endophyte *Neotyphodium lolii* endemic in New Zealand, and plants free of endophyte were grown in the glasshouse. The guttation droplets from both plant types were collected each morning for several days. The fluid was stored at -20°C until analysis. The analysis of the guttation fluid showed the presence of peramine in the fluid at approximately 50 ng ml^{-1} for only endophyte-infected plants. We did not observe any presence of ergovaline or lolitrem B in guttation fluid, which are other predominant fungal alkaloids in ryegrass Apart from this presence of peramine, the chemical profiles of the guttation fluid, such as for amino acids, were very similar for the endophyte-free and endophyte-infected plants. The exudation of peramine into guttation fluid by the hydathodes suggests the involvement of transporter proteins in the membrane of hydathodes that are able to relocate peramine across the cell membrane. We speculate that the exudation of the insect feeding deterrent peramine to the exterior of the plant on the leaf surface represents a further example of the symbiotic nature of the endophyte-grass association.

P 514 Rapid Identification of Major and Minor Constituents of *Harpagophytum procumbens* using HPLC-DAD-SPE-NMR

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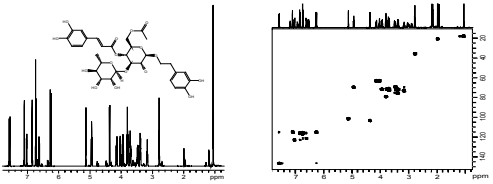

Figure 1. 600 MHz ^{1}H NMR and HSQC spectrum obtained in HPLC-DAD-SPE-NMR mode

The development of hyphenated techniques, particularly HPLC-NMR, has greatly increased the analytical capabilities in natural product research and has reduced the amount of time and resources required for structural characterisation of small amounts of natural products. The sensitivity of this technique has been further enhanced by the recent introduction of an on-line solid-phase-extraction (SPE) add-on. This enables multiple peak trapping and concentration of the eluted analytes into a small-volume NMR flow cell (30 μL). The increased sensitivity of this technique is displayed by the ability to acquire high-quality 1 and 2D NMR data following analytical-scale HPLC separation of extract constituents (Figure 1). The potential of HPLC-DAD-SPE-NMR is demonstrated by the identification of major and minor constituents of EtOH and PE extracts of *Harpagophytum procumbens* (Devil's Claw) (Pedaliaceae). *H. procumbens* is found in the north western parts of Southern Africa and is traditionally harvested from the wild. The plant is used locally to treat fevers, inflammatory and rheumatic conditions, and has gained international popularity for its potential as an anti-inflammatory agent. This study led to the identification of one unknown and six known compounds for the *H. procumbens* EtOH extract, and a series of closely related, previously unreported diterpenes in the PE extract. HPLC-DAD-SPE-NMR thus enables rapid identification of compounds in complex mixtures using only microgram quantities of crude plant extract. Not only is this technique applicable to the structure elucidation of known as well as unknown chemical constituents in plant extracts, it allows for the identification and quantification of secondary metabolites present in phytopharmaceuticals.

Fragmentation patterns of ergot and clavine alkaloids by electrospray ionization ion trap mass spectrometry

P 515

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Endophytic fungi in grasses produce a range of mycotoxins including the ergot alkaloids, which can cause serious toxicity to grazing animals. LC-MS-MS provides a powerful tool for the analysis of these mycotoxins in plant material by single or multiple reaction monitoring, particularly those lacking distinctive fluorescent properties. However effective use of LC-MS-MS to study ergot alkaloid biosynthesis and interpret data for novel compounds requires a good knowledge of the mass spectrometric fragmentation patterns and pathways of ergot alkaloids.
In this study we report detailed fragmentation pathways of several ergot peptides and clavines as determined with a linear ion trap mass spectrometer. Pure compounds and enriched fractions were infused into the mass spectrometer directly, and fragmentation pathways analysed in depth (up to MS^5). With these data we have been able to clarify previously ambigous analyses of fragmentation pathways based on either single or triple quadrupole mass spectrometry as the ion trap mass spectrometer yields more in depth fragmentation details.
The results show that in the fragmentation of the lygersic moiety of ergotamine, there are two pathways leading to fragment ion with 208 m/z, which yield different daughter ions. This shows that the two theoretical explanations of the occurrence of the 208 fragment are both correct. One mechanism is proposed by Lehner et al, and assumes that a violation of the even electron rule takes place by cleaving of CH_3 radical of the lysergic moiety (1). The fragments resulting from one 208 *m/z* fragment correspond to this theory. The fragmentation as proposed by Panaccione et al for lysergyl alanine, through a retro-Diels-Alder reaction losing CH_2NMe is correct as well (2). The use of appropriate selective mass fragmentation reactions to selectively detect ergovaline and several clavine alkaloids in endophyte-infected plant material is demonstrated

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Rapid SPE-guided Quantitative HPLC Determination of Cardenolides / Bufadienolides in Selected Homeopathic Mother Tinctures (2nd Communication)

P 516

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The selected homeopathic mother tinctures of *Digitalis* ∅, *Evonymus europaea* ∅, *Gratiola officinalis* ∅, *Strophanthus gratus* ∅, and *Scilla maritima* ∅ (Urginea) are monographed in the current German Homeopathic Pharmacopoeia (HAB 2004), however, only for *Strophanthus gratus* ∅ a quantitative determination is described. The chosen tinctures are utilised in homoepathy for a variety of disorders such as heart disturbances, headaches, or gastrointestinal complaints. In the context of establishing and harmonising current EU-monographs, it is necessary to create assays for efficient natural products, preferably recent HPLC-analysis.
In order to carry out a simple quantitative HPLC determination using reversed phase material and methanol / water mixtures as solvent systems, a rapid solid phase extraction (SPE, modified after [1]) for sample preparation is introduced [2]. The presented SPE procedures (sorbens: octadecyl-modified silicagel, C18ec; solvents: methanol, water, acetone) lead to samples which were directly or after evaporation used for HPLC.
The quantitative determination of the natural compounds was carried out by comparing retention times and AUC data with the values of authentic substances. The matching SPE fractions of the selected tinctures achieved satisfying mean, standard deviation *s*, and variation coefficient *v* values (e.g. Digitalis tincture/digitoxin: $s \approx 0{,}2$; $v \approx 1{,}6$).
The described rapid SPE-guided quantitative HPLC determination of natural products is little time consuming (< 2 h) demanding a small amount of material. It can easily be applied as a routine analytical method and might be considered as a possible quantitative determination in homeopathic pharmacopeia monographs.

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P 517 Determination of "Total Phenolics" in Echinacea Preparations: Comparison of the USP HPLC Method with the Folin-Ciocalteau Colorimetric Reaction

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The phytochemical composition of Echinacea preparations varies due to differences in the used species, the part of the plant used, the type of extraction and the time of harvest. (1) Due to this variability it is necessary to standardize and characterize the raw material and the preparations. Since there are different methods available for determination of "total phenolics" in plants, we studied their outcome and significance. Phenolic compounds like cichoric acid and echinacoside have been considered as markers of Echinacea and standardization of "total phenolics" has been suggested in USP. USP requires the identification and determination of phenolic constituents (chlorogenic acid, caffeic acid, echinacoside and cichoric acid) by high-performance liquid chromatography. In the Folin-Ciocalteu colorimetric method the content of total phenolics is expressed as gallic acid equivalents (GAE). We compared the two methods by analysing 11 different preparations from *Echinacea purpurea* MOENCH and *Echinacea angustifolia* DC. For the HPLC analyses the latest revision of "The United States Pharmacopeial Convention, Inc." with Chlorogenic acid used as reference standard was applied. Almost all extracts showed a higher content of total phenolics when using the colorimetric method. 9 of the 11 Echinacea preparations had an average content 30 times higher than determined by HPLC. Two preparations resulted in 0 % total phenolics by HPLC, but 0.07 % and 0.17 % by the colorimetric method. This shows that both methods do not lead to equivalent results. Since different compounds like sugars and alcohols adulterate the results of colorimetric analysis, the method of choice in the future should be the more specific HPLC determination as suggested also by Perry et al. (2) The term "total phenolics" should then be avoided because it is misleading.

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P 518 Isolation of ginkgolic acids from *Ginkgo biloba* leaves by centrifugal partition chromatography

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Extracts from the leaves of *Ginkgo biloba* L. belong to the most widely used phytotherapeutics [1]. Some alkylphenols (anacardic or ginkgolic acids, cardanols and cardols) have been identified as potential hazardous constituents of *Ginkgo* extracts. Ginkgolic acids found in *G. biloba* leaves mainly contain C13, C15 and C17 chains [2].
These compounds, besides strong allergenic properties, possess possible mutagenic and carcinogenic activity and do not contribute to the therapeutic of *Ginkgo* extracts. Accordingly a requirement for minimum concentration of these constituents has been included in the monographs of the EU and US pharmacopias by establishing a limit value of 5 ppm.
Recently, a simple HPLC-UV method has been developed for the quantification of ginkgolic acids in *Ginkgo* extracts [3]. In order to obtain the reference standards a pH-zone refining Centrifugal Partition Chromatography (CPC) using the unusual hexane/ethanol/water system has been carried out. Ginkgolic acids C17:1 and C15:1 were isolated from enriched fraction of *Ginkgo biloba* L. leaves.

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Preparative Purification of Natural Products using Centrifugal Partition Chromatography

P 519

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Centrifugal partition chromatography (CPC), a support-free liquid liquid partition chromatographic technique, has the advantage of no irreversible adsorption of a sample onto the solid matrix, and has been widely used in preparative separation of natural products. An efficient method for the preparative purification of lignans, an iridoid glycoside and a terpene lactone was developed using CPC. The two-phase solvent system composed of *n*-hexane-ethyl acetate-methanol-water (5:5:5:5) in a volume ratio was used to purify arctigenin and matairesinol from the crude extract of *Forsythia koreana*, a biphasic solvent system of *n*-hexane-methanol-water (10:7:3) showed efficient resolution for the purification of sauchinone from the *Saururus chinensis* extract. Geniposide, an iridoid glycoside, was purified with a two-phase solvent system composed of ethyl acetate-isopropanol-water (3:2:5, v/v) from 80% methanolic extract of *Gardenia* fruits in only one step. Bilobalide was purified with a two-phase solvent system composed of *n*-hexane-ethyl acetate-methanol-water (5:5:4:6 v/v). The results of our studies demonstrated that CPC is a useful method for the preparative separation of natural products.

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Diterpenes isolated from *Croton zambesicus* Muell Arg. inhibit the KCl-induced contraction on vascular smooth muscle

P 520

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The dichloromethane extract of the leaves of *Croton zambesicus* was purified using HSCCC and other chromatographic techniques to give 7 diterpenes: *ent*-trachyloban-3β-ol (**1**), isopimara-7,15-dien-3β-ol (**2**), *trans*-phytol (**3**), *ent*-trachyloban-3-one (**4**), *ent*-18-hydroxy-trachyloban-3β-ol (**5a**), 18-hydroxy-isopimara-7,15-diene-3-α-ol (**5b**), *ent*-18-hydroxy-trachyloban-3-one (**6**) (1,2).
ent-18-hydroxy-trachyloban-3-β-ol (**5a**) and 18-hydroxy-isopimara-7,15-diene-3-α-ol (**5b**) were isolated as a mixture. After treatement of the mixture with osmium tetroxide giving the hydroxylated derivative of the pimarane, we were able to separate the two compounds by preparative TLC.
As Croton zambesicus is used traditionnaly to treat hypertension, we analysed the effect of these compounds in vitro on KCl-induced contraction of rat aorta.
Compounds inhibited the *in vitro* contractility of rat aorta induced by KCl.
Their effect was analysed by cumulative concentration-response curves from 0.3 to 10 µg/ml on the rat smooth muscle.
ent-18-hydroxytrachyloban-3-one (**6**) and the mixture of *ent*-18-hydroxy-trachyloban-3β-ol (**5a**) and 18-hydroxy-Isopimara-7,15-diene-3-α-ol (**5b**) showed the highest activity with an $IC_{50} = 1.9$ µg/ml and 1 µg/ml respectively while *trans*-phytol did not show any effect.

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P 521 High yield isolation of destruxins A, B, D, E, and E-diol, the major depsipeptide derivatives of *Metarhizium anisopliae* by combining lipophilic Sephadex gel chromatography and HSCCC

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Destruxins (dtxs) are structurally closely related cyclic hexadepsipeptides, secreted by the entomopathogenic fungus *Metarhizium anisopilae*. Dtxs have been studied for more than four decades (1). Besides their role in fungal pathogenicity, a broad range of pharmacological activities– e.g. the prevention of osteoblast formation (2), the formation of ion-channels (3), or effects on heart muscle contraction (4) – have been reported. Common approaches for the isolation of dtx derivatives -involve column chromatography (CC) over silica gel or preparative RP-HPLC – both resulting in time consuming purification protocols. The need to obtain larger amounts of pure dtx derivatives as reference substances in analytical assays (5) and for pharmacological testing purposes, prompted us to investigate the possibilities of alternative purification protocols. A simple three step purification protocol combining liquid-liquid extraction, gel chromatography and HSCCC (high speed counter current chromatography) was established. The extraction of the fungal culture filtrate with DCM yielded a crude extract applied to Sephadex LH-20 CC. Chromatography with a step gradient protocol (DCM to DCM:acetone 1:1) yielded a mixture enriched with dtx A, dtx -B, and dtx E and additionally both dtx E-diol and dtx D as pure compounds (>95 % purity). The enriched fraction -was separated by HSCCC (solvent system: PE/EtAc/MeOH/H_2O) yielding dtx A, B, and E in >98% purity. The purity of the obtained derivatives was assessed by HPLC-DAD/MS and by 1D and 2D NMR experiments. With this experimental setup- a total of 519 mg dtxA (68 %), 309 mg dtx B (52 %), 402 mg dtx E (75 %), 22mg dtx E-diol, and 37 mg dtx D (% yields from the culture broth) were obtained from a 14 L submerged fungal culture broth batch within- six weeks.

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P 522 Essential oils of the aerial parts of three *Salvia* species from Jordan: *Salvia lanigera, S. spinosa* and *S. syriaca*

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Salvia is the largest and the most important genus of the family Lamiaceae. Plants belonging to this genus show high diversity in their secondary metabolites as well as in pharmacological effects. They are used for food, pharmacological and cosmetic purposes *(1)*. Here the composition of the essential oil of three spontaneous species of *Salvia* growing in Jordan has been reported, *S. syriaca* and *S. spinosa* which belong to the section Aethiopis Benth. and *S. lanigera* which belongs to the section Plethiosphace Benth.
S. lanigera is used by Bedouins as a condiment for tea (2), while *S. syriaca* is used as animal food (3), finally *S. spinosa* is used as medicinal plant against diarrhea, for pile, chest, stomach pain and against urinary disorder (4). The GC analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas. Eighty compounds were identified, accounting from 82.5% to 97.2% of the whole essential oils. Monoterpenes resulted the main class of the essential oil of *S. lanigera* (71.7%), followed by sesquiterpenes (21.7%) and phenylpropanoids (3.5%). Also in *S. syriaca* monoterpenes (68.0%) represented the main constituents. *S. lanigera* which is used as tea condiment contains thymol, this component with sharp smell gives characteristic taste for foods and drinks, beside thymol, other coumpounds are well known for their pleasant odor, such as cedrol (8.9%), methyl chavicol (3.5%), linalool (1.5%) and myrtenol (1.3%), these compounds are found as main component in the oil of some aromatic plants (Cedar wood, Basil, Coriander, Myrtle) which are used to prepare soft drinks, food additives and cosmetics *(1)*.

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Simplified isolation of major petasin derivatives from a lipophilic *Petasites hybridus* extract by a combination of HSCCC and chiral column chromatography over ~~-~~tribenzoyl-cellulose

P 523

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Petasites hybridus (Asteraceae), butter bur, is a widespread perennial herb in moist habitats throughout Central Europe. Its lipophilic erimophilane type sesquiterpene ester derivatives, especially petasin (=angeoyl-petasol) and congeners have been associated with most of the reported bioactivities. These include the application of petasin containing extracts against seasonal rhinitis or in migraine prophylaxis (1,2). Furthermore, the activity of petasin and S-petasin on a voltage gated Ca^{2+} channel hass been investigated recently (3). Currently procedures for the purification of petasin derivatives rely either on the use of column chromatography (CC) over silica gel or on straight phase preparative HPLC protocols (4-7). Since both approaches are known for substantial substance losses, alternative approaches to this promising substance class were pursued. A combination of high speed counter current chromatography (with hexane/ethanol/water as solvent system) as enrichment step and CC with tribenzoylated cellulose (CTB) as chiral stationary phase (methanol as mobile phase) as purification step allowed the reproducible isolation of petasin (227 mg, 8.1%), isopetasin (42 mg, 1.4%), and neopetasin (93 mg, 3.1%) from a commercially available CO_2 extract of *P. hybridus* leafs (PETZELL®). All constituents were of >95% purity and yields are calculated as percentage of ~~the~~ leaf extract. The purity of the obtained derivatives was assessed by rapid HPLC-DAD and HPLC-DAD/MS protocols (chromatographic cycle times < 10min) using a Phenomenex Synergy Max-RP column as stationary phase. Substance identity was proven by 1D and 2D NMR experiments and comparison with literature data (7).

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Purification of Soyasapogenols and Type-B Saponins by Kromaton Fast Centrifugal Partition Chromatography (FCPC)

P 524

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Centrifugal Partition Chromatography (CPC) is an effective yet underutilized tool for purifying metabolites produced by plants and microorganisms. CPC separates compounds based on differences in their partition coefficients for a given biphasic solvent system. This technique avoids traditionally encountered problems caused by solid chromatographic materials that adversely affect the purification and yields of labile compounds. A Kromaton FCPC was used to facilitate the purification of soyasapogenols (A, B, and E) as well as type B saponins. Under optimized conditions, slightly over a gram of a soyasapogenol mixture resulting from acidic hydrolysis, was injected into a 1-L Kromaton rotor and baseline separated into individual components. These soyasapogenols have very similar Rf values on silica gel, which makes their separation on this popular medium difficult to achieve. The collected fractions afforded 460 mg of soyasapogenol B, 380 mg of soyasapogenol A, and 30 mg of soyasapogenol E. In an unoptimized run, fractionation of 2.8 grams of a type B saponin mixture led to the purification of saponins B1 and B2 and to highly enriched fractions (50% – 80%) of saponins B3, B4 and B5. This method provides improved access to rare soyasaponins naturally occurring only at very low concentrations. The FCPC approach is well suited for isolating gram quantities of natural products often needed for pharmacological evaluations.

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P 525 Application of adsorption resin technology and FCPC chromatography for the recovery of natural antioxidants from olive oil mill waste water

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The high polyphenol content of the olive oil mill waste water (OMWW) is the major factor of the environmental problems caused by the olive mills. We designed and developed at pilot scale a system for the treatment of the OMWW with the aim to recover the polyphenols and reduce the environmental problems. The treatment system consists of four main successive individual wastewater treatment sections: The first section includes three successive filtration stages that aim at the gradual reduction of the wastewater suspended solids to 25 μm. The second section includes the pass of the filtered wastewater through a series of specialised adsorbent resins in order to achieve the deodouring and decolourisation of the wastewater and the removal/ recovery of the polyphenols and lactons content. The third section aims at the thermal evaporation and recovery of the organic solvents mixture, which has been used in the resins regeneration process, and finally the fourth section aims at the separation of the polyphenols and other contained organic substances using FCPC chromatography.
The final outcome of the whole procedure is

- an odorless yellowish wastewater with a 99.5% reduced content in polyphenols
- an extract rich in polyphenols and lactones with high antioxidant activity and high added value
- pure hydroxytyrosol

P 526 Biological comparative studies on three cyperus species growing in egypt

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A biological study was performed on the hexane extract of three *Cyperus* species tubers *viz. C. rotundus; C. papyrus* and *C. esculentus*, family Cyperaceae, growing in Egypt [(1)] to evaluate its hepatotoxicity and hepatoprotection activities using monolayer cultured rat hepatocytes technique [(2)]. In addition, comparative chemical analyses of its hydrocarbons and fatty acid constituents were performed using GC/MS. The results showed that *C. esculentus* was found to be nontoxic on hepatocytes and it possesses strong hepatoprotection activity. In spite of a strong hepatoprotection activity of *C. rotundus,* it possess highly hepatotoxic as same as *C. papyrus* that found to have a moderate activity. The unsaponifiable fractions of the three species were separately subjected to GC/MS. The results revealed that the main hydrocarbon, Cholestan-3-ol, 2-methylene ($C_{28}H_{48}O$, 0.17 %) was found in *C. rotundus* and the triterpenes; ß- Amyrin ($C_{30}H_{50}O$, 15.34 %) was found only in *C. papyrus* extract. The relative percentage of the unsaturated fatty acids were found to be 73.19%, 59.72%; and 49.37% in *C. esculentus, C. rotundus* and *C. papyrus* respectively. Linoleic acid (OMEGA-6) was found to be 27.52% in *C. rotundus*. However, it was 12.9% in *C. papyrus* and 9.95% in *C. esculentus*. Linolenic acid (OMEGA-3) was found in the three species where the high amount was detected in *C. papyrus* (2.80%) followed by *C. rotundus* (2.43%) and only 0.6% in *C. esculentus*. This is the first report of the presence of hepatoprotection activity and the GC/MS analyses of hydrocarbons and fatty acids constituents of tubers of the three *Cyperus* species growing in Egypt.

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Hydrodistillation-headspace solvent microextraction: a new and rapid method for essential oil analysis of *Lavandula angustifolia* Mill

P 527

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Lavender is one of the most useful medicinal plants. Commercially, the lavender provides several important essential oils to the fragrance industry including soaps, colognes, perfumes, skin lotions and other cosmetics. In food manufacturing, lavender essential oil is employed in flavoring beverages, ice-cream, candy, baked goods, and chewing gum. (1, 2).

At the present study, a new and rapid method involving concurrent headspace solvent microextraction combined with continuous hydrodistillation (HD-HSME) for the extraction and pre-concentration of the essential oil of *Lavandula angustifolia* Mill., into a microdrop is developed. A microdrop of *n*-hexadecane containing *n*-heptadecane (as internal standard) extruded from the needle tip of a gas chromatographic syringe to the headspace above the plant sample. After extraction for an optimized time, the microdrop was retracted into the syringe and injected directly into a GC injection port. The effect of extracting solvent, sample mass, microdrop volume and extraction time on HD-HSME efficiency were investigated and optimized. The constituents of the volatile oil were identified by calculation of their retention indices under temperature-programmed conditions for *n-alkanes* (C_6–C_{24}) and the oil on a DB-1 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature. (3) Quantitative data was obtained from FID area percentages without the use of correction factors. By this method, thirty-six compounds were identified. Linalool (32.8%), linalyl acetate (17.6%), lavandulyl acetate (15.9%), α-terpineol (6.7%) and geranyl acetate (5.0%) were found to be the major constituents. To the best of our knowledge this is the first report on using continuous headspace solvent microextraction coupled with hydrodistillation for investigation of essential oil components.

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The Chemical Composition of the essential oil from leaves and fruits of *Rhus coriaria*

P 528

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The dried fruits of *Rhus coriaria* L. used as pickles and spices in Iran. The essential oil obtained from the leaves and fruits of *R. coriaria* L. (sumac) (Anacardiaceae) by hydro-distillation were analyzed by GC & GC/MS. Identification of components was based on comparison of their mass spectra with those of authentic samples together with the Relative Retention Indices (RRI). Sixty-five constituents in the leaf oil (84.06%) and fifty-six constituents in the fruit oil (80%) were identified. The major components of the leaf oil were β-caryophyllene, caryophyllene oxide and cembrene, while major components in the fruit oil were β-caryophyllene, cembrene, (E, E)-2, 4-decadienal and α-terpineole.

P 529 Volatile Constituents of *Nepeta heliotropifolia* Lam.,*Menta mozzafarriani* Jamzad. and *Ziziphora persica* Bunge. three Labiatae Herbs Growing Wild in Iran

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Sixty-seven species of the genus *Nepeta* are found in Iran, thirty-nine of which are endemic (1). Some species of the genus are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge, emmenagogue and sedative agents (1). Many *Nepeta* species contain the diasteromeric nepetalactones, substituted cyclopentanoid iridodial derivatives, which are known as powerful attractants for cats (2). The main components of *Nepeta* spp. of Iranian origin are: 4a α, 7α, 7a α –*nepetalactone* (*N.cephalotes* and *N.racemosa*(3)), 4a α, 7α, 7a β –*nepetalactone* (*N.meyeri*), 1,8-cineole (*N.binaludensis, N.ispahanica*(4) and *N.denudata*), terpinen -4-ol (*N.asterotrichus*) and viridifloro (*N.makuensis*). Six species of the genus *Mentha* are found in Iran, Of which *M.mozaffariani* Jamzad is endemic plant.
The composition of the essential oils from three labiatae species of Iran: *Nepeta heliotropifolia* Lam., *Mentha mozaffariani* Jamzad., which are endemic to Iran and *Ziziphora persica* Bunge.obtaind by hydrodistillation were analyzed by GC and GC/MS.1,8- cineole (16.8%), 4a α 7α 7a β –nepetalactone (16.3%), *cis*-sabinene hydrate (16.1%) and linalool (11.9%) were the main components among the twenty-three constituents characterized in the oil of *Nepeta heliotropifolia* representing 92.8% of the total components detected. Twenty compounds were identified in the oil of *Mentha mozaffariani* representing 83.6% of the total oil with 1,8- Cineole (53.5%) was the major constituent. The oil of *Ziziphora persica* was characterized by higher amount of pulegone (27.8%), neomenthol (22.5%) and *p*-menth-3-en-8-ol (18.1%) among the thirty-four components comprising 94.7/% of the total oil detected. All three oils were richer in oxygenated monoterpenes than sesquiterpenes.

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P 530 The essential oil of *Portenschlagiella ramosissima* from Croatia, a rich source of myristicin

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Portenschlagiella ramosissima (Port.) Tutin [syn. *Portenschlagia ramosissima* (Port.) Vis.], fam. Umbelliferae, an aromatic plant from the Adriatic coast, has a limited occurence in Croatia and can only be found in Middle Dalmatia on limestone grounds. Previous investigations of the essential oil are limited (1-3) and afforded contradicting results concerning the content of phenylpropanoids in the oil obtained from the fruits. Hence it seemed to be interest to analyse the essential oil composition of a sample collected in Croatia in detail with special attention to myristicin and other phenylpropanoids. Isolation of the oil by hydrodistillation yielded 1.2 % from the ripe fruits. By GC-FID and GC-MS analyses on fused silica capillaries with polar and non-polar stationary phases, respectively, 16 compounds amounting to 97.1 % of the total oil could be identified. Major constituents were γ-terpinene (41.0 %) followed by myristicin (25.3 %) and p-cymene (14.5 %). Further phenylpropanoids, eugenolmethyl ether (0.7 %) and elemicin (0.2 %), were only present in small amounts. As a conclusion, our results confirmed the occurence of myristicin in *P. ramosissima* which is in contradicition to (3).
Aside from the psychotropic effect of myristicin, the major compound in nutmeg oil, additional effects such as a hepato-protective activity which could be partly attributed to inhibition of TNF-α release from macrophages (4) and an inhibition of benzo[a]pyrene-induced tumorigenesis (5) by myristicin have been reported.

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Determination of cysteine sulphoxides of *Allium* L. by a biosensoric flow injection method

P 531

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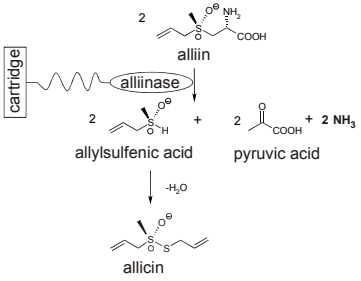

Figure. Enzymatic degradation of cysteine sulphoxides (*e.g.*, alliin) by immobilized alliinase. Enzymatically formed ammonia is detected by the fluorescent reaction with *o*-phthaldialdehyde.

Since ancient times onions, garlic and some other species of the genus *Allium* L. (leek) have been used as phyto-pharmaceutics, seasonings and vegetables. More recently, the medicinal benefits of these species were intensely investigated and lipid lowering, antibiotic, anti-atherosclerotic and anti-diabetic effects were discovered. Also a cancer-protective effect of onion and garlic was proven by a number of ethnic studies. The health benefits of *Allium* vegetables are mainly related to sulphur containing compounds. Precursors of all these sulphur compounds are cysteine sulphoxides. Therefore, wild *Allium* species containing a high amount of these compounds could be valuable medicinal plants. A newly developed biosensoric flow injection method, which is based on immobilized alliinase (Figure), allows a rapid determination of cysteine sulphoxides within 4.0 min. A set of bulbs collected in Iran was analysed by this method. Most remarkable were *A. xiphopetalum* and *A. scabriscapum*, both belonging to the subgenus *Rhizirideum*. They exhibited cysteine sulphoxide concentrations higher than 1 %, related to the fresh weight. Further on, two species, which could not be definitely determined yet, showed higher amounts than 0.6 % (*A*. sp. sect. *Codonoprasum*, *A*. aff. *griffithianum*).

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Determination and quantification of aromatase activity by TLC and phosphorimaging

P 532

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Aromatase found in a variety of tissues including fat and mannary tissue is responsible for estrogen synthesis. It is necessary for normal hormonal functions, but has also been implicated in mammary tumor growth. Aromatase inhibitors in plastics, plants and food have thus become well investigated as possible endocrine disruptors, on the other hand they are a basic treatment option in breast cancer and are investigated as chemopreventive agents. Quantification of aromatase activity usually depends on ^{3}H release using stereospecifically labelled substrates; with ^{3}H release instead of product formation measured and quantified. In order to directly detect the estrogen formation we have developed an alternative assay depending on the detection and quantification of estrogen or estrone separated from other steroids by thin layer chromatography and quantified by phosphorimaging. For test validation ^{3}H-labelled androstenedione (50 nM) was incubated with liver or placental microsomes; lipophilic compounds were extracted, dried and resuspended and the resulting mixture separated by thin layer chromatography. TLC plates were exposed to ^{3}H detecting phosphorimaging plates for up to 72 hours and the radioactivity in each spot quantified from the TLC plate readings. Product identity was confirmed with authentic standard compounds, the relative amount of each product was calculated from the signal densitiy after correction for background values. Since estrogen and other products are directly identified and quantified no specifically labelled substrate is necessary, other radiolabels like ^{14}C may be used in lieu of ^{3}H-labels which will further increase the sensitivity. The method is robust to interference by other enzymic activities, and assay results can be calculated directly from product formation. Additionally, use of TLC plates allows the parallel processing of multiple samples. We will present placenta aromatase enzyme characteristics determined by this method, as well as preliminary results from assay applications.

P 533 TLC analysis of free amino acids in *Valeriana officinalis*

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Valeriana officinalis L. is a perennial herbaceous plant, belonging to the *Valerianaceae* family. The drug Valerianae radix consists of subterranean parts of the plant including the rhizomes, roots and stolons, carefully dried below 40 °C. It contains volatile oil, iridoids, flavonoids, triterpenes, alkaloids, amino acids, salts, sugars and organic acids (1). Because there are only few data about free amino acids in this plant, a TLC as a rapid method was developed for its investigation. Leaves, stems, flowers and roots of *V. officinalis* were collected in the Pharmaceutical Botanical Garden "Fran Kušan" in June and October 2004. These plant parts were investigated in comparison with Valerianae radix from commercial origin. TLC method has been performed on cellulose and silica gel, using n-butanol – acetone – glacial acetic acid – water (35:35:10:20 V/V/V/V) as solvent system. Detection has been carried out by a ninhydrin reagent (2).
The TLC method proved that the amino acid composition depended on the plant part and its origin. Leaves and flowers collected in June contained more amino acids (alanine, phenylalanine, valine, tryptophan and tyrosine) in comparison with stalk and root. The root collected in October was richer in amino acids (glutamine, arginine, alanine and γ-aminobutyric acid) than the root collected in June, because in October the root became the deposit organ, in which the reserves of glutamine and arginine formed. The sample of root from commercial origin contained glutamine, arginine alanine and γ-aminobutyric acid.

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P 534 Quantification of total ginsenosides in ginseng powders by NIRS

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The potential of near-infrared reflactance spectroscopy (NIRS) for evaluating the total ginsenosides in the root powders of ginseng was examined as a possible alternative to high-performance liquid-cromatography (HPLC).
The analysis was performed using on a FT-NIR spectrometer (model MPA, Bruker.Optics) on a collection of 39 samples with known analyte concentrations obtained by HPLC, used as the reference method. Spectra were collected over the 800-2780 nm spectral range in diffuse reflectance using an integrating sphere equipped with a PbS detector. The calibration model was produced using partial least square (PLS) regression with 27 samples. Data was preterated using first derivative of log l/reflectance and multiplicative scattering correction in order to reduce physical effect of the scattering of the samples. The model was verified using cross-validation and an external set of 12 samples. Good calibration statistics are obtained for the predication of the different ginsenoside contents presenting suitable correlation coefficients (R^2=0,7), low root mean square errors of prediction (RMSEP < 0.43) and low root mean standard errors of cross validation (RMSECV) values. These investigations showed that NIRS can represent a promising alternative in the routine quality control of ginseng with several advantages over the traditional chromatographic methods such as rapidity and easiness of use, and not requirement of sample preparation.

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Mapping of *Allium* plants by NIR FT Raman microspectroscopy

P 535

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Raman spectroscopy principally allows to identify non-destructively various components in fresh plant material if characteristic key bands of the individual analyte molecules can be found in the spectrum [1,2]. Especially NIR FT Raman spectroscopy has been described as a valuable tool for *in vivo* investigations because fluorescence and thermal decomposition of the plant tissue can be reduced to a minimum. This paper demonstrates the special potential of vibrational Raman microspectroscopy for the study of *Allium* plants (*e.g., A. jesdianum* Buhse, *A. komarowii* Lipsky, *A. suworowii* Regel) to obtain detailed information about their microstructure and chemical composition. The Raman area mapping was done applying a suitable software package from Bruker, Germany, controlling an x-y stage directly connected with the Raman spectrometer. This technique allows to obtain 2-dimensional spectroscopic images of the investigated samples which can be matched with the corresponding visual image [3,4]. Raman mapping provides detailed information regarding the distribution of specific plant substances occurring in the investigated *Allium* bulbs, such as polysaccharides and the newly discovered dying compound dithiodipyrrole (2934 cm^{-1} and 1453 cm^{-1}). Based on these data not only qualitative but also semi-quantitative interpretation of the spectroscopic measurements is possible.

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Validation of spectrophotometric methods for determination of flavonoids in propolis

P 536

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The increasing use of propolis preparations now days requires adequate analytical procedures for quantitative determination of main active components (different polyphenols, including flavonoids and phenolic acids). Rapid spectrophotometric methods have been used for years and are useful methods for routine control of propolis samples.

The aim of this work was to improve and validate two spectrophotometric methods for determination of different groups of flavonoids in propolis. Flavones and flavonols form stable complexes with $AlCl_3$, while flavanones and flavanonols react with 2,4-dinitrophenylhidrazine (2,4-DNP). Both given methods were optimized using a mixture of flavonoids. Galangin was later used to make the calibration curve for the method with $AlCl_3$ (absorbance was measured at 415 nm), while pinocembrine was used as a standard for 2,4-dinitrophenylhidrazine method ($\lambda = 495$ nm). After the conditions were optimized, we have investigated the following validation characteristics: linearity, accuracy, repeatability, intermediate precision, stability, and selectivity. To prove that improved and validated methods are suitable for intended purpose, we have analyzed nine ethanolic propolis extracts from different regions of Croatia to establish the differences in their composition.

Investigated validation parameters for both methods satisfied the acceptance criteria for natural drug preparations (correlation coefficient ≥ 0.999; recovery – $AlCl_3$ method 99.17 – 101.25 % and 2,4-DNP method 98.67 – 101.46%; repeatability ($RSD_{(n=6)}$) 1.61% and 4.77%; intermediate precision ($RSD_{(n=18)}$) 3.21% and 4.03%). Tested colorimetric methods showed to be appropriate for establishing the differences in the composition of propolis samples (e.g. composition of propolis sample from peninsula Pelješac: mass concentration $\gamma_{(flavones, flavonols)} = 320.95$ μg/ml / RSD = 3.84%, $\gamma_{(flavanones, flavanonols)} = 546.49$ μg/ml / RSD = 3.28%; composition of propolis sample from central Croatia: $\gamma_{(flavones, flavonols)} = 54.87$ μg/ml / RSD = 1.93%, $\gamma_{(flavanones, flavanonols)} = 254.06$ μg/ml / RSD = 3.24%).

P 537 Determination of dietary fibre in some edible mushrooms from Macedonia

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Dietary fiber can play an important role in the prevention or treatment of various diseases and disorders i.e. obesity, diabetes, cardiovascular disease, colon cancer, diverticular disease and irritable bowel syndrome, constipation etc (1). Mushroom cell wall contains various indigestible components defined as mushroom dietary fibre. Little available informations mainly obtained by separately applied different methods intended for material of plant or animal origin greatly differ in their dietary fibre composition and content data. For that purpose, in several kinds of edible Macedonian mushrooms total dietary fibre (TDF) was isolated and determined by two parallel methods intended for material of plant (official AOAC method) and animal (by Hackman's method) origin. Analysis of the elements C, H and N on the TDF isolated components was performed by Pregl's and Dumas' procedures. Also, the chemical constitution of the both isolated components was investigated with the aid of infrared spectroscopy. The TDF contents of the mushrooms as measured by the AOAC method (av. 10.29% dry wt.) were considerably greater than those determined using the Hackmans method (av. 5.90% dry wt.). This survey indicates that about half kilo of fresh Macedonian mushrooms is more than enough to satisfy the daily dietary fibre intake (2). The content of the elements C, H and N of the TDF isolates, obtained according to two parallel-applied methods was very constant. C and H contents of both isolated products were very similar to chitin and cellulose. These products differ from cellulose as they contain N in lower quantities than pure chitin. The infrared spectra of the total dietary fibre isolates, obtained according to both applied methods, were similar and close to chitin. In all spectra cellulose is missing. Our finding for the absence of cellulose in mushrooms dietary fibre confirms the difference between the mushrooms and plants.

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P 538 Fructans in *Echinacea purpurea*: Determination of the degree of polymerisation (DP) by ^{13}C-NMR spectroscopy and silylation analysis

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Fructans are fructose-rich polymers derived from sucrose, and thus always contain one glucose residue. Besides starch, they are important storage polymers in numerous plant species (1). The structure and average degree of polymerisation (DP) of inulins, fructans with (2→1)-linked fructose residues, synthesized in storage organs of many species of Asteraceae is well documented for *Cichorium intybus* and *Helianthus tuberosus* (2,3), the main sources for inulin production. In many other species, however, fructan oligomers contain both, (2→1)- and (2→6)-linked fructosyl units and even some (2→1,2→6)-linked branch point residues (4). The types of fructan, their size and glycosidic linkages, vary markedly both between and within the families. Linear or branched fructans are found with DPs ranging from 3-12 in *Allium cepa*, 3-60 in *Cichorium intybus* (5) and up to 260 in *Phleum pratense* (4). Up to now, techniques commonly used for the determination of the DP of fructans include GPC, HPLC, enzymatic and different chemical methods (6). In our study, we used two methods, NMR and silylation analysis, for the determination of the DP, the latter convincing with the advantage of easy sample preparation and short analysis time. A highly water-soluble fructan was isolated from *Echinacea purpurea* roots by hot water extraction and precipitation with ethanol. It was found that the *Echinacea* fructan represents a linear inulin-type fructan. It has a (2→1)-linked ß-D-Fr*cf* backbone with only traces of (2→6)-linked ß-D-Fr*cf* residues. No branch point residues were detected. NMR data were confirmed by methylation analysis. The fructan shows an average DP of 20. Corresponding results were found with both methods demonstrating that these techniques provide sufficient sensitivity to determine not only the composition and structure but also the average DP of fructans.

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Binding studies of an arabinogalactan-protein (AGP) from *Echinacea purpurea* to leucocytes

P 539

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An AGP from pressed juice of the aerial parts of *Echinacea purpurea* is a high molecular weight glycoprotein (1.2x10^6 Da) with a highly branched carbohydrate moiety of >90% (w/w), mainly consisting of 3-, 6- and 3,6-linked ß-D-Gal*p* residues, substituted with terminal α-L-Ara*f* residues and terminal GlcA and has been shown to have complement-stimulating activities *in vitro* (1, 2). In order to get information about possible interactions of AGP with the immune system on a cellular level, we generated polyclonal antibodies against this AGP. Ara*f* residues form a key part of the antigen epitope for these polyclonal antibodies (3). Flow cytometric investigations show binding of the AGP to the cell surface of human leucocytes (lymphocytes, monocytes and granulocytes). Competition assays with two antibodies directed against CD4 and CD8 revealed no interaction of AGP with these receptors, leading to the conclusion that binding of AGP is mediated via different structures (4).

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Surface plasmon resonance (SPR) chip device for screening on lectins and binding of entire cells

P 540

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Lectins are proteins or glycoproteins from plants or animals, which are able to bind specifically sugar-residues of cell walls or membranes. This reaction changes the physiology of the cell wall and influences the metabolism of the cell [1]. Some lectins of plants stimulate the immune system by unspecific activation of T-cells or influence cell division; others cause agglutination of cells (e.g., erythrocytes) and are therefore of therapeutic interest [2].

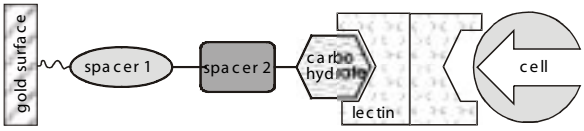

Figure. Scheme showing the different layers based on the gold surface, which were used for the immobilization of cells. The carbohydrate layer can be also used for the screening on lectins.

In a new approach, biomolecular interaction analysis (BIA) was utilized for a screening program on lectins. Such a BIA method is the so called "surface plasmon resonance" (SPR). SPR is a laser-based optical method, which allows the observation of binding processes and binding kinetics in a real time mode without any labelling steps. In a first step a lectin-binding carbohydrate was covalently immobilized on a surface of a thin gold layer (50 nm). A number of individual chips displaying complex carbohydrate surfaces, e.g., mannan, starch, hyaluronic acid and fucoidan, could be generated. Then test solutions containing different lectins were supplied and binding kinetics were recorded. Carbohydrate-lectin complexes were also successfully used for the immobilization of cells like cancer cells (Figure). Cells immobilized by this manner can be used for further pharmacological testing.

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P 541 Two newly developed test methods for the quantitative determination of cytotoxic activities of water soluble and not water soluble compounds by the use of cyanobacteria as test organisms

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Usual test systems for the detection of growth inhibiting or cytotoxic properties of natural and other compounds are diffusion tests carried out on agar plates or suspension test systems, carried out in test tubes or in microtiterplates alternatively. For the latter test systems, complex colouring procedures of the applied cell lines or organisms are in use. Photosynthesis prosecuting cyanobacteria represent expedient alternative test organisms in such test systems, because they are naturally of blue-green colour and cytotoxic activities of test compounds result in an easily recognizable decolourisation (1).
Against this background, at first a test system, carried out in microtiterplates, was developed for the detection of cytotoxic activities against cyanobacteria. Six cyanobacterial species, three filamentous and three unicellular strains, were screened as potential test organisms. Two of the filamentous species, namely *Arthrospira laxissima* and *Nostoc carneum*, were found to be suitable in particular. A decolourisation of suspensions of these organisms caused by cytotoxic concentrations of test compounds occurred within 48 h and was recognisable with the naked eye very well. Evaluation of the plates could be done also by the use of a standard microtiterplate reader. This solution test had one disadvantage: compounds, being poorly water soluble, could not be tested satisfactorily. Therefore, a second test system was developed, comparable to agar plate diffusion tests but much easier to perform: in this test a TLC plate was used as matrix and defined volumes of test compound solutions (containing organic solvents and/or water) were placed onto its surface by the use of a semiautomatic sample applicator (Linomat by CAMAG). After complete evaporation of the solvent the plate was sprayed with an aqueous suspension of the living cyanobacterial test organism and was kept moist afterwards. Cytotoxic concentrations of a test compound resulted in a regional decolourisation of the cyanobacterium. The species *Arthrospira laxissima*, *Nostoc carneum*, *Synechocysits aquatilis* and a *Synechococcus* species were found to be particularly suitable for this test. Substance caused regional decolourisations became visible within 48 h respectively 24 h in the case of *A. laxissima*.

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P 542 Determination of protein content in refined oil and allergenic potency

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No validated methods currently exist for measuring the total protein content of edible oils. The common oil refining processes lead to a high reduction of protein content by a factor 100 when compared with the crude plant oils (1). In our work proteins from refined oils were extracted by heating and mixing a saline solution; the protein content was determined by different methods after exhaustive dialysis. This approach was applied to different commercial edible seed oils including peanut, soy, maize and sun flower and soy and maize according to the requirement of Ph Eur. Electrophoretic analysis by 12 % SDS-PAGE (both Coomassie and silver stained) exhibited some peculiar bands corresponding to residual proteins extracted from each specific refined oil. The total protein content agreed (?) with that previously estimated ranging from 10 to 20 µg/100 ml of oil (2). From our findings, an higher protein content was obtained when we determined it by using the amino acid analysis. This method was fully validated and resulted highly sensible and specific. Moreover the amino acid analysis resulted more accurate in protein content determination with respect the standard colorimetric assays. Those results suggest that some peptides (ranging from 3500 to 10000 Da) are present in the oil in addiction to the 12 % SDS-PAGE showed proteins. Besides, amino acid patterns revealed a specific contribution for each component in different oils, suggesting the possibility of a sort of amino acid fingerprint. Since some allergic reactions (anaphylaxis) can be triggered by compounds contained in our samples (1,3), an immuno-recognition was performed by using sera with specific higher level of IgE in a protein Western-blotting detection. Preliminary data strongly support the idea that refined oils contain residue and immunoreactive proteins able to be specifically recognized by sera of susceptible patients.

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Study of the Phytochemical Variability of the Essential Oil of *Hypericum perforatum* in Relation to Vegetative Stage

P 543

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The composition of secondary metabolites of Saint John's wort (*Hypericum perforatum*, SJW) varies according to growth conditions and vegetative stage. We determined the quantity and qualitative composition of SJW oil by GC-MS at three vegetative stages: before flowering, at full flowering, and at the time of fruit formation.
Results: Whereas fresh and dry yield of biomass per hectare was highest at the time of fruit formation, SJW in full flowering gave the highest amounts of essential oil (0.35 ml/100 g of dry matter) as compared to earlier (0.12 ml/100 g) respectively later vegetative stages (0.16 ml/100g). Major constituents of the essential oil of SJW of all vegetative stages are bicyclogermacrene, α-cadinol and spathulenol. Bicyclogermacrene shows a distinct peak at full flowering as compared to early or late stages. β-Caryophyllene and γ-muurolene are only present in major amounts in early flowering, whereas α-cadinene is indicative for full flowering, and β-bisabolene respectively globulol for late flowering stages. γ-Eudesmol sharply decreases after full flowering. Longifolene is not present in full flowering, but present in very high amounts (18.71 respectively 21.99%) in early respectively late flowering. It can be used as a quality parameter for essential oils from SJW. The analytical results would suggest the stage of full flowering as the optimal harvesting time, in accordance to traditional use of SJW.

Stability Studies of *Bacopa monniera* Ethanolic Extract

P 544

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Bacopa monniera L. has been used for cognition enhancing and improving memory in Ayurvedic medicine. Its major constituents are triterpenoid saponins such as Bacopaside I, Bacopaside II, Bacopaside III, Bacopasaponin A, Bacopasaponin B, Bacopasaponin C, Bacopaside A_3. The alcoholic extract of B. monniera was used in many pharmaceutical formulations. However, the stability study of the extract has not been reported. In this study, we aimed at the stability studies of B. monniera extract in various temperatures using a saponin glycoside, Bacopaside I, as a marker for quantitative analysis. The quantity of Bacopaside I in the extract was analyzed using HPLC techniques. The experiments were conducted at the temperature of 5°C, 40°C, 60°C, and 80°C under the controlled relative humidity of 75% for 28 days. The heat-cool cycle was conducted at 4°C for 48 hours, then changed to 40°C for 48 hours at the total of 7 cycles under the controlled relative humidity of 75%.
The results revealed that at the temperature of 5°C and 40°C, the amount of Bacopaside I remained unchanged, while at 60°C and 80°C, the amount of Bacopaside I decreased gradually down to 83% and 45% after 28 days, respectively. The water sorption study at 5°C, 40°C, 60°C, and 80°C under the controlled relative humidity of 75% showed that the ethanolic extract adsorbed moisture slower at lower temperature, while at 80°C, the extract adsorbed the moisture up to the maximum level at 56% within 28 days. In conclusion, the ethanolic extract of B. monniera was highly hygroscopic which can take up moisture up to 56%. Keeping the extract in low temperature can significantly decreased the moisture uptake.

P 545 Amaranth Dye Adulteration in a Bilberry Extract

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HPLC analysis of a commercial Chinese Bilberry extract (*Vaccinium myrtillus*) certified as containing ~25% anthocyanins indicated a much lower anthocyanin content with a profile not consistent with that of bilberry. The major component present in the extract was identified after subsequent isolation and characterization as the dye Amaranth (FD&C Red NO 2, EU E 123).
Amaranth is a red to dark-red brown sulphonic acid based naphthylazo dye principally of synthetic origins but originally derived from the plant of the same name. Various uses of this compound have been as a colouring agent for food products and medicines, as a dye and as a chemical indicator. Use of amaranth in food products,drugs and cosmetics is banned in the USA and several other countries due to links to several health-related problems.
The sophistication of the adulteration is evident in two ways. Firstly a routine UV-VIS analysis of this extract yields a result consistent with the material having an anthocyanin content of ~25%. Secondly the UV-VIS scan profile of Amaranth and this adulterated extract closely mimics the profile of an anthocyanin-containing solution.
This example of deliberate adulteration and the UK recall (February 2005) of food products containing contaminated chilli powder adulterated with the carcinogenic food dye Sudan 1 reinforces the importance of Quality Control testing using appropriate methods rather than non-specific spectrophotometric assays.

P 546 Great Variability of Flavonoids and Furanocoumarins in Commercially Available Grapefruit Juices: A Source of Inconsistancy in Grapefruit-Drug Interaction Studies

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Grapefruit juice (GFJ) contains a variety of compounds that may both reduce atherosclerotic plaque formation and inhibit cancer cell proliferation [1-4]. However, GJF interacts with several oral prescription medications leading to elevation of their serum concentrations which have been associated with an increased frequency of dose-dependent adverse effects [5, 6]. The predominant mechanism for this interaction is the inhibition the cytochrome P-450 3A4 in the small intestine by flavonoids (naringin and naringenin) and the furanocoumarins (bergamottin and 6',7'-dihydroxybergamottin) derivatives. [7-11]. Therefore, the amount of flavonoids and furanocoumarins contained in juices may be an important factor influencing the mechanism and magnitude of the grapefruit-drug interaction. We investigated the contents of the flavonoids (naringin and naringenin) and furanocoumarins (bergamottin and 6'7'-dihydroxybergamottin) in commercially available and fresh squeezed grapefruit juices as well as in different tissues of grapefruit (peel, albedo, pulp, and seeds) by HPLC with UV detection. We did not detect naringenin in any of the grapefruit juices. However, a great variability of naringin (from 174.09 ± 4.72 μMol L^{-1} to 1491.78 ± 76.23 μMol L^{-1}), bergamottin (from 0.97 ± 0.01 μMol L^{-1} to 38.47 ± 1.46 μMol L^{-1}) and 6',7'-dihydroxybergamottin (from 0.220 ± 0.002 μMol L^{-1} to 28.47 ± 1.10μMol L^{-1}) in fourteen different brands of grapefruit juice was detected. The white grapefruit showed the highest concentration of naringin and both furanocoumarins located in the albedo and in the peel when compared with ruby red grapefruit. Findings from this study demonstrate the great variability of the polyphenolic profile in different brands and also lots of commercially available grapefruit juice and suggest the importance to consider the profile of compounds in the used juices before performing the interaction studies in order to correlate the effect observed to the concentration of each putative component responsible for grapefruit juice-drug interaction. *Acknowledgements:* CAPES, Brazil, for financial support

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Analysis of resveratrol containing extracts and botanical dietary supplements

P 547

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The stilbene derivative *trans*-resveratrol **1** has been shown to have chemopreventive activity against cardiovascular diseases and a variety of cancers in model systems (1, 2). Natural sources of resveratrol include grape skins, red wine and roots of *Polygonum cuspidatum* Siebold & Zucc.(Japanese Knotweed, Polygonaceae). Currently numerous extracts with specified amounts of resveratrol and botanical dietary supplements which are prepared thereof are on the market. However, roots of *P. cuspidatum* have also been reported as a rich source of free anthraquinones (emodin **2**, physcion **3**) (3). Hence, in our ongoing studies it seemed to be of interrest to analyse different extracts and preparations for the major stilbenes and for the occurence of anthraquinones. *Trans*-Resveratrol **1**, ε-viniferin **4** and the relevant anthraquinones, **2** and **3** were analysed by LC/PDA/MS methods. A commercial extract from grape skins contained above all **1** and **4** whereas other stilbene oligomers were present only in smaller amounts, anthraquinones were absent. Commercial extracts from *P. cuspidatum* and an extract of unspecified source contained **1**, but additionally **2** and **3**. Anthraquinones were identified by TLC (detection with ethanolic potassium hydroxide solution, Bornträger reaction) as well as by LC/PDA/MS analysis. The amounts of hydroxyanthracene derivatives in *Polygonum* extracts ranged from 148.7 mg/g to 266.8 mg/g. Hydroxyanthracene derivatives could also be detected in two botanical dietary supplements containing 1.9 and 2.8 mg of **2** as well as 0.3 mg and 0.4 mg of **3** per capsule, respectively. For comparison, as a laxative, doses of 20 – 30 mg hydroxyanthracenes are used (4). As a conclusion, there is a risk of hydroxyanthracene intake when resveratrol preparations made from *P. cuspidatum* (or from unknown sources) are used. Obviously no risk arises from resveratrol preparations made from grape skin extracts.

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Safety of genistein (Bonistein™): results from safety studies in dogs and phase I clinical trials

P 548

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Genistein possesses various pharmacological properties. Mainly, due to its tyrosine inhibiting and estrogen receptor modulating effects, it is currently under investigation as cancer preventive agent and therapeutic option for postmenopausal bone-loss and climacteric symptoms. Bonistein™ (synthetic genistein), is in development to provide a phytomedicinal alternative to classical hormone replacement therapy (1). The subchronic and chronic safety of Bonistein™ was evaluated in beagle dogs including a 4-week and a 52-week safety study with a 13 week interim sacrifice (2). Bonistein™ doses of 50, 150 and 500 mg/kg/day were administered orally in capsules. In conclusion, Bonistein™ was well tolerated and did not result in systemic toxicity. Effects of genistein on the reproductive tract at very high doses (♀ increased uterus weight, ♂ atrophy of testes and prostate gland) were functional in nature and are of a type that would be expected in view of the relatively weak estrogenic activity of genistein and, therefore, not considered to be adverse effects. In the 4-week study, the no observed adverse effect level (NOAEL) was considered to be >500 mg/kg/day and the no observed effect level (NOEL) be 150 mg/kg/day. For the 52-week study the NOAEL was >500 mg/kg/day; the NOEL 50 mg/kg/day. Until today, three clinical phase I Bonistein™ studies were conducted including 81 volunteers (43 f, 38 m): two studies with young healthy volunteers (n=69, age 18 to 40 yrs), and one study in healthy postmenopausal women (n=12, age 45 to 70 yrs). At all 53 adverse events (AE) were reported. Neither of the AEs were serious nor unexpected (unexpected means published as AE in a human intervention trial with a comparable, genistein containing product). 85% of the AEs were of mild and 15% of moderate intensity. 49.1% of the AEs were judged as likely related to *Bonistein™* administration. Most common AE was headache and slight fluctuation of pancreas enzymes lipase and amylase (only observed during repeated dosing). Both are well-know effects of highly concentrated, purified isoflavones and were also reported in other clinical studies (2, 3).

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P 549 Antioxidant Activity and Quality Control by HPLC Method of Constituents from the Rhizome of *Boesenbergia pandurata*

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Three flavanone, pinostrobin (**1**), pinocembrin (**3**) and alpinetin (**5**), a charcone, cardamonin (**4**) and β-sitosterol (**2**) were isolated from rhizome of *Boesenbergia pandurata* (Zingiberaceae). Physicochemical properties and spectroscopic techniques were performed for compound characterization. The crude ethanolic extract was determined the total phenolic content using caffeic acid as standard to be 10.6 ± 0.2 %w/w, tested for the radical scavenging activity showing EC_{50} values was 67.7 ± 2.6 μg/ml, however, was found to be related to the amount of **3** presented in the rhizome (EC_{50} = 297 μg/ml). The percent inhibition of autoxidation by linoleic acid assay showed to be 64.4%. An analytical method was developed to determine 4 flavonoids (**1**, **3** – **5**) and crude ethanolic extract, using HPLC technique. With an ODS column (RP-18) and diode array detection, the analytical condition was optimized in terms of resolution (Rs), capacity factor (k'), selectivity factor (α), and tailing factor (T_f). With isocratic mobile phase consisting of 0.5% acetic acid-acetonitrile (45:55), at a flow rate of 1.0 ml/min, detection at λ 280 nm, the compounds were well separated in a total analysis time of less than 30 min. Intra-day and inter-day of the method were 2 % and 3% RSD, respectively. The quantity control demonstrated an HPLC determination of major constituents (**1**), as maker. This method would allow for a fast and accurate standardization method for the main ingredients. Five different sources of dry rhizome of *B. pandulata* were then analyzed and evaluated for their antioxidation using DPPH assay.

Acknowledgements: Prince of Songkla University, Thailand
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P 550 Efficient determination of cysteine sulphoxides in onion (*Allium cepa* L.) applying new biosensor and HPLC-MS^2 methods

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Onion cultivars (*Allium cepa* L.) can be found in many regions of Europe, Asia, North America and Africa. It is one of the most important horticultural crops with an annual world-wide production of around 44 million tonnes [1]. It is known that cysteine sulphoxides (CSO) which are mainly present in the onion bulb act as flavour precursors generating the typical taste and odour in presence of the enzyme alliinase. In case of onion, the CSO isoalliin is most important.
Generally, CSO play an important role in the biochemistry of *Allium* plants. That is why efficient analytical methods are needed to determine the individual content of these compounds. In this study numerous cultivars of *Allium cepa* were analysed applying two different rapid methods. One of these is a biosensoric method developed by Keusgen [2]. In this context immobilized alliinase is used to initiate the alliinase-reaction. Pyruvate and ammonia are by-products formed during this process. The amount of ammonia built during the enzyme reaction is proportional to the level of cysteine sulphoxides and can be therefore used for indirect quantification of these sulphur constituents.
Furthermore, a new HPLC-MS^2 technique was developed to measure the content of the individual CSO (methiin, isoalliin, propiin), present in onion bulbs. Using this method it is possible to determine the CSO contents without prior pre-column derivatization. Based on the MS-MS ions which are formed by fragmentation of the individual analyte molecule ions, a very high selectivity and reliability can be achieved. Both methods were applied to 26 cultivars of *A. cepa* giving total CSO contents between 0.2 and 0.7 %, related to freeze dried material of bulbs.

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Considerations on the quality control of *Harpagophytum*

P 551

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Extracts and preparations of *Harpagophytum spp.* are used in treatment of rheumatic complaints, osteoarthritis, loss of appetite, dyspepsia and the relief of lower-back pain (1). Originally, *H. procumbens* was used to prepare extracts and its main characteristic constituent harpagoside (HS) for quality control and standardization. Recently, also *H. zeyheri* is used to prepare extracts, this contains both HS and 8-*p*-coumaroylharpagide (8-PCHG) as major constituents. The latter component has been used in the past to determine the purity of extracts and preparations (2,3). It is assumed that most commercially available extracts are mixtures and that both HS and 8-PCHG may be involved in biological activity (4). This makes 8-PCHG a valuable additional component for quality control. The European Pharmacopoeia monograph on *Harpagophytii radix* (5) uses HPLC to determine the HS concentration, based on an indirect method, using the chromophore of methyl cinnamate, which is equal to the chromophore of HS. In an analogue approach, we synthesized the 8-PCHG chromophore, *p*-hydroxycinnamic acid methyl ester and its structure was proven by ^{1}H-NMR-, IR- and mass spectrometry. We are currently validating the HPLC method using the HS and 8-PCHG chromophores, to evaluate its feasibility for determining both the HS and 8-PCHG concentration in *Harpagophytii radix*.

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High sensitive detection of natural active ingredients in complex mixture- a requirement for bioavailability test and product quality control

P 552

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In nutritional supplements and functional foods natural ingredients are often combined on a theoretical basis for their investigated and published efficacy as single substances without demonstrating if the actives are still bioavailable in a complex mixture. One important step to determine the bioavailability is to develop analytical methods for the identification and quantification of those active ingredients within complex mixtures. Natural ingredients are often analytically very complex. This complexity normally enlarges with the number of ingredients, concentration, polarity, different responses and the level of interference. High performance chromatography is the most used method to carry out analysis of non-volatile natural ingredients[1-5]. A suitable sample preparation is ultimatively important to reduce overall complexity and ensure reliable results. Analytical methods for two complex mixtures were developed to determine overall ingredient bioavailability and product quality. Methods for three different mixtures including seven natural ingredients were developed (lutein-ester, natural vitamin E, conjugated linoleic acid, green tea extract, passionflower extract, grape seed extract and red clover extract). The bioavailability study was determined in collaboration with BioTeSys GmbH, Esslingen, Germany. These developed analytical methods are additionally important tools for any quality-control of final products.

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P 553 An estimation of the assumed amount of catechins, xantines and theanin of several commercial representative types of teas

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The potential health benefit of tea infusions are widely known, nevertheless, up to now, no systematic analysis has been carried out to measure the amount of the bioactive molecules directly assumed with a cup of tea prepared according to tradition and immediately consumed after preparation. All the samples were analysed by HPLC/DAD/MS within 20 min from the infusion preparation. The highest concentrations of caffeine were near 80 mg/400 mL). The total catechin content was in the range 5-100 mg/400 mL of infusion with the highest amounts in the green teas from China and Japan. The main phenolic antioxidant, EGCG, was particularly abundant in the Gyokuro, a valued Japanese green tea. After pulverization it is called MatCha and it is consumed "*in toto*". For this reason an exhaustive extraction with solvent was applied only for this sample, obtaining a concentration up to 70 mg/g of total catechins, several times higher than other green teas. On the fermented samples the teaflavin content was also evaluated and resulted in a very low concentration. The highest amounts of the AA theanin were detected in the white and green teas.
In literature the total catechins in the infusion are often higher with respect to our results. The main reason of this discrepancy can be related to longer times (10 to 30 min) if compared with those applied in this work (few min). Finally also "washing" procedures and a moderate increase of the infusion times have been applied.

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P 554 Variation of total curcuminoid content in *Curcuma longa* growing in Thailand

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Curcuma longa L. (turmeric) is a popular medicinal herb of Thailand for spice and a coloring agent. Medicinal uses of the rhizome come from volatile oil as a carminative and antifungal activity (1) and from yellow curcuminoids as anti-oxidative and anti-inflammatory properties (2). In Thailand, *C. longa* is mainly used as capsule/tablet of turmeric powders for herbal medicine and used the extract in herbal cosmetics. So, the quality assessment of this plant is needed to control the limit of volatile oil and total curcuminoids. This study was undertaken to evaluate the content of essential oil and total curcuminoids in dried powders and total curcuminoid content the ethanolic extract of *C. longa* rhizome collected from 10 locations from the North, North East, Central and South of Thailand during January to April 2005. The highest content (9.00 $\pm$1.41% v/w) of essential oil was found in the samples collected from the North where the climate is cool while the lowest oil content (7.00 $\pm$ 0.00 % v/w) was found in the samples from the South. The highest of total curcuminoid content (8.99 $\pm$ 0.83% w/w) was found in the Southern samples while the lowest content (5.99 $\pm$ 0.89% w/w) was in the Central samples. In ethanolic extract, total curcuminoids were found in the limit of 14.14 $\pm$ 0.87 to 26.76 $\pm$ 0.17 %w/w. The maximum content (25.46 $\pm$1.85 % w/w) was found in the samples from the South while the minimum content (19.10 $\pm$ 3.37% w/w) was in the Central samples.

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Pharmacovigilance and Literature Case Reports – Some Considerations regarding the Causality Assessment of Herbal Medicinal Products

P 555

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Growing interest in herbal medicinal products and the extensive scientific investigation of botanicals has led to an increased sensitivity for possible adverse events which may occur during the use of herbal medicinal products. Within the European Union, legislation regarding pharmacovigilance also focusses on reports of adverse events or safety issues published in the literature(1). Pharmaceutical companies are obliged to regularly review literature databases for case reports and make adequate risk benefit assessments of their products on an ongoing basis. Expedited reporting is required in serious cases. Aditionally, all literature case reports have to be included in the periodic safety update report (PSUR).
Generally, the assessment of adverse events of herbal products should follow the same standards as established for chemically defined products(2). Basically, the same causality assessment criteria apply to all kinds of products used for the treatment of patients. However, in the context with herbal products some specific issues should be kept in mind with regard to the causality assessment, for instance using another algorithm(3). To our experience the WHO system leads frequently to a "possible" assessment because a reasonable time relationship qualifies for this classification. The difficulties may be even greater in fixed combination products. Some examples of published case reports shall be discussed.

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Subcutaneous toxicity of the antileishmanial lead compound PX-6518 in mice and dogs

P 556

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PX-6518 is a triterpenoid saponin isolated from the leaves of the Vietnamese plant *Maesa balansae* showing a strong and selective *in vitro* and *in vivo* action against the intracellular protozoan parasite *Leishmania* [1,2]. Since this drug candidate must be administered parenterally and within the knowledge that saponins are potentially toxic, subcutaneous dose-escalation toxicity studies were performed in mice and dogs. Daily dosing of mice at up to 5 mg/kg for 14 days produced no clinical symptoms, except for an inflammatory reaction at the injection site. A marginal increase of the liver enzymes ALP, ALT and AST together with mild histopathological findings (granulopoiesis, Kupffer cell proliferation, occasional single cell necrosis) revealed that the liver was the target organ for toxicity. In the dog, single-dose escalation at 2-week intervals (0.1 » 0.2 » 0.4 mg/kg) resulted in increased liver enzyme levels at 0.4 mg/kg, which slowly returned to normal over the next period of about 4-6 weeks. Toxicokinetic monitoring revealed that the plasma concentrations, even after 0.1 mg/kg, exceeded by far the 20 ng/ml *in vitro* IC_{50} against intracellular amastigotes[1], suggesting the possibility of lowering the projected therapeutic dose and enhancing an adequate safety margin.

Acknowledgements: WHO-TDR (Geneva, Switserland), DGOS (Brussels, Belgium), Tibotec (Mechelen, Belgium)
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P 557 Safety of ethanolic kava extract: Results of a study of chronic toxicity in rats

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Kava and kava derived products are generally considered as very safe. In 2002, the German health authorities banned kava extract containing products based on the suspicion of a potential liver toxicity, as derived from adverse effect reports. From the case reports and the sales figures of kava extracts, an incidence rate of one potential case in 60 to 125 million patients was deducted (1-3). Clinical, pre-clinical and toxicological studies have so far failed to show toxicity for kava preparations and their constituents.
The potential toxicity of a three month respectively six month oral application of 7.3 versus 73 mg/kg body weight of ethanolic full kava extract was tested in rats. The animals were examined for changes in body weight, hematogogical and liver parameters, and macroscopical and microscopical histological changes in the major organs. No signs of toxicity could be found. These results are in accordance with the medical experience for the use of kava preparations and the long tradition of kava drinking in the South Pacific island states. Specifically, the results do not back the suspicion of potential liver toxicity.

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P 558 Problems of safety of *Spirea ulmaria* and *Salix alba* extracts in botanical supplements related to pediatric diseases

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There is a rapidly growing trend in the consumption of herbal remedies in industrialized and developing countries. Users of botanical supplements are at risk of toxicity and adverse effects of herbal preparations. Some problems are related to the safety of natural herbal remedies, mostly considered as food. Here we present the case of a child, four years old, who presented melena, hematemesis and hypovolemic shock on the first day of administration of a herbal syrup. He received five milliliters of the syrup for three times, starting from the morning. In the evening he presented dark stool, the mother thought related to the colour of the product. In the night he started vomiting fresh blood and became lethargic. At the admission in the Emergency Department he presented hypovolemic shock. Endosocopy was performed and revealed multiple erosions of the esophagus and that the child suffered for a gastroesophageal reflux. We suppose that the acute esophageal hemorrage was related to the salicylates contents of the product. The chemical analyses have been carried out on the botanical supplement, a syrupy liquid, which contains *Spirea ulmaria* (*Filipendula ulmaria)* and *Salix alba* extracts. According to Ph.Eu.5 ed., salicylic acid precipitation by Fe^{3+} coagulation was evaluated positively. The salicylic acid concentration was then determined by the reduced fluorescence intensity of salicylic acid in presence of Fe^{3+} . It is noteworthy that is it absolutely needed and indispensable to check for an analytical control of herbal commercial remedies, mainly if related to children diseases.

Formation of aromatic amines during coffee roasting

P 559

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Aromatic amines belong to these chemical compounds, which structural and molecular mechanism of carcinogenicity is best known. Aromatic amines may form during roasting of coffee, as a result of decarboxylation of aromatic aminoacids, the products of decomposition of proteins and peptides in high temperature conditions. The aim of this work is the determination of aromatic amines content in selected market brands of roasted coffee. The study material was natural coffee, roasted and ground.
For the determination of aromatic amines content in coffee, a distillation-spectrophotometric method was applied. The analysis was performed using a water vapor distillation system and a UV/VIS spectrophotometer. The method of determination of total aromatic amines content is based on the measurement of absorbance of solution containing the color product of reaction of diazonium derivatives of aromatic amines with α-naftol. As a result of this reaction, a compound of yellow color is formed, with characteristic absorption band at λ=570 nm. The distillate obtained from coffee by water vapor distillation was used in preparation of the colorful solution.
It was found that the content of aromatic amines in roasted coffee was variable and depended on the degree of roasting. In medium-roasted coffees, the content of aromatic amines was 2.65-9.26 µg/kg on average, whereas in well-roasted coffees it amounted 71.99-140.58 µg/kg. It is worth noting that high content of aromatic amines was found in coffee with high degree of roasting and accompanying very dark color.

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Content of bioactive compounds in black tea

P 560

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Tea is an aqueous infusion of dried unfermented or fermented leaves of *C. sinensis*, for which numerous biological activities have been reported, including antimutagenic, antibacterial, antioxidant and cancer preventing properties. It is commonly assumed that catechins are responsible for these health benefits of tea. Recent research indicates that tannins express antioxidant properties, similarly to flavonoids and anthocyanins and they may be used in the prophylactics of civilisation diseases (1). Caffeine, called theine in tea, is a plant alkaloid occurring in coffee, tea or cocoa. Caffeine, like catechins, exerts strong effect on the human organism.
The aim of this work is estimation of the content of catechins, like: (+)-C, (-)-EC, (-)-EGC, (-)-ECG, (-)-EGCG, caffeine and tannins in tea imported to Poland from China, India, Kenya and Vietnam. Varian HPLC system with quaternary pump and diode-array detector was used. Catechins and caffeine identification from chromatograms of examined teas' extracts (1g of tea in 100mL boiling water) were performed by comparison of retention times with respective standarts purchased from Sigma Aldrich, whereas caffeine was identified by comparison of its retention time to the standard Caffeine from Fluka Chemie GmbH (2).The determination of tannins was performed utilising a method based on formation of insoluble tannins salts with copper (II) cation (3).
The content of catechins in examined tea samples was very different and varied by country of origin and catechin type. The highest content of catechins was detected in samples of tea from Kenya (22.54 mg/100ml) and the lowest in Chinese tea (10.23 mg/100ml). Among the studied compounds, (-)-EGCG was detected in highest quantities. Its content ranged from 2.41 to 10.88 mg/100ml for Chinese and Kenyan tea, respectively. The content of caffeine in examined teas was very different too. The least of caffeine was contained in tea from India (17.8 mg/100ml) and the most – in tea from Kenya (17.8 mg/100ml). The content of tannins ranged from 70.85 mg/g in samples from Vietnam to 219.83 mg/g in samples from Kenya.

References: 1. Chung, K-T. et al. (1998) Crit. Rev. Food Sci. Nutr. 38: 421-464. **2.** Khokhar S, et al. (1997) Cancer Lett. 114: 171-172. 3**.** Śmiechowska, M., Dmowski, P. (2004) Food Chemistry, (in print).

P 561 A simplified but effective system to identify medicinal plants by TLC

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Quality control of herbal medicines is critically important to ensure that the client gets what is stated on the label and to ensure that no contamination or adulteration is present. Thin layer chromatography of herbal extracts still seems to be the most promising method. A valuable TLC Atlas compiled from work of different authors uses 17 extractants, 41 TLC solvent systems and 44 spray reagents to yield fingerprints of most important western herbal medicines (1).
We investigated whether it would be possible to use fewer parameters to yield unique TLC fingerprints. We selected 5 commonly used medicinal plants containing a variety of compounds [*Agnus castis*, *Silibum marianum*, *Ginkgo biloba*, *Hypericum perforatum* and *Piper methysticum*] and compared the quantity extracted by five British Herbal Pharmacopoeia methods with acetone (2). Acetone gave a much better yield, took less time and yielded a similar TLC pattern. We compared five general spray reagents and found that *p*-anisaldehyde-sulphuric acid gave the best results.
We developed three TLC solvent systems that would separate compounds in a wide polarity range using different pH's to provide additional separation parameters for basic and acidic plants components. The system developed gave unique TLC fingerprints when analyzing 81 samples of 52 different herbal products. Fingerprints of the same species from different sources were similar. A simplified method can therefore be developed to identify plant extracts based on their TLC profiles using low cost apparatus.

Acknowledgements: Biomox Pharmaceuticals provided samples and funding. NRF and THRIP also provided funds.
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P 562 Herbal Profiling by Thin Layer Chromatography-BioLuminescence Detection

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ChromaDex Analytics is developing a biosensor based method for rapid-screening of complex mixtures such as herbals. The proposed methodology, BioLuminex, is a kit system designed to support material identity, detect toxins and chemical adulterations, identify potential bioactive compounds, and control manufacturing procedures. This technology directly couples bioluminescence to thin-layer chromatography (TLC) providing a unique and effective way of monitoring complex mixtures. Complex mixtures are first separated by TLC. The TLC plate is then coated with bioluminescent bacteria, which identifies single toxic compounds as dark zones on a luminescent background. Results occur within seconds and can be documented by video imaging or photographically with typical limits of detection for toxic substances in the picomol range. This technology is kit compatible thus providing a rapid and inexpensive analysis of many commodity samples.

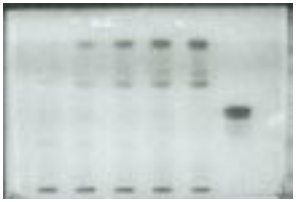

Figure 1. Chromatographed rhizome extracts of *Actaea racemosa* adulterated with various amounts of *Actaea pachypoda* leaf and analyzed by BioLuminex.

The Role of Gum Arabic in alleviating toxicity of tropical medicinal plants

P 563

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Gum arabic, *Acacia senegal*, (1) is the natural hydrocolloid of choice for a wide variety of food and pharmaceutical industries worldwide. There is a long standing tradition of using gum arabic (GA) infusions for calming down symptoms of various ailments such as malaria, diabetes and kidney malfunctions. Recent reports have shown that this tradition is not lacking in integrity. One such report showed that GA has a role in alleviating kidney malfunctioning and supplementation of diet with GA fiber lead to lowering of serum urea-N in patients suffering from chronic renal failure (2). Several tropical medicinal plants have been associated with human and animal toxicity such as nephrotoxicity with *Aristolochias* (3), and hepatotoxicity with the Heliotropes (1). The work presented here is directed towards finding out a possible role for GA towards reducing toxicity of some of the known toxic medicinal plants. Ethanol extracts of *Aristolochia bracteolata*(AB), *Heliotropium curassavicum*(HC), *Nerium oleander*(NO), *Azadirachta indica* (AI), *Calatropis procera* (CP), *Sesuvium verrucosum* (SV), *Cassia italica* (CI), *Citrullus colocynthis* (CC) and *Acanthospermum hispidum* (AH) were used for this purpose. Lethal concentrations (LC_{50}'s) to brine shrimp (*Artemia salina*) of these plant extracts were determined in triplicate at three different concentrations (10,100 and 1000 ppm) before and after adding GA. Results show that the toxicity of these plants extracts and that of known toxic chemicals such as $CuSO_4$(Cu-LC_{50} 2.88 ppm) and the fish poison rotenone(LC_{50} $1.23x10^{-2}$ ppm) have been significantly reduced with values of $p \leq 0.05$ obtained using one-way analysis of variance (ANOVA) on these results.

References: **1**. Rizk , A., (1986). Phytochemistry of The Flora of Qatar. Kingprint, Richmond. **2.** Bliss, Z. et al. (1996) Am. J. Clin. Nut. 63: 392-398. **3**. Steward, M.J. Steenkamp,V. (2001) Therap. Drug Monitor. 23(6): 698-706.

P 564 A Simple Method for the Preparation of 14β-Hydroxyprogesterone

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14β-Hydroxyprogesterone was synthesized in 1987 from progesterone and later from 3β-acetoxypregn-5,16-diene-20-one in 1992 (1,2). It was patented for its cardiotonic action. Here we present a simplified method for its preparation which was developed using a saponin from *Caralluma umbellata* (Asclepiadaceae). As first step, the steroidal glycoside Carumbelloside-I was hydrolysed using emulsin. One of the reaction products is the genin 3β, 14β-dihydroxypregn-5-ene-20-one. Subsequent Oppenauer oxidation of this intermediate led to 14β-hydroxyprogesterone in an overall yield of 25%. The method is very simple and consists of only two steps. 14β-Hydroxyprogesterone exhibited free radical scavenging activity when tested in the DPPH assay (IC_{50} = 106 ng). It also exhibited antifertility activity when tested in mice (at 50 mg/Kg intraperitoneal as a suspension in water using CMC).

References: 1. Templeton, J.F., Shashi Kumar, V.P., Cote,D., Bose,D., Elliot,D.,Kim R.S. and La Bella, F.S., (1987), *J. Med. Chem*, **30**, 1502-1505. **2**. Templeton, J.F. and Yulin Yan.(1992), Org.Prep.Proced.Int. **24**,159-163.

P 565 Synthesis of new Disaccharide Analogs of Daunorubicin and Adriamycin

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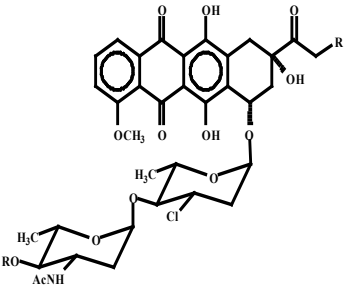

Over the last two decades the anthracycline antibiotics Adriamycin and Daunorubicin have proved to be important cancer chemotherapeutic drugs for the management of hematologic malignancies and a large variety of solid tumors. However a dose cumulative cardiotoxicity and the development of resistance in chemosensitive tumors (MDR) have restricted their extensive use in chemotherapy. Earlier reports have shown that 3′-deamino-3′-halo derivatives were more active than adriamycin against resistant tumor cells, suggesting that the increased lipophilicity of the sugar moiety of anthracyclines could be related to the ability to overcome MDR (1, 2). Unfortunately, these derivatives exhibited reduced potency compared to Adriamycin, when evaluated against sensitive leukemia cell lines and a panel of solid tumors (2). Having in mind the above mentioned considerations, we have synthesized two new anthracycline derivatives having disaccharide unit. The first sugar possess a lipophilic chlorine substitution and the last one bears an acetamido substitution at position C-3.

References: 1. Aligianis, N. et al. (1996) Bioorg. Med. Chem. Lett. 6: 2473-2476. **2.** Aligianis, N. et al. (2000) Chem. Pharm. Bull. 48: 150-153.

Carnation (*Dianthus caryophyllus*) natural flavonols: structural analogies and defensive role

P 566

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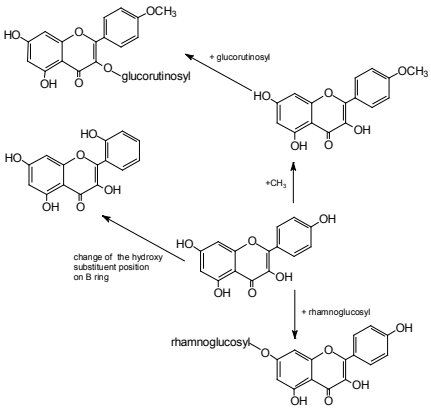

Three flavonoids have been isolated from as many carnation (*Dianthus caryophyllus*) cultivars, constitutively highly resistant to *Fusarium oxysporum* f. sp. *dianthi* (*Fod*). These compounds were extracted, purified and identified according to already published protocols (1) and, when assayed for their antifungal effects, they proved to be active against *Fod* starting from 50 μM concentrations (2). Their molecular structure is shown in the table. From the results, the following conclusions can be drawn:

(1) the detected flavonols are somehow involved in the defensive processes of carnation towards *Fod* ;
(2) the selective pressure forced the carnation species to follow a biosynthetic pattern leading to an antifungal molecule shared by different genotypes;
(3) this common structure is represented by the 5,7-dihydroxy-flavonol, the main skeleton of which undergoes different substitutions depending on the different carnation cultivars.

References: 1. Curir, P. et al. (1996) Phytochemistry 41: 447-450. **2.** Curir, P. et al. (2005) J. Phytopathol. 153: 63-67.

Secondary metabolites from insect infected leaves of *Quercus ilex* L. (Fagaceae)

P 567

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It has been suggested that non-glandular leaf hairs may have a significant role in plant defence against insects (1). However, 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, insect infected leaves were analyzed for their flavonoid content. 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 glycosides of quercetin and myricetin, as well as acylated glycosides of kaempferol: tiliroside and kaempferol-3-O-(2′,6′-di-p-coumaroyl)-glucoside.
The structures of the isolated compounds were established by means of NMR [^{1}H-^{1}H-COSY, ^{1}H-^{13}C-HSQC, HMBC, ROESY] spectral analyses.

References: 1. Levin D. A., (1973), Quart. Rev. Biol.: 43, 3.

P 568 *Dioscorea villosa* as a source of antiyeast steroidal saponins

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Dioscorea villosa L. belongs to the Dioscoreaceae family in which the steroidal saponins are fairly widespread. This plant is shown to be an important source of diosgenin and is used in alternative medicines to minimize post-menopausal symptoms and for the treatment of low progesterone level (1). As part of our ongoing search for biologically active steroid saponins as potent antifungal agents (2, 3), a phytochemical investigation of the rhizome of *D. villosa* has led to the isolation by several chromatographic steps on normal and reversed phase silica gel of six known steroidal glycosides (**1**-**6**). They were identified as protodioscin (**1**) methylprotodioscin (**2**), parrisaponin (**3**), dioscin (**4**), prosapogenin of dioscin (**5**) and progenin II (**6**) by comparison of their spectral data obtained from 2D NMR experiments (COSY, TOCSY, HSQC and HMBC) and FAB-MS with literature data. In addition, the antifungal activity of these compounds was tested against three human pathogenic yeasts (*Candida albicans, C. glabrata and C. tropicalis*). Spirostanol saponins (**3**-**6**) presented antifungal activity with MICs values between 6.25 and 200 μg/ml whereas compounds (**1**-**2**) were inactive. Regarding the aglycone structure, we observed the presence of antifungal activity only with the spirostanol derivatives whereas none was observed with the furostanol derivatives.

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P 569 Fumigant and contact activities of some Thai native plant extracts against rice weevil (*Sitophillus oryza*)

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Contact and fumigant activities of four ethanolic crude extracts of Thai native plants i.e. *Acorus calamus* Linn., *Eugenia caryophyllus* (Spreng.) Bullock et Harrison, *Mammea siamensis* (Miq.) T. And. and *Stemona curtisii* Hk. f. were investigated for their insecticidal properties against rice weevil (*Sitophillus oryza*). Consequently, crude extracts which have the highest efficiency of both fumigant and contact activities against rice weevil were chosen for the isolation and identification of their main compounds. For the fumigant activity , *A. calamus* produced strongest efficiency with the LC_{50} value of 3.17 mg/cm^2 at 72 hrs after application, followed by *E. caryophyllus, M. siamensis* and *S. curtisii* with the LC_{50} value of 3.24, 4.26 and 9.58 mg/cm^2, respectively. Whereas *M. siamensis* expressed the highest activity in the test of contact activity with the LD_{50} value of 70.92 μg/mg insect at 24 hrs after application, followed by *A. calamus*, *E. caryophyllus* and *S. curtisii* with the LD_{50} value of 106.21, 114.47 and 218.34 μg/mg insect, respectively. *A. calamus* and *E. caryophyllus,* both the best results from the fumigant test, and *M. siamensis* from the test of contact activity were isolated and identified for their main compounds using GC-MS and spectroscopic method. Volatile substances, asarone and its derivatives were the main compounds of *A. calamus* and eugenyl acetate was of *E. caryophyllus*. Non-volatile substances, surangin B and surangin C, were found as the main compounds of *M. siamensis* .

Acknowledgements: We are grateful to the National Research Council of Thailand (NRCT) and Ministry of Natural Resources and Environment for supporting this project.

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Is the bioavailability of water- and fat-soluble actives derived from plant extracts influenced by a simultaneous administration?

P 570

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Plant extracts are at present in the focus of anti-aging and health care products. They are widely used as food additives, food supplements, and as cosmetic ingredients. Especially the epicatechins and catechins, which occur in grape seed or green tea extracts, are of great interest for anti-aging products. However, it is little known about the influences between different bioactive compounds concerning their bioavailability when administered simultaneously. In the present study we investigated the bioavailability of different plant extracts with water- and fat-soluble active ingredients for CaCo-2 cells *in- vitro*. We further investigated whether the concomitant supplementation with combinations of these plant extracts has some influence on the bioavailability compared to the single extract. Plant extracts from grape seed, passiflower, and red clover as well as vitamin E and lutein esters were added as single supplements to CaCo-2 cells. Grape seed extract was also supplemented as a mixture with passiflower and as a mixture with red clover, vitamin E and lutein esters. The uptake of each extract was monitored by RP-HPLC analysis after extraction of cells. The marker substances belong to the classes of flavanols, flavonols, isoflavones and to the fat-soluble substances tocopherol and lutein. The study describes the cellular uptake of single extracts and complex formulations thereof after an incubation period of up to 24 hours.

Phytophtora nicotianae Control by some Medicinal Plants Active Substances

P 571

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Essential oils of *Menthae piperita, Carum caraway,* and *Foeniculum vulgar* were tested for antifungal activity. In this study phythopathogenic, *Phytophtora* sp. that accumulated from umbelli form locust tree, used as test organism. The essential oils of peppermint and cumin at 2ppm concentration provided inhibitory activity against tested phythopathogenic organism (*Phytophthora* sp.) on in vitro conditions. *Phytophthora* sp. were completely inhibited at 2ppm of peppermint and cumin, while in control and other treatment, there were not any anti-fungal activity. The essential oil of peppermint had highest activity between all treatments.

P 572 Effect of Essential Oils of some Medicinal Plants on Control of Greenhouse Whitefly (*Trialeurodes vaporariorum*)

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The insecticidal activities of the essential oils of five different medicinal plants (cumin, thyme, peppermint, fennel and rosemary) were investigated on control of greenhouse whitefly (*Trialeurodes vaporariorum*). The activity of the essential oils were evaluated on basis of fatalities percent of whitefly after three days from application. Results in this study, shown that the most effective essential oils were thyme (*Thymus vulgaris* L.) cumin (*Carum caraway*) and fennel (*Foeniculum vulgar*) with 8, 7 and 5 ppm concentration, respectively.

P 573 Bioactive polysaccharides are present in white cabbage, a plant used both as food and medicine

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Cabbage leaves are used both as food and as a remedy for promoting the healing of wounds in traditional medicine. Previously, immunostimulating polysaccharides have been isolated from other plants used for this purpose or for other ailments where stimulation of the innate immune system may be beneficial. Examples are *Plantago major* (1, 2) and *Echinacea purpurea (3, 4)*. In many cases, these polysaccharides are of pectin or arabinogalactan nature which are polysaccharide types that seem to be present in all higher plants (5). Due to the universal occurrence of pectin type polysaccharides, there are reasons to believe that such structures are present in food plants as well as in medicine plants.
In order to obtain more knowledge about the presence of bioactive polysaccharides in vegetables, we wanted to test for and isolate bioactive polysaccharides in white cabbage leaves.
Polysaccharide fractions were isolated from white cabbage (*Brassica oleracea* var. *capitata*) extracts by ion-exchange and size exclusion chromatography. The fractions were characterized as pectin type polysaccharides containing homogalacturonan regions, rhamnogalacturonan I and arabinogalactan type II. The complement-fixation test revealed that several polysaccharide fractions were active, but with lower activity than PMII (1, 6), a well-characterized pectin fraction from *Plantago major* leaves.
This study has shown that white cabbage contains pectic type polysaccharide fractions that are active in the complement-fixation test.

References: 1. Samuelsen, A. B., Paulsen, B. S., et al., (1996) Carbohydrate Polymers 30: 37-44.**2**. Samuelsen, A. B., (2000) Journal of Ethnopharmacology 71: 1-21.**3**. Steinmuller, C., Roesler, J., et al., (1993) International Journal of Immunopharmacology 15: 605-614.**4**. Alban, S., Classen, B., et al., (2002) Planta Medica 68: 1118-1124.**5**. Albersheim, P., Darvill, A. G., et al. (1996) Pectins and Pectinases. Elsevier Science. Amsterdam.**6**. Michaelsen, T. E., Gilje, A., et al., (2000) Scandinavian Journal of Immunology 52: 483-490.

Sequestration of furostanol saponins by *Monophadnus* sawfly larvae from host plants of Ranunculacae family

P 574

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Sawfly larvae of the tribe Phymatocerini (Hymenoptera: Tenthredinidae) are specialised on toxic plants in the orders Liliales and Ranunculales, and they can release a hemolymph droplet when attacked by a predator (1). Within the INCHECO E.U. project the hypothesis of a chemical defence based in the sequestration of host plant secondary metabolites is here investigated in four species of *Monophadnus*, namely *M. monticola, M. latus, M. alpicola,* and *M. pallescens*, which feed on *Helleborus, Pulsatilla,* and *Ranunculus* ssp. (Ranunculaceae). Comparative analyses of *n*-butanol plant extracts and ethanol hemolymph extracts were carried out by HPLC/ESI/MS and the presence of furostanol saponins, both in the plant and in the hemolymph, was detected. The isolation and characterization by NMR spectroscopy of four furostanol saponins from *Helleborus viridis* was carried out (2). The results of the analyses HPLC/ESI/MS showed that among the saponins present in the host plants, *Monophadnus* larvae are able to sequester selectively (25*R*)-26-[(α-L-rhamnopyranosyl)oxy]-22α-methoxyfurost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-[6-acetyl-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranoside. This compound occurs at a 100 to 1000 fold higher concentration in hemolymph than in the plant.

References: 1. Duffey, S. S. (1980) Annu. Rev. Entomol. 25:447–477. **2.** Braca, A. *et al*. (2004) Phytochemistry 65:2921-2928.

Anthraquinones and Iridoids from *Rubia peregrina* L.

P 575

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Rubia peregrina (Rubiaceae) grows in Northwest Anatolia in Turkey (1). The underground parts of *R. peregrina* have been used as a dye in Anatolia (2). This study describes the isolation and structure elucidation of the anthraquinones, anthraquinones glycosides and iridoid glycosides from *R. peregrina*. The powdered undergrounds parts of *R. peregrina* were extracted with methanol. The concentrated extract was suspended in water:methanol mixture (9:1) and partitioned with chloroform and ethyl acetate. The chloroform extract gave three anthraquinones by means of a series chromatographic techniques: 1-Hydroxy-2-methyl-9,10-anthraquinone, 1,3-dihydroxy-2-methyl-9,10-anthraquinone and 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone. The aqueous phase gave two anthraquinone glycosides and two iridoid glycosides using several and repeated chromatografic techniques: Rubiadin 3-0-β-primeveroside, lucidin 3-0-β-primeveroside, asperulosidic acid and deacetylasperulosidic acid. The structures of the compounds were elucidated by means of spectral (^{1}H-NMR, ^{13}C-NMR and EI-MS) methods .

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P 576 Etiological study of redness on St.John's worth plants and maize in Serbia

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Redness on St.John's wort (*Hypericum perforatum* and *H.barbatum*) was manifesting in Serbia during last few years, but redness of maize had been observed in our country for last several decades. The diseases could be very severe and destructive on St.John's wort (Pavlović, 2004) and on maize (Šutić, et al., 2003) in some years. As the ethiology of this diseases have been variously interpreted, study was conducted to identification of causal agents by using electron microscopy of ultrathin sections made of vessels' zone of infected plants and serological reactions with antiserum produced from diseased plants. To prove infective nature of those diseases by transplantation fragments of infective plants to healthy one was done. The results indicate the presence of fastidious bacteria in leaves and roots on St.John's wort and leaves, seeds and adventitious roots on maize. The symptoms were successfully reproduced on artificially inoculated St.John's wort plants, so infective nature of redness was proved. Serological tests indicated that redness causal agents belong to the same group of pathogens.

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P 577 Sclerotinia blight of marshmallow (*Altea officinalis* L.)

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Slerotinia blight is common disease of sunflower (1). During 2001 it was isolated from marshmallow which was grown in plantation in Bela Crkva vicinity (Serbia) where the previous crop was sunflower. Marshmallow diseased plants wilted and died before the end of vegetation. Plants for artificial inoculations were planted in autumn and kept under greenhouse condition. Next year they were inoculated with suspension of blended mycelia and sclerotia at the base of flowering-age plants. Plants were kept for 3 days in a dew chamber and then incubated in greenhouse at 25°C. Pathogen development in vitro and sclerotia formed were studied at four media at 5-30°C.Sclerotia collected in the field were kept for one month in refrigerator at 4 °C, and then incubated on wet filter paper at 25 °C, afterwards formation of apothecia was observed. The white cottony mycelia and characteristic symptoms were appeared 5 days after inoculation. Pathogen grew well at all temperatures with optimum being 20-25°C. Mycelial development was best at PDA. The abundant sclerotia 1-2 mm in diameter were formed at the culture periphery at malt extract agar (MA) and Sabouraud dextrose agar (SDA), but largest and scattered one were formed in the centre of culture on PDA. Apothecia started to be formed after 2 months. Almost all (95%) four-year-old sclerotias formatted apothecia after 2-4 months. Morphology and the size of asci and ascospores agreed with literature data for *Sclerotinia sclerotiorum* (2). Rotation for marshmallow plantation should be more than 7-8 years after sunflower with sclerotia blight symptoms.

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When Ecological Observations Lead to Medicinal Discoveries: Chemical Constituents of North American *Hypericum* L. (Clusiaceae) Active Against Bacterial and Fungal Diseases

P 578

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The Lise-Meitner-Programm, funded by the Fonds zur Förderung der wissenschaftlichen Forschung (FWF), provides a unique opportunity for foreign researchers to conduct innovative research in Austria, and encourage collaborations between Austria and other countries. The primary objective of our proposal was to test extracts and purified compounds from selected flowering plant species of *Hypericum* L. (St. John's Wort; Clusiaceae) against diseases affecting both humans and bees. A specific class of compounds, phloroglucinols, was selected due to their unique structures and demonstrated antibacterial, antifungal, antiviral, antimalarial, anti-inflammatory, anti-HIV, and antidepressant activities[1]. Of particular interest is the ecological relevance of phloroglucinol derivatives, which are produced only by a limited number of plant families, including Clusiaceae. The ecological observation that these compounds are often collected by certain species of solitary bees in South America for use in nest construction and may protect the hive against pathogens[2], requires further investigation in the context of Northern Temperate species of *Hypericum*. Here, we present the results of our bioassay-guided fractionation and purified compound testing conducted through collaborators in North American and European research institutions, and discuss the proposed future directions of the project.

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Effect of different phenolic compounds on α-amylase activity: investigation of influence of molecular structure on inhibitory potency

P 579

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In our studies we investigated different phenolic compounds of plant origin and derivatives with regard to inhibit α-amylase activity related to their structural features. In previous studies we established a kinetic assay using p-nitrophenylmaltopentaoside as substrate (1) to determine the inhibitory effect of plant compounds and extracts on enzyme activity.
Experiments showed that some caffeic acid derivatives are able to inhibit the enzyme in a significant way. Interaction of diverse structural characteristics in the molecules are responsible for their inhibitory potency. A glycosidic component was not essential for the inhibiting effect. This fact suggested that the inhibitory mechanism is not based on a competition against the enzyme (as the mechanism of Acarbose effect) but a rather unspecific binding site in a non-competitive manner. IC_{50}-value of dihydrocaffeic acid was three times higher than that of caffeic acid. This fact demonstrated that the double bound in the propionic acid rest seems to be decisive for inhibitory potency. Caffeic acid derivatives with free hydroxyl groups and because of it the ability to form quinones (2) showed higher inhibition rates than the derivatives with methylated hydroxyl groups. By comparison of IC_{50}-values of chlorogenic acid (1.4 ± 0.03 mM) and isochlorogenic acid (0.56 ± 0.027 mM) we could demonstrate that the arrangement of hydroxylgroups in the same plane led presumably to a more pronounced effect. As positive control and well established inhibitor of α-amylase activity acarbose was used.

Acknowledgement: We thank the Bayer AG for providing Acarbose and Lichtwer Pharma GmbH for financial support.
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P 580 Synthesis and Structure-Activity-Relationship Studies of Natural Bioactive Diterpenes from Natural Sources

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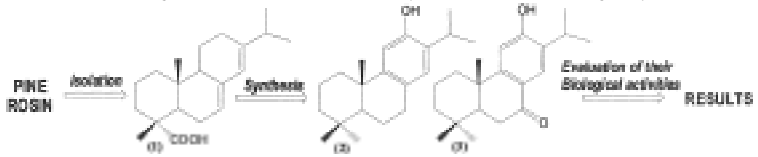

The synthesis and the isolation of a wide variety of metabolites from natural sources have been reported. Following our interest in the correlation between these bioactive compounds and their biological activities: e.g. antibiotic[1], antitumor[2], anti-virus[3] and antimalarial[4], the purpose of our research was synthesized a variety of natural products such as ferruginol **(2)** and Sugiol **(3)** from abietic acid **(1)** as well as biological assays, that will serve as tools to understand the structural requirements that are responsible for the pronounced "bioactivities" and to possibly address the questions regarding the structure-activity relationship.

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P 581 Metabolic study of sanguinarine and its congeners in HepG2 cells

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Sanguinarine (SA), a benzo[c]phenanthridine alkaloid isolated from a plant of the Papaveraceae, Fumariaceae, and Rutaceae families displays phytoallexin function and a broad spectrum of biological activities (1). A decrease of SA toxicity was observed in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 3-methylcholanthrene, or β-naphtoflavone treated rat hepatocyte and HepG2 cell cultures (2). We studied the effect of SA and its derivatives - dihydrosanguinarine (DHSA) and oxysanguinarine (OXYSA) in HepG2 cells. The cell cultures were treated with 5 nM TCDD for cytochrome P450 1A1 induction or DMSO (vehicle) for 72 h and then incubated (1 and 4 h) with 0.2 and 2 μM SA, DHSA or OXYSA. The compounds were dissolved in DMSO before the addition to the cultures; final DMSO concentration was 0.5%. The modified HPLC method (3) was applied for SA, DHSA and OXYSA determination in culture medium and cell sediment. The alkaloids were found predominantly in the cell sediment fraction, their content in culture medium was 3 to 30 times lower. When SA alone was added to the cell culture, DHSA was found in the cell sediment together with SA (ratio from 3.5:1 to 20:1 SA:DHSA), which could be the result of reversible reduction of SA to DHSA by NADH or NADPH *in vitro* (4). Pure culture medium did not cause reduction of SA to DHSA. When DHSA was added to the cell culture, SA was determined in the cell sediment and culture medium together with DHSA (1:3 to 1:1 ratio of SA:DHSA) apparently as the consequence of aerobic cultivation conditions. Decreased concentrations of tested compounds were found in TCDD treated HepG2 cells in comparison to nontreated cultures. Levels of analytes were below the detection limit in cells treated with TCDD and 0.2 μM SA, DHSA or OXYSA. In case of 2 μM SA, DHSA or OXYSA cell treatment by TCDD lowered the compounds concentrations by 25 % for OXYSA, by 55 – 70 % for SA or DHSA. We were not able to resolve if the decrease is due to the alkaloids transformation or tight binding to cell structures.

Acknowledgements: Financial support by the Ministry of Education (grant No. MSM 6198959216) is gratefully acknowledged.

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A new hypersensitivity therapy specific phytocomplexes-based

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Acids cause tooth erosion and increase dentinal permeability by inducing the tubules diameter augment. The aim of this study was to evaluate the erosive potential on human dentine of different dietary fruit acids. The effect of fruit acid on dentinal permeability (Lp) has been studied, also in presence of experimental treatment phytocomplexes-based (Rhubarb+Spinach 1% spray solution + carnauba wax 0.4% W). Dentin discs were exposed for 3 minutes to fruit acids (like juice or homogenate) and then to phytocomplexes spray solution for 3 minutes and finally to H_3PO_4 for 90 seconds; after each treatment the Lp was measured and relate to Lp max obtained with EDTA application for 5 minutes. Lp data were statistically analysed by LSD and Bonferroni test. SEM analyses completed the study. Resulting data of Permeability Tests are reported in the table. **T**he study demonstrated that treatment with Rhubarb+Spinach 1% spray solution (+carnauba wax 0,4% W) statistically reduce dentinal permeability caused by dietary fruit acids by occluding dentinal tubules by means of acid resistant oxalate micro-crystals formation.

Treatments	Lp mean attack (and Lp increase %) after fruit acid	Lp mean (and Lp reduction %) after treatment with spray	Lp mean (and Lp increase %) after H_3PO_4 attack
STRAWBERRY	107,7 ±11,9 (+ 7,7)	57,9 ± 15,2 (- 49,8)	88,7 ±12,0 (+ 30,8)
LEMON	102,5 ± 9,1 (+ 2,5)	56,3 ± 10,3 (- 46,2)	81,3 ±10,6 (+ 25,0)
GREEN APPLE	109,4 ± 19,0 (+ 9,4)	61,2 ± 16,8 (- 48,2)	86,6 ±14,0 (+ 25,4)
KIWI	105,4 ± 13,6 (+ 5,4)	61,5 ± 15,9 (- 43,9)	83,0 ±15,6 (+ 21,5)
ORANGE	106,9 ± 17,7 (+ 6,9)	56,6 ± 13,8 (- 50,3)	89,2 ±16,5 (+ 32,6)

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