

OP-1

Antiphlogistic properties of newly isolated diprenylated flavone from *Morus alba* L.

J. Hošek¹, K. Šmejkal¹, V. Závalová², P. Kollár², M. Bartoš^{1,3}

¹Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Brno, ²Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Brno, ³Genex CZ, Brno, Czech Republic

A white mulberry (*Morus alba* L., Moraceae) is used in the traditional Chinese medicine for a treatment of some inflammatory diseases. The inflammation is a part of innate immunity, but the uncontrolled inflammation can seriously damage the body. Thereby, production of new antiphlogistic drugs represents an important issue in the pharmacotherapy development.

Diprenylated flavone MA-13 was selected on the basis of screening of compounds isolated from *Morus alba*. This compound showed the highest capability to attenuate *in vitro* transcription of TNF α in macrophages (prepared from the human cell line THP-1) stimulated by bacterial lipopolysaccharide (LPS). Anti-inflammatory action of MA-13 was further investigated in detail as a change of transcription of the genes for pro-inflammatory cytokine CCL2, enzymes NOS2 and COX2 and anti-inflammatory protein ZFP36. Transcription of TNF α was correlated with its translation. The ability of MA-13 to reduce the translocation of NF- κ B from cytoplasm to nucleus was measured because most of the selected genes are under its transcription control. Obtained results have shown the ability of MA-13 to significantly decrease transcription of cytokine TNF α . Attenuation of TNF α translation was also observed; MA-13 declined the total amount of this cytokine in the medium obtained from LPS-stimulated macrophages by a factor of 10. Transcription of CCL2 was also decreased, but with no statistic significance. Also the production of NOS2 and COX2 mRNAs was reduced upon the MA-13 treatment. Expression peak of the only anti-inflammatory gene – ZFP36 – was delayed 2 hours. The ability of MA-13 to slow the nuclear translocation of NF- κ B proves the observation of decreased pro-inflammatory genes expression. In all studied parameters, MA-13 was more effective than indomethacin, which is used as a therapeutic anti-inflammatory agent and served as an experimental control. Thus, newly isolated compound MA-13 could represent a promising molecule for the development of new non-steroid anti-phlogistic agent.

OP-2

The effects of *Eryngium maritimum* l. Extract on nitric oxide synthesis, oxidative stress and phagocytosis in acute experimental inflammation

S. Suci¹, A.E. Pârvu²

¹Department of Clinical Pharmacy, Faculty of Pharmacy, Vasile Goldis Western University of Arad, 310119 Arad, ²Department of Pathophysiology, Faculty of Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

The aim of this paper is to evaluate the effects of the 1:5 (w/v) ethanolic extract of *Eryngium maritimum* aerial parts growing in Romania, on acute inflammation induced by turpentine oil in male Wistar rats. Results of the 1:10 and 1:50 dilutions of ethanolic extracts were compared to those from a control group that received only sterile saline and also to a group that received diclofenac, 15 minutes after inflammation was induced. Effects were evaluated by measuring serum nitrites and nitrates (Griess reaction), total oxidative status (TOS), total antioxidant activity (TAR) and index of oxidative status (ISO), *in vitro* phagocytosis, total leukocytes count and differential leukocytes count. Both dilutions of the *Eryngium maritimum* extract showed anti-inflammatory effects because they reduced nitric oxide synthesis, oxidative stress and phagocytes activity. The effects on oxidative stress were caused by the antioxidative properties. Compared to the anti-inflammatory control group, *Eryngium maritimum* extract had a better anti-inflammatory and antioxidative effect.

OP-3

Extracts of *Juniperus excelsa* inhibit NF κ B regulation involved in cancer

K. AlMaarri¹, L. AlAmir¹, W. Van den Berghe², G. Heagemann², L.L. Zhou³, D.Y. Xie³

¹General Commission of Biotechnology, Damascus, Syria, ²Gent University, Fac. of Science, LEGEST, Gent, Belgium, ³NCSU, Department of Plant Biology, Raleigh, USA

Transcription factor-oriented screening serves an effective approach to identify novel anticancer natural products from plants. NF κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF κ B is involved in many cellular responses to different stimuli including stress, cytokines, free radicals, UV irradiation, LDL oxidation, and bacterial or viral antigens. However, a mistaken regulation of NF κ B likely causes cancer and other diseases including inflammatory and autoimmune disease. Multiple evidences have clearly demonstrated that NF κ B is involved in regulation of cell proliferation and apoptosis. In this report, we show inhibitory results of methanolic extracts of leaves and berries of *Juniperus excelsa* on the growth of cancerous cells. Different concentrations of crude extracts of leaves and berries were prepared for *in vitro* anticancer tests. The fibrosarcoma cells were used in our experiments and NF κ B reporter gene assays were performed to test inhibitory activities of methanolic extracts. Our preliminary results revealed that extracts at concentrations from 0.5-1mg/ml showed strong inhibitory activities on NF κ B-associated gene expression. In addition, MTT assay found that these *Juniperus* extracts did not show cytotoxic effects from 0 up to 1 mg/ml. We used HPLC-mass spectrum-based profiling to analyze metabolites from extracts and identified epicatechin, dimeric procyanidins, and other oligomeric procyanidins, which most likely are the major phytochemicals providing anticancer activities in our experiments. Anticancer activities of juniper extracts will be discussed in our presentation

OP-4

Cytotoxic activities of several geranylated and prenylated flavonoids

K. Šmejkal¹, J. Svačinová², T. Šlapetová¹, K. Schneiderová¹, S. Dall'Acqua³, G. Innocenti³, V. Závalová⁴, P. Kollár⁴, S. Chudík¹, R. Marek⁵, O. Julínek⁶, M. Urbanová⁶, M. Kartal⁷, K. Doležal², T. Bárta^{8,9}, A. Hampel^{8,9}

¹Department of Natural Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Brno, ²Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Olomouc, Czech Republic, ³Department of Pharmaceutical Sciences, University of Padua, Italy, ⁴Department of Human Pharmacology and Toxicology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, ⁵National Center for Biomolecular Research, Faculty of Science, Masaryk University, ⁶Department of Physics and Measurements and Department of Analytical Chemistry, Institute of Chemical Technology, Prague, Czech Republic, ⁷Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey, ⁸Department of Biology, Faculty of Medicine, Masaryk University, Brno, Kamenice 5, CZ-625 00, ⁹Department of Molecular Embryology, Institute of Experimental Medicine, v.v.i., Academy of Sciences of the Czech Republic, Brno, Kamenice 5, CZ-625 00, Czech Republic

Polyphenols as natural compounds are secondary metabolites of many plant species. According to the basic skeleton, they can be divided into several categories with common structure feature – phenolic hydroxyl. Especially flavonoids, chalcones, xanthenes, stilbenes, coumarins, quinones, phtalides and aromatic organic acids are included. The biological activity of polyphenols is varied and is often modified by presence of different substitution on basic skeleton. In the present time, our attention is focused on the group of prenylated polyphenols. These compounds arise from crossover of different biosynthetic pathways, one of them represents terpenoids. There are many possibilities of prenylation; and the type of prenyl connection and modification affect the biological activity of modified polyphenolic compound. Prenylated polyphenols show wide spectrum of biological effects, including antioxidative, antiphlogistic, antimicrobial, anticancerogenic and estrogenic. Their influence on metabolism of sugars and lipids is also described.

Polyphenolic compounds isolated from *Paulownia tomentosa* fruits and *Morus alba* roots were tested on their cytotoxicity for *Nicotiana tabacum* BY-2 cell line, chosen human cancer cell line and normal human fibroblasts. Cytotoxicity was determined based on fluorescent vital staining, calcein AM assay and erythrosin B staining, in dependence on cell line tested. Geranylated compounds were compared with known simple flavanones, phenylpropanoids and cytotoxicity standards olomoucine II, diaziquone, oxaliplatin, cytarabine and camptothecin. Effect of structural parameters as the geranyl substitution and oxidative pattern of flavonoid skeleton, and possible metabolism of compounds is discussed. The effect of chosen compounds was also tested on cell cycle progression. Flavanone with modified geranyl side chain showed ability to interfere with cell cycle through on the cyclin-dependent mechanism.

OP-5

Anti-Malassezia furfur activity and potential use in anti-dandruff shampoo of Asparagus racemosus

N. Waranuch^{1,2}, C. Onlom³, S. Thirach⁴, K. Ingkaninan³

¹Cosmetic and Natural Products Research Center, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, ²Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, ³Department

of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, ⁴Department of Microbiology and Parasitology, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000, Thailand

Asparagus racemosus is an herb employed in traditional medicine in many parts of the world. It also has been recognized by European Commission as a cosmetic ingredient of anti-microbial, emollient and skin conditioning activities. In the present study, the extracts of *Asparagus racemosus* were further investigated in their activity against *Malassezia furfur* which is a major cause of human scalp dandruff. The extract showed some degree of activity against *Malassezia furfur*. The inhibitory effect of the extract against *Malassezia furfur* was compared to standard anti-dandruff agents. The biological stability of the extract under stress condition of different temperature was also tested to evaluate it potential to be used in the development of anti-dandruff shampoo.

Acknowledgement

This work has been supported by National Nanotechnology Center (NANOTEC), NSTDA, Ministry of Science and Technology, Thailand.

OP-6

The effect of sage (*Salvia officinalis* L.) And oregano (*Origanum vulgare* L.) On selected biochemical parameters of piglets

J. Poracova¹, M. Blascakova², M. Zahatnanska³

¹Centre of Excellence of Human and Animal Ecology, ²Department of Biology, Faculty of Humanities and Natural Sciences, ³Faculty of Humanities and Natural Sciences, Presov University, 01, 17th November Street, 081 16 Presov, Slovakia

Phytogenic feed additives are one of the options to replace the use of synthetic substances in nutrition and prevention of livestock diseases. Sage (*Salvia officinalis* L.) has a disinfecting, antibacterial, antioxidant, anti-stress, spasmolytic effects, it is used in difficulties during climacterium, therapy of mental and nervous diseases. Oregano (*Origanum vulgare* L.) has antifungal, antibacterial, antioxidant, analgesic, anti-inflammatory, immunostimulating and anticancer effects.

The experiment was carried out in the Slovak white noble breed x pietrain, weaning when piglets were aged 28 days. Piglets were divided into the control group (CG = 7 pc) and the experimental group (EG = 7 pcs), for which essential oils from sage (Cineol 15%, Thujone 24%, Borneol 18%) and oregano (Carvacrol 65%, Thymol 16%) were applied into feed (ČOS 1 and ČOS 2) at a concentration of 0.05% / kg.

Pigs were kept in cages, in the normal manner by the mother. Microclimate was secured by electrical brooders and ventilation. During the experiment, we monitored piglets' mortality, morbidity, and we sampled blood in the control and experimental group for biochemical determination of low-molecular substances (triacylglycerides, urea, creatinine). By comparing selected biochemical parameters between control and experimental groups, we found statistically significant differences in triglyceride concentration (CG = 0797 ± 0322, EG 0255 = ± 1.25, P < 0.05), urea (CG = 9:49, EG = 5.20, P < 0.01) and creatinine (CG = 6:00, EG = 8:45, P < 0.01).

Acknowledgement

My participation at the 6th CMAPSEEC is supported by the Agency of Ministry of Education SR for the Structural Funds

of the EU, the project: ITMS 26220120023.

OP-7

The glycemic elemental profile of *Trichosanthes dioica*: a libs-based study

P. K. Rai¹, N. K. Rai², A. K. Rai², G. Watal¹

¹Alternative Therapeutics Unit, Drug Discovery & Development Division, Medicinal Research Lab, Department of Chemistry, University of Allahabad, Allahabad- 211002,

²Laser Spectroscopy Research Laboratory, Department of Physics, University of Allahabad, Allahabad- 211002, India

Antidiabetic efficacy of medicinal plant can't be ignored in order to develop drugs without toxicity and side effects. Hence, present study deals with the Laser Induced Break Down Spectroscopy, a recent laser technique, based validation of glycemic elements present in *Trichosanthes dioica* fruits. The dose of 1000 mg/kg bw showed the maximum fall of 23.8 % and 19.1 % in BGL (blood glucose level) during FBG (fasting blood glucose level) and GTT (glucose tolerance test) studies respectively of non diabetic rats. In case of sub and mild diabetic models, the same dose showed reduction in BGL of 22.0 and 31.4% during GTT. The study, also involves the first use of Laser Induced Breakdown Spectroscopy (LIBS) as a sensitive analytical tool to detect the elemental profile responsible for the antidiabetic activity of aqueous extract of *Trichosanthes dioica* fruits that exhibits the antidiabetic activity. The decreasing blood glucose level and improving glucose tolerance test significantly showed that the higher content of Mg & Ca in *T. dioica* as well as other plant already reported by our research group.

OP-8

Results regarding seed morphology of some medicinal plant varieties

M.M. Duda¹, M. Fălticeanu², S. Vaida¹, B. Duda¹

¹Department of Plant Growing, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine, 400372, Cluj-Napoca, ²Vegetable Research-Development Station, 600388 Bacau, Romania

In the research on conventional and unconventional introduction to the culture of certain varieties of perennial medicinal species uses have led MMS (Mass of 1000 seeds) to 50 varieties of 38 species from 22 sorts. Counting has been done with an electronic seed counter type Sadkiewicz. The MMS was obtained by multiplying with 10 the arithmetic mean of the 8 repetitions, if the repetition was not a difference of more than 6% of the MMS – if it exceeded 25 g - or 10% if the MMS was less than 25 g. Between species and even varieties there is a great variability regarding the MMS of seeds. So the largest MMS occurred in *Lupinus polyphyllus* – L. Bacău (242,5 g), and the smallest in the species *Digitalis purpurea* - Dp-20/06 (1,6 g). Between the two species of *Agastache* (*A. mexicana* and *A. foeniculum*) were also major differences of 10,7 g. In studies *Echinacea purpurea* dominated with 10 varieties. The average MMS of these was 27,2 g, with amplitude of $\pm 12,7$ g.

OP-9

Fusarium oxysporum on medicinal plants in Serbia

S. Stojanović¹, S. Pavlović², D. Jošić³, M. Starović¹

¹Institute for Plant Protection and Environment, T. Drajžera 9, ²Institute for Medicinal Plant Research "Dr J. Pančić", T. Košćuška 1, ³Institute of Soil Science, T. Drajžera 7, Belgrade Serbia

Polyphagous and cosmopolitan fungi *Fusarium oxysporum* is common pathogen on cultivated medicinal plant in Serbia. It was isolated from all green parts of valerian, from seeds and root of marshmallow, coneflowers, yellow gentian and St. John's wort, from seed of lemon balm, lavender and chamomile, from root and stem of basil, from stolon of mint, from seeds and basal stem of basil, from stem of *E. angustifolia* and from flower head of *E. purpurea*. From total of 68 isolates, 33 were obtained from seeds, 25 from root, and one from stem, flower head and seedlings of investigated plants. *Fusarium oxysporum* significantly reduced germination of seed and this reduction depend of host from which isolates were obtained. The pathogen caused root rot, and stem and flower withering of infected plants. Morphology of fungus isolates (appearance of mycelia, pigmentation on medium, morphology of macro- and micro-conidia and chlamidospores) was strongly variable on potato dextrose agar (PDA). There were a little difference of mecelial growth of tested isolates at 25° and 30° C, but the growth at 25° was faster. According to the point of isolation, pathogenic species were dominant in seeds of medicinal and aromatic plants and in lesser extent in roots and over-grounds organs.

OP-10

Ecological impacts on cardenolide accumulation in different endemic *Digitalis cariensis* lines collected from antalya region

B. Yücesan, E. Ađlıç, T. Aydın, İ. Eker, S.K. Verma, E. Gürel

Abant İzzet Baysal University, Faculty of Science and Literature, Department of Biology, 14280 Bolu, Turkey

Digitalis cariensis Boiss. ex Jaub. & Spach (foxglove) belonging to the family Scrophulariaceae is an endemic plant species of the Turkish flora. Members of the genus *Digitalis* are medicinally and economically important plants as they contain cardiac glycosides that strengthen the cardiac diffusion and regulate the heart rhythm. Digoxin is one of the most widely used cardiotoxic drugs and found in predominantly in this plant species. Our HPLC studies showed that digoxin content in the leaves varied depending on plant vegetation, location, altitude, etc. For the Alanya line, in terms of digoxin level, June was found the most productive season producing 188.7 mg/kg DW, while it was the lowest in September (69.6 mg/kg DW), which is the seeding season for the species. The Manavgat line contained the highest amount of Digoxin in their leaves collected in August as well (224.32 mg/kg DW). In order to underline certain ecological parameters as an impact on cardenolide accumulation, total phenolic content of the leaves was also calculated by spectrophotometric methods. This preliminary study emphasizes the possibility of the large scale production of bioactive compounds from natural sources with alternative plant breeding techniques and plant propagations.

Acknowledgments

The authors are grateful to the Scientific and Technological Research Council of Turkey (TUBITAK) for financial support.

OP-11

Fusarium verticillioides on medicinal plants in serbia

S. Pavlović¹, G. Vuković², S. Stojanović³, T. Stević¹, M. Starović³

¹Institute for Medicinal Plant Research "Dr J. Pančić", ²Institute of Public Health, ³Institute for Plant Protection and Environment, Belgrade, Serbia

Fusarium verticillioides (Sacc./ Nirenberg) was isolated from the seed of marshmallow, coneflowers, St. John's wort, yellow gentian and sage, as well as from roots and stem of the mallow and the root of the coneflower. *F. verticillioides* is one of the most prevalent fungi that produce fumonisins, a group of closely related polar metabolites, before harvest, during the time between harvesting and drying, and in storage of medicinal plants. The purpose of this experiment was to identify range of symptoms of the insufficiently investigated diseases of tested medicinal plants and etiology of the causal agents in Serbia. Thirty-seven samples of *Gibberella moniliformis* were isolated from the seeds of diseased plants and from the root and stem of the mallow and the root of the coneflower, at different localities, during the 2005-2008 year period. The fungus was isolated from the parts of the plants below and above the ground using standard procedures and the following selective media: PDA, SNA, CA and CLA. A modified method given by Leslie was used to isolate the teleomorph stadium of the development. The symptoms and the pathogenesis were also described. Root rotting followed by dwarfism and asymetric growth, wilting and chlorosis of leaves were the symptoms present on the tested plants. This disease can significantly reduce the yield of these commercially important medicinal plants.

OP-12

Influence of biofertilizers on behavior changes of dtpa- extractable cationic micronutrients content in soil and their impact on biomass production of *Stevia rebaudiana* bert.

R. Dang¹, K. Das², T.N. Shivananda³

¹Al-Ameen College of Pharmacy, Hosur Main Road, Bangalore- 27, ²St. John's Pharmacy College, #6, R.P.C. Layout, Vijayanagar, Bangalore-40, ³Principal Scientist, Indian Institute of Horticultural Research, Hessaraghatta, Bangalore -80, India

The field experiment was conducted for the period of six month at Shimoga (Dist: Karnataka), India, to study the effect of biofertilizers in sole or in combination, on behavior changes of cationic micronutrients content in acidic soil and their combined effects on fresh biomass production of *Stevia rebaudiana* Bert. The results revealed the cationic micronutrient (Fe, Mn, Cu and Zn) contents in soil significantly increased up to three months and being highest (19.42, 6.15, 0.36 and 0.62 mg/kg respectively) with combined application of three biofertilizers. Further the same have been found to be decreased gradually up to sixth month of plant growth (9.98, 2.70, 0.17 and 0.38 mg/kg respectively) than that of sole applied biofertilizers and control treatment. Even though the total yield in terms of biomass production has been found to be periodically increase with the applied biofertilizers in sixth month treatment and being highest (928 g) in the treatment where combined three biofertilizers were applied by maintaining constant soil fertility. Finally the results revealed the importance of micronutrients have significant role in presence of biofertilizers to increase the healthy growth of *Stevia* plant in acidic soil condition.

OP-13

Researches on *Amaranthus* sp. seed production in pedoclimatic conditions of Somesan Plateau, Romania

T. Rusu¹, D.I. Marin², A.I. Pop¹, P.I. Moraru¹, M. L. Soptorean¹,

I. Pacurar¹, L.I. Pop¹

¹University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, 400372, Cluj-Napoca, ²University of Agronomical Sciences and Veterinary Medicine Bucuresti, Faculty of Agriculture, 011464, Bucuresti, Romania

The *Amaranthus* genotype species are cultivated as „pseudo cereals” because of their high content of carbohydrates, proteins and fats, comparable or even superior to cereals themselves. Testing of 12 varieties of *Amaranthus* sp. (table 1), for the purpose of their integration in the agricultural technologies of Somesan Plateau, has been done on stagnic argic phaeozem, in the following climatic conditions: annual precipitations value of 523 mm and annual average temperature of 9.4°C.

Statistical processing of seed production data shows significant positive results, compared to the experiments average (2530.36 kg/ha), for the varieties: Golden, Plenitude, Amont, Hopi Red Dye (with positive differences ranging between 18.97-196.81 kg/ha). The variance analysis of density factor showed very significant positive differences for all variants planted at 100000 plants/ha, the crop yield registered being higher with 307.03 kg/ha from the variants planted at 70000 plants/ha.

OP-14

A good model for domestication of medicinal plants in Turkey: kekik

N. Şekeroğlu

Kilis 7 Aralık University, Art and Science Faculty, Biology Department, 79000 Kilis, Turkey

Most of the medicinal plants used and traded have still been collected from nature in all over the world. Some of these plants that have economic value have recently been cultivated under controlled conditions. Nevertheless, production of the number of cultivated medicinal and aromatic plants has been limited because of handicaps related to domestication and agricultural techniques. The main restriction in domestication of these plants is their wild characteristics. The second one is changes in phytochemical contents and compositions in cultivated crops by comparing to collected ones from natural habitats. Thirdly, farmers who cultivate these plants do not have enough technical information about these exotic crops. As a result high content of secondary metabolite with desired composition can be hampered by cultivation and industrial applications. Fourthly, chemical agricultural practices which increase the yield are rejected for them, because these crops are used in food and medicinal purposes. Lastly, harvest time and postharvest practices such as drying, processing, packing and storing have vital importance for the yield of secondary metabolites with high quality. In this mean, our topic is domestication of kekik, which is the first domesticated aromatic and medicinal plant with high economical value. Kekik is a general name for *Origanum*, *Thymus*, *Thymbra*, *Satureja* and *Coridothymus* species in Turkey. Kekik has been cultivated in the last two decades, therefore, , domestication of kekik, mainly *Origanum* spp., was clarified step by step in this present study. We are hoping that domestication story of kekik in Turkey would be a good model for any other wild collected medicinal plants in the world. Thus biodiversity of these plants would be conserved in their nature and standardized medicinal and aromatic plants would be offered to market.

Table 1: The influence of *Amaranthus* sp. variant on seed production yield obtained on stagnic argic phaeozem from Cluj-Napoca, 2009

Variety	Crop production, kg/ha	Yield, %	Difference, ±	Significance of differences
Witness (average)	2530.36	100	0	MT
Alegria (<i>Amaranthus cruentus</i>)	2425.83 c	95.9	-104.53	000
Amont (<i>Amaranthus cruentus</i>)	2670.17 j	105.5	139.81	***
Pleisman (<i>A. hypochondriacus</i> x <i>A. hybridus</i>)	2497.17 g	98.7	-33.19	000
Golden (<i>Amaranthus hypochondriacus</i>)	2727.17 k	107.8	196.81	***
Mercado (<i>Amaranthus hypochondriacus</i>)	2411.00 b	95.3	-119.36	000
Burgundi (<i>Amaranthus hypochondriacus</i>)	2511.00 h	99.2	-19.36	000
Hopi Red Dye (<i>A. hypochondriacus</i>)	2549.33 i	100.7	18.97	***
Chihuahuan (<i>Amaranthus cruentus</i>)	2472.83 d	97.7	-57.53	000
Opopeo (<i>Amaranthus hypochondriacus</i>)	2488.83 f	98.4	-41.53	000
MT3 (<i>Amaranthus cruentus</i>)	2404.17 a	95.0	-126.19	000
Plenitude (<i>Amaranthus</i> ssp.)	2727.00 k	107.8	196.64	***
Intense Purple (<i>A. hypochondriacus</i>)	2479.83 e	98.0	-50.53	000

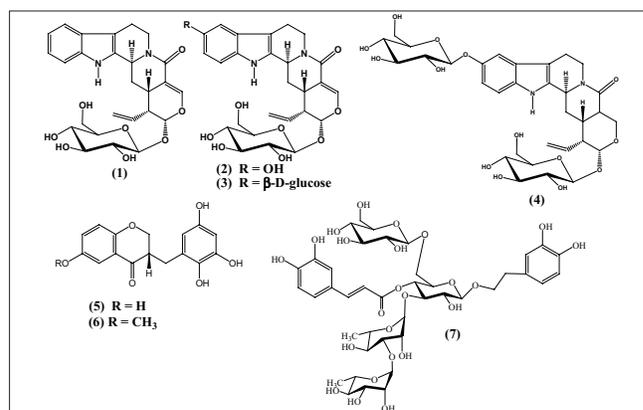
DL 5%= 6.77 kg/ha, DL 1% = 9.22 kg/ha, DL0,1% = 12.40 kg/ha

Note: *significant positive, osignificant negative; a, b, c...j, k – classification after Duncan test

OP-15**Novel GST inhibiting natural products from medicinally important plants****A. Ata**

Department of Chemistry, The University of Winnipeg, 515 Portage Avenue, Winnipeg MB, R3B 2E9 Canada

Glutathione S-transferase (GST) is a phase II detoxification enzyme that functions in the conjugation of endogenous and exogenous toxic substances to glutathione (GSH) producing a less toxic conjugate for excretion. Some GST isoforms have been discovered to be associated with multiple drug resistance in cancer and antibiotic chemotherapy. This is possibly by virtue of this derivatization reaction. As a result, a rationale was established to use chemical compounds that can inhibit GST as adjuvant in chemotherapy. Several compounds have been modeled after GSH to inhibit GST. Recently, we initiated a project to discover naturally occurring GST inhibitors from medicinally important plants and we screened crude methanolic extracts of 10 plants for GST inhibition activity. We discovered that crude methanolic extracts of *Naucllea latifolia*, *Barleria priontis* and *Caesalpinia bonduc* were active in our bioassay. Our bioassay-guided fractionations on the crude methanolic extracts of these plants resulted in the isolation of several new natural products (1-7) and these compounds exhibited GST inhibition. During this presentation, structure elucidation of these new bioactive natural products, their bioactivity data and structure-activity relationships (SAR) will be discussed.

OP-16**Anti-inflammatory and anti-hyperalgesic effects from a benzoquinone in *Ardisia crispata* thunb d.C.****A. H. Roslida¹, K. H. Kim²**¹Department of Biomedical Sciences, Faculty of Medicine & Health Sciences, Universiti Putra Malaysia, Serdang, 43400 Selangor; ²Department of Physiology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

The genus *Ardisia* is the largest in family Myrsinaceae and approximately 500 species of evergreen shrubs and trees are found throughout the subtropical and tropical regions of the world). There were a few of *Ardisia* species that have been studied and reported for its medicinal properties. It has also been reported to produce several groups of biologically active phytochemicals including saponins, coumarins and quinines. Bioassay guided fractionation and isolation of the dried root of this plant (ACR) yielded a bioactive compound from the *n*-hexane fraction (ACRH) namely AC2, identified as 2-methoxy-6-undecyl-1,4-benzoquinone. The fraction, ACRH and its compound, AC2 were shown to be pharmacologically active for its anti-inflammatory and anti-hyperalgesic effects by using carrageenan induced oedema for its anti-inflammatory properties and carrageenan induced hyperalgesia by using Plantar test for its anti-hyperalgesic properties. We found AC2 to possess both activities as its ED₅₀ of anti-inflammatory effect is 2.5-fold less potent compared to ED₅₀ of ACRH respectively, while for the anti-hyperalgesic effect, ED₅₀ of AC2 is 3.4-fold more potent compared to ED₅₀ of ACRH. Based on the skeleton structure of AC2, we postulated that both its anti-inflammatory and anti-

hyperalgesic effects might be due to its 5-lipoxygenase (5-LOX) inhibiting action. Therefore, further investigation should be done to confirm whether AC2 might also be possible to act as a 5-lipoxygenase inhibitor.

OP-17

Antioxidant, antiinflammatory and xanthine oxidase inhibitory activity of *Plumbago zeylanica*

S.H. Nile, C.N. Khobragade

Biotechnology Research Laboratory, School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded- 431606 (MH), India

The possible effect of methanolic extract of *P. zeylanica* shoots was evaluated *in-vitro* for its antioxidant, anti-inflammatory and xanthine oxidase inhibitory activities. Antioxidant activity was measured using ABTS, DPPH, FRAP and ORAC methods, while anti-inflammatory activity was measured by Diene-conjugate, HET-CAM and β -glucuronidase methods. The enzyme inhibitory activity was tested on the cow milk xanthine oxidase. The average antioxidant activity of *P. zeylanica* shoot extract in the concentration range 1-2 $\mu\text{g/ml}$ in the reacting system revealed significant antioxidant activity viz 42.2 (ABTS), 28.7 (DPPH), 36.5 (FRAP) and 25.6 percent by ORAC assay. The anti-inflammatory activities as recorded by Diene-conjugate, HET-CAM and β -glucuronidase assay methods were 45.40, 10.50, and 70.50 percent respectively. Screening of Xanthine oxidase inhibitory activity by extract in terms of kinetic parameters revealed noncompetitive mode of inhibition, where in the K_m and V_{max} values in presence of (25 to 100 $\mu\text{g/ml}$) *P. zeylanica* methanolic shoot extract is 0.20 μg and 0.035, 0.026, 0.023 and 0.020 ($\mu\text{g}/\text{min}$) while for control K_m and V_{max} is 0.21 μg and 0.043 ($\mu\text{g}/\text{min}$) respectively. These findings suggest that *P. zeylanica* shoot extract may possess prominent medicinal properties and can be exploited to treat the diseases associated with free radical formation, oxidative stress, xanthine oxidase activity and inflammation.

OP-18

Pharmacological evaluation of the wound healing potential of *Olea europaea* l. Leaf extracts by *in vivo* experimental models

U. Koca¹, I. Pesin Süntar¹, E. Küpeli Akkol¹, D. Yılmaz¹, M. Alper³

¹Department of Pharmacognosy, Faculty of Pharmacy, Gazı University, 06330, ²Department of Pathology, Dışkapı Yıldırım Beyazıt Education and Research Hospital, Ankara, Turkey

Fruits and leaves of *Olea europaea* had been used as laxative; hypotensive, cardiogenic, antiarrhythmic anticholesterolemic, antidiabetic, antirheumatic, emollient, in skin ulcers, cracks and speed healing of inflammatory wounds in folk medicine. For the assessment of the wound healing potential of the leaves, *n*-hexane, and water extracts were prepared from the dried leaves of *Olea europaea* L. grown in northwest part of Turkey. The activity of the extracts was evaluated by *in vivo* linear incision and circular excision wound models, comparatively with the reference ointment Madecassol[®]. The group of animals, which were treated with the aqueous extract of *O. europaea* L., demonstrated 87.1 % contraction on excision and a significant increase (34.8 %) in wound tensile strength on incision wound model as compared to other groups. The data obtained in the histological investigation, confirm the results of *in vivo* wound models as well. Moreover, *in vitro* antioxidant activity assay demonstrated

that aqueous extract of the leaves has higher scavenging ability than the *n*-hexane extract. The experimental data demonstrated that aqueous extract of *O. europaea* leaves displayed remarkable wound healing activity. Oleuropein (4.6059 %), a secoiridoid, was identified as the major active compound according to HPLC analysis of the aqueous extract.

OP-19

Steroids from the *Erythrina variegata* plant of Indonesia and their anti-cancer properties against breast cancer cell t47d

T. Herlina¹, Nurlelasi¹, R. Maharani¹, U. Supratman¹, U.M.S. Soedjanaatmadja¹, Z. Udin², H. Hayashi³

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Jatinangor 45363, Sumedang, ²Research Centre for Chemistry, Indonesian Institute of Sciences, Bandung 40135, Indonesia, ³Laboratory of Natural Products Chemistry, Division of Applied Biological Chemistry, Graduate School of Agriculture and Life Science, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan

The *Erythrina variegata* (Leguminosae) were commonly used as traditional medicine for anti-cancer. In the course of our continuing search for novel anticancer compound from *Erythrina* plants, the methanol extract of the leaves and stem bark of *E. variegata* showed significant anti-cancer activity *in vitro* toward breast cancer cell lines T47D using the Sulphorhodamine B (SRB) method. The methanol extracts were separated by bioassay-guide fractionation. By using the anti-cancer activity to follow separation, the ethyl acetate fractions were separated by combination of column chromatography to yield three active compounds (1-3). The chemical structure of active compounds (1-3) was determined on the basis of spectroscopic evidences and comparison with those previously reported and identified as steroid derivative. The active compounds (1-3) showed anti-cancer activity against of breast cancer cell lines T47D with IC_{50} of 6.5, 5.3, and 3.2 $\mu\text{g/ml}$, respectively. These results strongly suggested that compounds (1-3) are promising sources for anti-cancer agents.

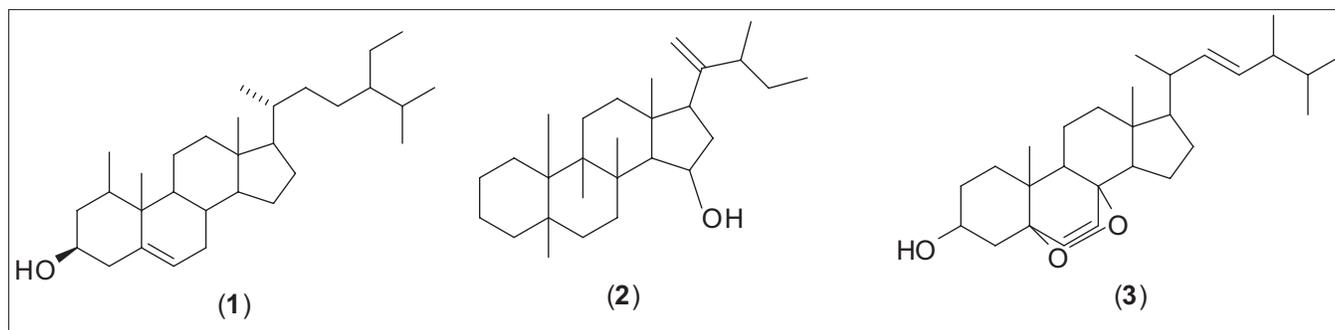
OP-20

Modern technologies of herbal alkaloids receipt

M. Meskheli¹, N.Gagua¹, A.Bozhadze¹, R. Gotsiridze², V. Vachnadze³, A. Bakuridze¹

¹Tbilisi State Medical University, Tbilisi, ²Shota Rustaveli State University, Batumi, ³Ivlev Kutateladze Institute of pharmacochemistry, Tbilisi, Georgia

Alkaloids are characterized by multilateral pharmacological activity, broad spectrum of therapeutic action and hypotoxicity. Receipt of herbal alkaloids is difficult technological process connected with heavy financial expenses. Together with it, there are used organic solvents polluting environment and finished product. The purpose of our research was the development of alternative and safe technologies of herbal alkaloids making. The objects of the research were above-ground parts of the following of plants: *Phellodendron amurense* Rupr., var. *lavallei*, *Vinca herbaceae* Waldt et Kit, *Vinca minor* L., *Chelidonium majus* L., *Glaucium flavum* Crantz; Extraction of alkaloids from medicinal herbal raw material was realized with diluent gas, with recirculation of the latter in closed system. Fractionation was realized by alkaloid substitute alcohol extracts run through reusable fluoroplastic microfiltering membrane plants resistant to rust, alkali and acidity. Extraction of alkaloids with diluent gas in comparison



with standard extraction by fluid in fluid method and membrane technologies gave us opportunity to extract biologically active substances with more concentration by means of their natural combination retention. Consequently, pharmacologically active sums and individual substances output considerably increased (for example, output of sum total from above-ground parts of *Vinca minor* L. as compared with fluid in fluid extraction increased twice as much).

Phellodendron amurense Rupr., var. *lavalleyi* in relation to delivered technologies is exception in the context of the fact that the extraction of crushed cortex with diluent gas in this case was conducted with the purpose of for extraction, as a result of which there was fluid in fluid extraction of herbal raw material released from lipophilic substances with the purpose of alkaloid berberin secretion, output of which for each 100 g of air-dry crust made up 2.5 g, and total medicine with antidiabetic and anticholinergic action – 10 g. Maximum output of plants *Vinca herbaceae* Waldt et Kit, *Vinca minor* L., *Chelidonium majus* L., *Glaucium flavum* Crantz is reached at the time of extraction with diluent gas, and its use is reasonable for *Phellodendron amurense* Rupr. crust for extraction.

OP-21

Use of chemotaxonomic markers of problematic medicinal plants used in traditional medicines

M. Ahmad, M A. Khan, M. Zafar, S. Sultana

Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan

Onosma hispidum Wall. & G. Don. (Boraginaceae) is important medicinal plant, used in Unani system of medicine as cardiac drug. In Unani, Ayurvedic and traditional system of medicines, the drug is marketed under the trade name rattan jot. In herbal market, this drug is misidentified, confused and adulterated with local available drug obtained from another species, *Geranium wallichianum* D. Don. ex Sweet. In order to ensure the use of only genuine and uniform material of such herbal drug, work on chemotaxonomic authentication assumed vital significance. Chemotaxonomic study has, therefore been carried out, covering detailed morpho-anatomical, palynological (SEM), features of crude drug (roots) include organoleptic tests, response to UV & IR light exposure and results of TLC fingerprinting (flavonoids) evaluated in this work would serve as standard reference for correct identification in commercialization at global trade.

OP-22

Chemical composition of algerian endemic plant *Euphorbia guyoniana* Boiss. and Reut.

H. Haba¹, H. Harkat¹, C. Lavaud², A.A. Magid², L. Marcourt³, M. Benkhaled¹

¹Laboratoire de Chimie et Chimie de l'Environnement (L.C.C.E), Département de Chimie, Faculté des Sciences, Université de Batna, Batna 05000, Algérie, ²CNRS FRE 2715 Laboratoire de Pharmacognosie, BP1039, 51097 Reims Cedex 2, France, ³Centre de Recherche sur les Substances Naturelles, UMS CNRS 2597, 3 rue des Satellites, BP 94244, 31432 Toulouse, France

In a continuation of our work on search for bioactive compounds from Algerian plants, present study was carried out on *Euphorbia guyoniana* Boiss. and Reut. of the family Euphorbiaceae. It is an endemic species of Algeria growing in sandy and desert habitat. It is used in the Algerian folk medicine against the venomous bites and it is known as a wart remover. Like the other species of the genus *Euphorbia*, it is characterized by the presence of milky irritant latex. This latex is very rich in isoprenic compounds as diterpenes and triterpenes which showed interesting pharmacological activities. The aerial parts of this plant have already investigated and macrocyclic diterpenoids type jatrophae have been reported. We now present the results of the phytochemical investigation of the chloroform extract of the roots of *E. guyoniana* and describe the isolation and structure elucidation of 24 secondary metabolites including 8 diterpenoids among them 4 are new, 11,16-epoxy-*ent*-abieta-8,11,15-triene-13,14-dione (1), 11-hydroxy-*ent*-abieta-8,11,13-trien-15-one (2), 8,11,13-*ent*-abietatriene-11,16-diol (3) and 4,12-dideoxy- (4 α) phorbol-13-hexadecanoate (4) and 16 triterpenoids among them 1 is new named 24-methylenecycloartane-3,28-diol (5). The known compounds are jolkinolide E (6), jolkinolide A (7), 3 α -hydroxy-*ent*-atis-16(17)-ène-2,14-dione (8), 3-benzoyloxy-5,15-diacétoxy-9,14-dioxojatropha-6(17),11-diène (9), 24-methylenecycloartanol (10), 3-hydroxycycloart-24-one (11), cycloeucaenol (12), cycloartenol (13), 24-epimers 24,25-epoxycycloartenol (14), lanosterol (15), euferyl (16), butyrospermol (17), obtusifoliol (18), multiflorenyl acetate (19), multiflorenyl palmitate (20), peplusol (21) 3 β -hydroxytaraxer-14-en-28-oic acid (22), β -sitosteryl-3 β -glucopyranoside-6'-O-palmitate (23) and β -sitosterol (24). Structures of the isolated compounds were established mainly by 1D and 2-D homo and heteronuclear NMR (¹H, ¹³C, COSY, HSQC, HMBC, NOESY) and mass spectrometry EI-MS and ESI-MS and by the comparison with literature data.

OP-23

Acrosomal status and sperm viability of mice spermatozoa fed aqueous extract of *Phyllanthus amarus*

S. Sukardi, R. Abdul Hamid, Z. Ahmad

Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti

Putra Malaysia, Serdang, Selangor, 43400, Malaysia

Acrosome reaction in spermatozoa is a prerequisite for fertilization and fresh ram spermatozoa has been shown to stay viable *in vitro* two hours after acrosomal exocytosis (Sukardi *et al.*, 1997). Research on the effects of *Phyllanthus amarus* on the male rodent reproductive system has shown its potential as a contraceptive (Shah *et al.*, 1996 and Rao *et al.* 1997). The present study was carried out to determine the effects of feeding aqueous extracts of the whole plant of *Phyllanthus amarus* on the acrosomal status and sperm viability of Balb/C mice. Seventy male mice were randomly allocated to four groups i.e., first group (7 mice without treatment – preliminary group), second group (21 mice as control - distilled water), third group (21 mice - 100 mg/kg extract) and the fourth group (21 mice - 400 mg/kg extract). Treatment was continued for 60 days. The first group was sacrificed at the beginning of the experiment as control for all groups. Meanwhile, seven mice were sacrificed from each group on days 20, 40 and 60. Observation of the spontaneous acrosome reaction was carried out by incubation of cauda epididymal sperm suspensions in a water bath at 39°C for 2 hours. Sperm suspensions were taken for fluorescent labeling at 0, 30, 60, 90, and 120-minute intervals. Fluorescent staining was performed using propidium iodide and fluorescein isothiocyanate conjugated (FITC) *Pisum sativum agglutinin* for sperm viability and acrosomal status respectively. Slides were observed under fluorescence microscope equipped with FITC filters. The percentages of live and dead acrosome reacted sperm cells were recorded. The results showed no significant difference between the percentages of live and dead acrosome-reacted sperm cells in groups 3 and 4 as compared to control for all the days of sperm collection. Sperm viability percentages also return a non-significant difference between groups 3 and 4 as compared to control. The results suggests that the concentrations of *Phyllanthus amarus* aqueous extract given for 60 days did not exert any effects on the acrosome reaction and sperm viability in mice spermatozoa.

References

- Rao, MV., Shah, KD and Rajani, M (1997). *Phytotherapy Research*, 11:469-71
- Shah, KD., Rajani, M., Patil, GR and Rao, MV. (1996). Proceedings of International Symposium on Male Contraceptive: Present and Future. AIIMS, New Delhi. Abstract no. 88. PP:52.
- Sukardi, S., Curry, MR and Watson, PF. (1997). *Animal Reproduction Science*, 45:89-96.

OP-24

Discovery of natural specific enzyme inhibitors for the treatment of malaria and other neglected diseases

A. Khalid¹, S.A. Mustafa¹, M. Galal¹, M.I. Choudhary², S.T. Prigge³

¹Medicinal and Aromatic Plants Research Institute, National Center for Research, P.O. Box: 2404, Khartoum, Sudan, ²Dr. Panjivani Center for Molecular Medicine and Drug Research, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan, ³Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe Street, Baltimore, MD 21205, USA

Enzymes are attractive targets for drug discovery. Recent reports show that about 50% of small molecule drugs inhibit enzymes as their molecular targets¹. On the other hand, natural products yield unorthodox and often unanticipated chemical structures that offer novel leads for molecular modifications leading to clinically useful drugs. Previous studies have demonstrated that the hit rate of natural products is on average 3-10%, as compared

with ~ 0.03% of that of compounds from synthetic origin².

Our enzyme inhibition research target several tropical and neglected diseases-related enzymes including malaria, diabetes, tuberculosis, trypanosomiasis and few other parasitic diseases. We have recently conducted a research study to discover new natural antimalarials that inhibit *Plasmodium falciparum* Enoyl-ACP reductase (PfENR). Inhibition of the activity of this enzyme is believed to be promising therapeutic targets and a major goal in malaria research^{3,4}. During this study we have screened several hundreds of natural, synthetic and microbial biotransformed compounds against PfENR. More than 30 compounds have inhibited PfENR activity in low micro molar concentrations. This talk will give an overview of our research on the inhibition of neglected disease related enzymes by natural and synthetic compounds. Some of our newly-discovered natural PfENR inhibitors will be presented to demonstrate how theoretical computational medicinal chemistry could be utilize along with classical experimental enzymology to investigate the molecular basis of enzyme-inhibitor interactions.

References

1. Kinghorn AD. In: Gullo V, editor. *The Discovery of Natural Products with Therapeutic Potential*. Stoneham: Butterworth-Heinemann; 1994. p. 81-108.
2. Koch MA, Wittenberg LO, Basu S, Jeyaraj DA, Gourzoulidou E, Reinecke K, et al. Compound library development guided by protein structure similarity clustering and natural product structure. *Proc Natl Acad Sci U S A* 2004;101(48):16721-6.
3. Kapoor M, Dar MJ, Surolia A, Surolia N. Kinetic determinants of the interaction of enoyl-ACP reductase from *Plasmodium falciparum* with its substrates and inhibitors. *Biochem Biophys Res Commun* 2001;289(4):832-7.
4. Biagini GA, O'Neill PM, Nzila A, Ward SA, Bray PG. Antimalarial chemotherapy: young guns or back to the future? *Trends Parasitol* 2003;19(11):479-87.

OP-25

Antimicrobial potential and antioxidant potential in the shell extract of *Cocos nucifera* against *Candida* and *Trichophyton* species from diabetic patients

N. Thebo¹, M.I. Bhangar², M.A. Memon³, M.H. Nizamani³

¹Free Radical Research Laboratory, National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamsboro 76080, ²Mycology Research Laboratory Institute of Botany, University of Sindh Jamsboro, ³Liaquat University of Medical Health and Science Jamsboro Sindh Pakistan

Tinea infection as one of the most dermatomycoses is caused by species of pathogenic fungi such as *Epidermatophyton*, *Trichophyton* and *Microsporum*. Opportunistic diseases cause substantial morbidity, necessitate toxic and expensive therapies, result in the survival of diabetic patients. The potential of biotechnology provides health protection through medicinal plants because it also facilitates the earlier detection of various ailments by new biotechnological techniques.

Coconut considered very healthy in Ayurveda and *posses* a variety of beneficial properties affecting most notably cancer, high blood pressure; infectious disease and diabetes. Coconut oil also helps in treating various skin problems including psoriasis, dermatitis, eczema and other skin infections. In our study, we have used shell extract of *coconut* for the evolution of antioxidant, antimicrobial potential and therapeutic efficacy against isolated pathogenic micro-organisms. Fifty five patients with fungal lesions attending the different departments of Liaquat University Medical Health and Science (LUMHS) were screened for *Candida*. Forty six (61.3%) *Candida* strains were isolated from the oral

lesions. The extract was prepared by Soxhlet method and TPC in the extract was determined by spectrophotometrically. The antioxidant activity of crude extract of the coconut shell was evaluated by using the DPPH, (2,2-diphenyl-1-picrylhydrazyl) radical scavenging system and (MIC) of extract was determined by well method against isolated pathogenic causative organisms for confirmation the possibility of short term therapy only 15 days. The total antioxidant activity varied from 93.32 to 94.40 % and total phenolic content was found 3.422 mg/gm in coconut shell extract. Shell extract of coconut was found to be most effective as an antifungal and antibacterial agent against human pathogenic *Candida* species, *Trichophyton*, *Actinomyces*; *Fusarium* and *Aspergillus niger*. The bacterial species were examined; *E. coli*, *staph aureus*. Finally the results provide a therapeutic potential for microbial infections.

OP-26

Determination of antioxidant activity and inhibition of xanthine oxidase of ethyl acetate extract from snake fruit (*Salacca edulis reinw.*) Variety of Bongkok

L.H.A. Priyatno¹, E.Y. Sukandar², S. Ibrahim³, I.K. Adnyana²

¹Food Technology Department of Pasundan University, ²Pharmacology-Clinical Pharmacy, School of Pharmacy-Institute Technology, ³Pharmaceutical Chemistry Research Group, School of Pharmacy-Institute Technology Bandung, Indonesia

Antioxidant activities of ethyl acetate extracts of snake fruit (*Salacca edulis Reinw.*) variety of Bongkok has been determined using 2,2-diphenyl-1-picrylhydrazil (DPPH) reagent. Ethyl acetate extract of snake fruit gave antioxidant activities, it has IC₅₀ was 33.92 µg/ml. Ascorbic acid is used as reference substance. IC₅₀ of ascorbic acid was 3.18 µg/ml.

Inhibition of xanthine oxidase by ethyl acetate extract of snake of Bongkok was determined *in vitro*. The value of IC₅₀ to inhibit xanthine oxidase was 24.75 µg/ml. Allopurinol was used as reference substance. IC₅₀ of Allopurinol was of 0.92 µg/ml.

OP-27

Threats to community based conservation of medicinal plants in bastar hills of Chhattisgarh: Issues of protecting intellectual and cultural property rights of Shamans

R. N. Pati

Consultant, UNDP Project, State Medicinal Plants Board, Jail Road, Raipur, Chhattisgarh, India

The General Agreement on Tariffs and Trade (GATT) and Trade Related Aspects of Intellectual Property Rights agreement (TRIPs) fail to address the challenges encountered by tribal shamans and herbalists in developing countries of the world. The imposed intellectual property right regime overlooks the customary rules and cultural values governing sustainable use of medicinal plants. The paper is based on one year field investigation focused on developing viable mechanism for documenting traditional botanical knowledge, promoting organization of shamans, herbalists and developing legal framework for patenting rights of the shamans. The biological resources in the indigenous territories of Chhattisgarh consist of various natural sources of agricultural, medicinal, ecological, veterinary and cosmological potencies which ensure equilibrium between local environment and social health of the tribal communities in forest villages.

Very often the development planners overlook rules, roles of these communities as regard to identification of plants and

habitat of plants, methods of planting and extracting medicinal components guided by traditional knowledge system.

OP-28

Survey of composition and bioactivity of essential oils and extracts of aromatic plants grown in Colombia

E. Stashenko

Research Center for Biomolecules, CIBIMOL-CENIVAM, Industrial University of Santander, AA 678, Bucaramanga, Colombia

820 Botanical accessions of aromatic plants (herbs, shrubs, trees) were collected in 20 expeditions to various Colombian regions (2005-2009). Essential oils were isolated by steam distillation, conventional, or microwave-assisted hydrodistillation. Absolutes and concretes were obtained from flowers and floral scents were studied using *in vivo* and *in vitro* HS-SPME techniques. The various volatile fractions and essential oils were analyzed by GC-FID and GC-MS techniques, using mass spectra, retention indices and standard compounds. Essential oils were subjected to biological assays to examine their antifungal, antibacterial, anti-*Leishmania*, anti-Chagas, anti-tuberculosis, anti-viral, and anti-oxidant activities. Significant antioxidant capacity was found in 16% of essential oils tested with the ABTS and DPPH assays. Approximately half (87) and one fourth (35) of 181 essential oils examined showed activity against *T. cruzi* and *L. chagasi*, respectively. A total of 33 and 28 essential oils were active against *M. tuberculosis* H37Rv and *M. chelonae*, respectively. The most active oils were obtained from *Swinglea glutinosa*, *Achyrocline alata* (bacteriostatic activity), *Salvia aratocensis* and *Turnera diffusa* (bactericidal activity). The results of this extensive *in vitro* study constitute a solid basis for further research into the sustainable use of Colombia's large biodiversity. Agroindustrialization of 8 promising aromatic species is currently under way.

OP-29

Opportunities to exploit the variation in the essential oils of herbs and spices- epca's experience in Albania

E. Bazina¹, X. Hysenaj², A. Ibraliu^{3,4}

¹ProMali Program funded by the Danish Ministry of Foreign Affairs and SNV- the Netherlands Organization for Development, ²Chairman of EPCA (Exporters, Essences producers and Cultivators Association); Administrator of Xherdo Company, ³Department of Crop Production, Faculty of Agriculture and Environment, Agricultural University of Tirana, Koder Kamez, Tirane, Albania, ⁴Institute of Plant Breeding, Seed Science, and Population Genetics, University of Hohenheim, 70593 Stuttgart, Germany

The use of the essential oils of Herbs and Spices is widespread in most Western Countries thus increasing pressure on natural resources of these plants. Albania continues to be a sound supply source of dried and processed herb and spice products; Sage being the major export item. Statistics of USDA 2009 indicate Albania has the biggest market share of Sage export to the US market accounting for about 55%. EPCA, the only truly vibrant industry association, focuses on industry development to go hand in hand with suitability issues. EPCA also addresses key factors that affect the success for commercialization of herb and spice products such as variation in the essential oils. This research study supports the above providing evidences on the variability of essential oil content and composition of wild populations of *Salvia officinalis*, *Satureja montana*, *Origanum vulgare*, *Thymus capitata* from different regions of Albania analyzed using GC/FID and GC/MS.

OP-30**Chemical composition and antioxidant activity of essential oils extracted from Egyptian medicinal plants**A. E. M. Abdalla^{1,2}¹Food Science Department, Faculty of Agriculture, Saba Basha, Alexandria University, ²Vice Dean, Faculty of Specific Education, Alexandria University, Egypt

The ancient Egyptian culture clearly documented their use of medicinal plants in their hieroglyphic records which are being used as pharmaceutical ingredients, and in food supplementation. Several medicinal plants have been reported to exhibit antioxidant activity.

The present study aimed to evaluate the chemical composition of essential oils extracted from Egyptian artemisia, juniper, rosemary, sage and thyme and investigate their antioxidant activities in different test systems as well as in soybean oil.

The results showed that rosemary, sage and thyme extracts had the highest amount of polyphenols. Essential oils from medicinal plants were found to contain 25 – 30 components at different concentrations. Camphor, α -thujene and artemisia ketone were the major components of artemisia, α -Pinene and β -phellandrene of the juniper, camphor, α -terpineol and 1,8-cineole of the rosemary, α -thujene and camphor of the sage and carvacrol and p-cymene of the thyme. Generally, rosemary, sage and thyme essential oils showed highest antioxidative activities in test systems and were found to be the most effective in retarding the oxidation of soybean oil under both accelerated (60°C for 18 days) and ambient temperatures (22 \pm 2°C for 180 days). Antioxidative activities of different essential oils could be related to their polyphenol contents and chemical composition.

OP-31**Induction of volatile terpene from spruce seedlings and their role in protecting against oxidative damage**G. Zeneli^{1,2}¹Max Planck Institute for Chemical Ecology, Hans-Knöll Str. 8, D-07745 Jena, Germany, ²Current: Faculty of Integrated Studies with Practice. University "Aleksander Moisiu" Durres, Albania

Oxidative stress is a common physiological stress that often challenges plants. Reactive oxygen species (ROS) are major factors in oxidative stress that significantly affect plant cell growth and secondary metabolism. Terpene metabolites are frequently implicated in chemical defense mechanisms against pathogens in conifers but, while the non-volatile resin components have often been suggested to have a role in protecting the tree against herbivores and pathogens, the function of the volatile portion of the resin is less clear. Although it has been hypothesized that they could protect plant cells against oxidation *in vivo*, this hypothesis has been less studied. To test this hypothesis, Norway spruce seedlings were treated with chemical elicitors. The treatment caused an increase in terpene emission while formation of (ROS) produced in the photosynthetic membranes lead to production of peroxidation of lipids as showed by the production of MDA. Compared with control samples, MDA levels increased three fold in stressed seedlings. This action is typical of other isoprenoids but we speculate that volatile monoterpenes provide a more dynamic protection mechanism as it is synthesized few hours after treatment. Use of advanced methods (e.g., metabolic profiling, use of modified tree genotypes etc) will give might better insight in the role of volatile terpenes against oxidative stresses.

OP-32**Determination of variability of essential oil and agricultural characterization of *Satureja hortensis* L. On date of harvest during flowering period in the Çukurova condition**M. Aşçı¹, S. Kirici², M. Turk³, M. İnan⁴¹Department of Field Crops, Institute of Natural And Applied Sciences, Çukurova University, 01330 Adana, ²Department of Field Crops, Faculty of Agriculture, Çukurova University, 01330 Adana, ³Department of Chemistry, Faculty of Letter and Science, Çukurova University, 01330 Adana, ⁴Department of Field Crops, Kahla Vocational School, Adyaman University, Kahla/Adyaman, Turkey

This study was conducted to investigate determination of variability of essential oil and agricultural characterization of *Satureja hortensis* L. in the flowering stage, at experiment area of Field Crops Department, Çukurova University, Agriculture Faculty, during 2006- 2007 and 2008-2008 under Çukurova ecological conditions. In the plant height, number of branch, fresh herbage yield, dry herbage yield, dry leaves yield, essential oil content of dry leaves, and composition of essential oil by GC-MS were determined during eight cutting.

In the research, plant height, number of branch, fresh and dry herbage yield in the experiment years varied 28.1- 31.0 cm, 22.5-22.9 per plant, 790.5-1085.0 kg/da and 345.4-455.7 kg/da, respectively. Dry leafyields were 261.6 and 338.1 kg/da in the first and second year. Mean of essential oil content was varied between 3.15 % and 2.30 %8. The main constituents of essential oil were determined as γ -terpinene, carvacrol and p-cymene in the each cutting and both years. The most suitable cutting time in terms of content of essential oil was beginning of flowering stage.

OP-33**Organic energy saving production and processing of *lavandula angustifolia* mill. In republic of Moldova**

B. Filip

Institute of Applied Physics, Academy of Sciences, 2028, Kishinen, Republic of Moldova

Soil and climatic conditions in Republic of Moldova are favorable for many aromatic plants. The main aromatic culture was, is and will be the lavender. In the middle of the 80's lavender oil year production was near 80 tons. Now there are only 8-10 tones per year. The used technological cultivation and processing procedures are energy-intensive and needs a lot manual labor, the costs of lavender oil is close to its selling price. Since 2004 we have started to implement some efficient environmentally friendly agro-technological procedures. The mechanized two phases harvesting is used. Continuous processing of dried raw materials (16-20% humidity) by superheated steam countercurrent to the raw material motion and efficient recovery of heat was implemented. For superheated steam production is used biomass (dry lavender wastes). We found that distillation process is accelerated more than 3 times, energy consumption is reduced by 4-5 times, significantly is increased the quality of the final product. For example - in 2004 we have produced 4.0 tons of lavender oil with content of linalyl acetate 46.8% and 39.2% of Linalool – the average from other manufacturers is 32.0-35.0% and 28.0-37.0% respectively. Since 2004 our technology and lavender oil are organic certified by SGS Moldova. The costs of lavender essential oil obtained by this technology are about 2.5 times lower than the price. At the same time these technological

procedures may be used for processing the other aromatic plants.

OP-34

The new introduced medicinal plants and their usage in extracts and final product preparations

I. Salamon

Excellence Centre of Human and Animal Ecology, Presov University in Presov, 01, 17th November St., SK-081 16 Presov, Slovakia

Over the last few years, the introduction of new research & application tools has dramatically changed the field of natural products, in an effort to keep pace with growing consumer demand. Alternative food ingredients, preservation materials and pharmaceutical compounds mainly from medicinal and aromatic plants, such as, natural antioxidants, bioflavours, biopreservatives, natural colorings and fragrances are being increasingly utilized by the industry today. In regard to a huge number of these natural components their part of single medicinal & aromatic plant species and their relationship to ecological growth conditions is a basic call to eco- biological research and summation.

The large-scale cultivation of medicinal plants in Eastern Slovakia belongs to the special agricultural production. It is the only way to supply the contracted volume and high quality of this plants raw material. The university field with an area of 0.75 hectares at Presov University in Presov, belonging to the Department of Ecology, was used mostly for growing vegetables and fruit in previous times. The present agro-technology research & development included medicinal and aromatic plant introduction. The introduction of further medicinal plants – *Stevia rebaudiana* Bertoni, *Tribulus terrestris* L., *Rhaponticum carthamoides* Ilja. - to the university field is in progress after it has been proved that it has the potential for research, cultivating and educational activities. A leader of this production in Slovakia is the company CALENDULA, Co., which is situated in Nova Lubovna. It has been establishing medicinal plant processing (the essential oil, extract production, food and cosmetic products) since 1999. Our mutual research includes dry and liquid extracts of *Rhaponticum carthamoides* Ilja., *Tribulus terrestris* L., *Stevia rebaudiana* and STEVIANA preparation. The main components of all these items were determined by chemical and analytical methods.

Acknowledgements

My participation at the 6th CMAPSEEC is supported by the Agency of Ministry of Education SR for the Structural Funds of the EU, the project: ITMS 26220120023.

OP-35

Evaluation adaptability of *Rosa damascena* mill. Genotypes in different province of Iran (Esfahan)

H. Zeinali¹, S.R. Tabaei Aghdai², Z. Miri¹, L. Safaei¹

¹Esfahan Agriculture Research Center, Esfahan, ²Research Institute of Forests and Rangelands, Iran

This experiment was carried out in order to study of relationship between flower yield and flower yield components in *Rosa damascena* genotypes. Thirty seven *Rosa damascena* genotypes collected from different regions of Iran were cultivated in randomized complete block design with three replications at Kashan Desert Research Station. The traits evaluated were flower yield per plant, number of flower per plant, number of petal per

flower, wet and dry flower weight, and dry matter percentage of flower, wet petal weight per flower, and weight of wet petal to flower weight, plant vigor, plant height and canopy. Analysis of variance showed significant differences among genotypes for all the studied traits. Significant differences observed among years for all traits except for dry weight of flower. Result of analysis of variance for interaction year × genotype was significantly different for all characters. Factor analysis revealed four factors which justified 85.60 percent of the total variation among characters. In the first factor, fresh and dry weight of flower, fresh petal weight per flower had high loading factor and were named weight of flower factor. In the second factor, canopy and plant vigor had high loading factor and named plant vigor. The third factor showed that flower yield per plant and flowering period traits had high loading factor and were named flower yield factor. In the fourth factor, dry matter percentage of flower, plant height and canopy had high loading factors and named time flowering factor. Based on cluster analysis, genotypes were classified in 5 groups. Also the result of this study showed the genetic variation is not related to geographic diversity.

OP-36

Development of a sustainable agri-silvicultural system by integrating medicinal plants

K. Bimlendra, D.P.S. Nandal

Department of Forestry, CCS, H.A.U Hisar, India

In the northwestern parts of India, poplar tree has gained popularity among the farmers as an important agro forestry tree because of the added advantages associated with the tree. Poplar tree is known to cause a negligible effect on the intercrops during the first three years of tree growth. There was a significant (40-45%) decrease in the yield of traditional rabi and kharif (wheat and fodder crops) crops when taken with poplar after 3rd year of tree planting. In order to make up for this loss if some shade loving and perennial medicinal plants are introduced in the system after 3rd year onwards, it can prove to be a very viable and beneficial option, for the small farm holders, who are interested to grow poplar as a short rotation commercial tree. Since the medicinally important plant resource in the natural forests has considerably diminished during recent years because of continuous increasing demands of the rare medicinal value plant stock. According to IUCN, World Conservation Union, the global market for medicinal and aromatic plants is estimated to be worth US dollars 800 billion a year. International trade is dominated by China which exports 1,21900 tones a year, India exports about 40,000 tones in toto. The natural forests have shrunk in size and to meet such demand they will not be sufficient in near future. No new land areas can be converted into natural forests because of urbanization and increasing demand of ever growing population for food and housing. To reduce the pressure on the natural forest the only alternative left is to encourage the farmers to integrate trees on their farm lands to meet the requirement of tree products such as timber, small wood and paper pulp etc. The cultivation of trees along with agricultural crops and medicinal crops can be a viable option in the coming times. Taking these into consideration six medicinal plants viz mulahati, shatawari, ashwagandha gwarpatha, giloe and kwanch were planted with poplar trees. Poplar trees have been planted at three spacing i.e 5x4, 10x2 and 18x2x2m and replicated

thrice. During first year of growing the effect of spacing of poplar trees on all the medicinal plants was negligible on growth parameters. The medicinal crops such as mulahati, gwarpatha, giloe and shatawari grew significantly better (in terms of growth parameters such as plant ht., collar diameter and branch no. etc.) in close spacings of poplar i.e. 5x4&10x2m as compared to the wider spacing of 18x2x2m and control. Quality parameters such as the total crude glycyrrhizin content for shatawar, total alkaloid content for Ashwagandha, the mucilage % and Aloin content for Aloe, the total bitter content for Giloe, Saponin content for Shatawar and L-DOPA content for Kwanch will be worked out at maturity of all the crops, for the entire tree spacing treatments. The research work still continues as most of the medicinal plants included in the study are perennial, the quality and yield parameters will be analyzed after two years of growth and economic viability of these systems will be worked out. The inclusion of such perennial medicinal plants in to the poplar based agri-silvicultural systems from 3rd year onwards will prove a highly sustainable and economically beneficial system advocated to the farmers of the region.

OP-37

Hypericin from *Hypericum triquetrifolium* in wild and under cultivation: Variation revealed by genetic distance

M. Al-Rifae¹, T. Aburjai², N. Haddad³

¹National Center for Agricultural Research and Extension (NCARE), Field Crops Directorate, P.O.Box. 639, Baq'a 19381, ²Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, ³Department of Horticulture and Field Crops, Faculty of Agriculture, Jordan

Hypericum triquetrifolium (Guttiferae family) is a medicinal plant growing wild in Jordan and known as Roja, Halawa, Aran, and Orayna. Hypericin content and genetic diversity were studied in 27 wild populations of *Hypericum triquetrifolium* in Jordan. The wild populations were explored from arid to semi-humid areas (176 to 582 mm), growing in varied altitude (341 to 1577 masl). Hypericin content significantly varied among wild populations (0.03 to 0.14 %), and negatively correlated with rainfall indicating increased percentages of hypericin in arid environments. Five populations with high hypericin contents were introduced for cultivation in 2006 and 2007. They were behaved differently and categories into no increase in hypericin content over the wild, continuous increase and decrease then increase above the wild hypericin content. Genetic diversity among the wild populations was high, which distinguished populations into 5 clusters according to their geographical origins. A cultivated two years old Ramtha population significantly contained the highest hypericin content (0.156 %). Ramtha superiority alleviated genetically by its clustering pattern and its genetic distance from the other populations. *Hypericum triquetrifolium* wild populations are potentially important source for hypericin that encourage their improvement and cultivation.

OP-38

Alternative approach for bioactive alkaloid production by bulgarian medicinal plants

I. Ivanov¹, V. Georgiev¹, S. Berkov², A. Pavlov¹

¹The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, ²AgroBioInstitute, 8 Dragan Tzankov Bld., 1113-Sofia, Bulgaria

From time immemorial, humans have been highly dependent on plants as sources of proteins, carbohydrates and fats. Furthermore, the volume and range of phytochemicals used by modern society (inter alia as drugs, nutrients, cosmetic additives and biopesticides) are continuously expanding. Currently, most commercially important secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is not economically feasible. These high demands are driving efforts to develop new ways to produce plant-derived metabolites. Plant *in vitro* techniques, in which plant cells, tissue and organs are cultivated in sterile conditions totally offer such alternatives for producing important metabolites. The advantages of this technology over the conventional agricultural production are as follows: independence of geographical and seasonal variations and various environmental factors; insurance of the continuous supply of products with uniform quality and yield; possibility to produce novel compounds that are not normally found in parent plants; independence of political interference; efficiency in downstream recovery and product; etc. The presentation will attend to production of alkaloids using Amaryllidaceae plant *in vitro* systems as biofactories.

Acknowledgement

The investigations, subject of this presentation, have been done under financial support of National Science Fund of Bulgaria (Contracts N: TK-B-1605/2006 and DO-02/105-2009).

OP-39

Biofungicide for powdery mildew control

S. Rajković¹, M. Tabaković-Tošić¹, M. Marković¹, L. Rakonjac¹, M. Ratknić¹, D. Mitić²

¹Laboratory of Institute for Forestry, 11030, Belgrade, ²IRITEL a.d., 11000, Belgrade, Serbia

Because of the desire for reducing the negative consequences of applying chemicals, biological control is becoming increasingly important. For biological control of plant disease causing fungi used biofungicides. AQ-10 is a new biofungicide that contains fungal spores of *Ampelomyces quisqualis* for the control of powdery mildew by parasiting and killing the fungal organisms that cause the disease. AQ-10 is approved for the efficient and biotical use of Powdery Mildew. AQ-10 is not selective for specific strains of Powdery Mildew but parasites the fungal organisms on various crops. The efficacy of biofungicide AQ10 becomes better by adding the polymer during its application. Aim of this work is to verify the effect of biofungicide AQ10 and in combination with polymer Nu-film 17 in controlling powdery mildews oak. Preliminary examination were performed by standard OEPP methods PP1/69 (2) (OEPP / EPPO, 1997) on the oak seedlings infected by parasitic fungus *M. albitoides*. Treatments were carried out in 4 repetitions, in the period June - September 2009. Infection list is evaluated by the method of EPPO/PP1/69 (2), Volume 2, P100-102. The intensity of infection was calculated by the method-Towsend-Heuberger, and performance by Abbott.

OP-40

Unusual patterns of direct somatic embryogenesis in *Digitalis trojana* Ivan: effects of exogenous auxin and auxin transport inhibitor

S.K. Verma, B. Yücesan, G. Şahin, E. Gürel

Abant İzzet Baysal University, Faculty of Science and Literature, Department of Biology, 14280 Bolu, Turkey

The effects of exogenous auxin and polar transport inhibitor on somatic embryogenesis in *Digitalis trojana* cultures were investigated. *D. trojana* Ivan, commonly known as “Helen of Troy Foxglove” (Truva Yüksükotu in Turkish), belongs to the family of Scrophulariaceae, an Turkish endemic medicinal plant. Vertical hypocotyls sections from 3 weeks old seedlings of *D. trojana* were used to examine the effect of Indole-3-acetic acid (IAA) on direct somatic embryogenesis. Although direct somatic embryogenesis was obtained with all IAA concentration (0.1, 0.5 and 1 mg/l) on the top of hypocotyls explants, optimum results were obtained with 1 mg/l IAA. The transport inhibitors, 2,3,5-triiodobenzoic acid (TIBA), block morphological transitions to subsequent stage; for example, they cause the formation of enlarged globular and oblong embryos. We observed in morphologically abnormal embryos induced on media supplemented with auxin or auxin polar transport inhibitors (0.1-2 mg/l IAA with 1-5 mg/l TIBA). Inhibitors of auxin polar transport disrupt normal embryogenesis and thus specific spatial auxin distribution due to auxin movement may be important in establishing embryonic pattern formation in plants.

OP-41

***In vitro* germination of *Bituminaria bituminosa* (L.) C.H. Stirt**

H. Çölgeçen¹, T. Körüklü², H.N. Büyükkartal²

¹Karadokuz University, Faculty of Arts and Science, Department of Biology, 67100 Zonguldak, ²Ankara University, Faculty of Science, Department of Biology, 06100 Tandoğan, Ankara, Turkey

Bituminaria bituminosa (L.) C.H. Stirt. is a perennial pasture legume, widely distributed in Mediterranean and African areas. The natural population belongs to the three taxonomic varieties *bituminosa*, *albomarginata* or *crassiuscula*. *B. bituminosa* is a long-day plant which flowers and sets seeds mainly in the spring. The plant has a high pastoral value and high palatability and is eagerly grazed by cattle, sheep and goats. No cultivars are commercially available as of 2009. There are four furanocoumarins (psoralen, angelicin, xanthotoxin and bergapten) present in leaves, stems and/or fruits of this species. Furanocoumarins are stated to result in photosensitization in grazing animals. However, there are not any evidence in the literature or stated by farmers that supports the photosensitization effect or any other health problems in grazing animals associated with *B. bituminosa*. The seed is covered by the fruit (fruits beaked). About 70% of seeds are hard and do not imbibe. The plant seeds collected from the field have undergone different experimentations. Germinations were conducted in Hormone-free MS medium. No germination was observed in beaked fruits and cut beaked fruit. A small scalpel cut that can reach the seed coat improved imbibition and a germination rate of 85% was obtained.

Acknowledgments

The authors are grateful to the Scientific and Technological Research Council of Turkey (TUBITAK) for financial support.

OP-42

Micropropagation and antioxidant potential in *Silybum marianum*

B.H. Abbasi, M.A. Khan

Department of Biotechnology, Quaid-i-Azam University, Islamabad-45320, Pakistan

Successful protocol for regeneration of medicinally important plant *Silybum marianum* L. was established and free radical scavenging activity of regenerated material was assessed and compared with wild grown plant material by using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Callogenesis and shoot organogenesis occurred from wild-grown leaf explants inoculated on media supplemented with Benzyladenine (BA), Gibberellic acid (GA₃) and α -Naphthalene acetic acid (NAA). Maximum explant response was observed on MS medium supplemented with 5.0 mg L⁻¹ BA after 20 days of culture. 25.5 \pm 2.0 shoots *per* culture flask was induced on MS medium supplemented with 2.0 mg L⁻¹ GA₃ and 1.0 mg L⁻¹ NAA after 30 days of sub-culture. Furthermore, best shoot length was observed on MS medium supplemented with 0.5 mg L⁻¹ BA and 1.0 mg L⁻¹ NAA. Optimized rooting response was recorded for MS basal medium. DPPH antioxidant activity of *in vitro* and *in vivo*-grown tissues was evaluated as gross parameter of medicinal efficacy. Callus showed significantly higher antioxidant activity than other regenerated tissues and wild-grown plantlets. This efficient *in vitro* production system developed in this study provided consistent tissues for investigation of bioactivity and germplasm conservation of *S. marianum*.

OP-43

Densitometric HPTLC analysis of 6-shogaol in *Zingiber officinale* extract and ginger-containing dietary supplements, teas and commercial creams

H. Yusufoglu

Department of Pharmacognosy, College of Pharmacy, P. O. Box 2457, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

Ginger root powder is widely used as a spice as well as a dietary supplement and flavoring agent in foods and beverages. High performance thin layer chromatographic (HPTLC) densitometric method for analysis of 6-shogaol has been developed to determine the quantity of 6-shogaol in *Zingiber officinale* extract and ginger-containing dietary supplements, teas and commercial creams on aluminum-backed silica gel 60 F₂₅₄ plates with n-hexane:ethyl acetate 70:30 (*v/v*) as mobile phase. This system was found to give a compact spot of 6-shogaol at retention factor (R_f) value of 0.33 \pm 0.02. The calibration curve shows good linear relationship with correlation coefficient of 0.9933 in the concentration range of 100–500 ng/spot. The proposed method was found cheap, selective, precise and accurate and can be used for routine quality testing of ginger extracts as well as ginger containing herbal formulations.

OP-44

Chemical and biological evaluation of Indonesian dipterocarpaceae

E.H.Hakim, Y.M.Syah, L.D. Juliawaty, D.Mujahaidin, S.A.Achmad

Department of Chemistry, Faculty of Mathematic and Natural Sciences, Institut Teknologi Bandung, Jalan Ganeca 10, Bandung 40132, Indonesia

The Dipterocarpaceae is one of large family of plant, comprising 16 genera and about 600 species, which are distributed primarily in South East Asia including Indonesia. Plants of this family elaborate mainly oligostilbenoids, the biological effects such as chemopreventive, hepatoprotective, inhibitors of 5 α -reductase, scavengers of superoxide, anti-inflammatory, fungicidal activities, phytoalexin, inhibition of topoisomerase II, histamine release

and gastric ATPase have been reported. As part of an ongoing search for novel natural products of plant origin with potential biological activity, a number of species of Dipterocarpaceae endemic to Indonesia have been investigated. In this paper, a review of our recent work that has led to the isolation of many types of oligostilbenoids, such as diptoindonesins A, B, D, F, and G and their cytotoxic activity will be presented.

References

- Syah YM, Aminah NS, Hakim EH, Aimi N, Kitajima M, Takayama H, *et al*. Two oligostilbenes, cis- and trans-diptoindonesin B, from *Dryobalanos oblongifolia*. *Phytochemistry* 2003;63:913-7.
- Atun S, Achmad SA, Hakim EH, Syah YM, Ghisalberti EL, Juliawaty LD, *et al*. *Biochem Syst Ecol* 2004;32:1051-3.
- Tukiran A, Hakim SA, Makmur EH, Sakai I, Shimizu K, Syah K, *Biochem Syst Ecol* 2005;33:631-4.
- Sahidin, Hakim EH, Juliawaty LD, Syah YM, Din LB, Ghisalberti EL, *et al*. *Naturforsch* 2005;60c:723-7.
- Juliawaty LD, Sahidin, Hakim EH, Achmad SA, Syah YM, Latip J, *et al*. A 2-Arylbenzofuran derivatives from *hopea mengarawan*. *Nat Prod Commun* 2009;4:947-50.

OP-45

Phenolic acid and flavonoid composition of *Sideritis lycia* and *Sideritis libanotica* subsp. *linearis*

C. Dinçer¹, A. Topuz¹, İ. B. Çam¹, İ. Tontul¹, K.S. Özdemir¹, H. Şahin¹, R. S. Göktürk², S. Tuğrul Ay³

¹Department of Food Engineering, Faculty of Engineering, Akdeniz University, 07059, Antalya, ²Department of Biology, Faculty of Arts and Sciences, Akdeniz University, 07059, Antalya, ³West Mediterranean Agricultural Research Institute, Antalya, Turkey

The genus *Sideritis* belongs to the family Lamiaceae and includes many species which are generally known as “dağ çayı or yayla çayı (mountain tea)” in Turkey. The present study was carried out to determine the phenolic acids and flavonoids composition of *Sideritis lycia* L. and *Sideritis libanotica* subsp. *linearis* L. Both plant samples were harvested from three different locations nearby Antalya province during flowering period and dried by using two different methods; conventional drying under shade (natural convection) and freeze drying. Dried samples were extracted in methanol/water solution and these extracts were then analyzed by using HPLC system.

It has been determined that p-coumaric and caffeic acids were the main phenolic acids for both species. The major flavonoids identified in *S. lycia* samples were hesperitin, quercetin and morin, whereas; apigenin was the principal flavonoid, followed by hesperitin, myricetin and morin in *S. libanotica* subsp. *linearis*. Moreover, the phenolic acid and flavonoid compositions of both species did not change significantly depending on the drying methods.

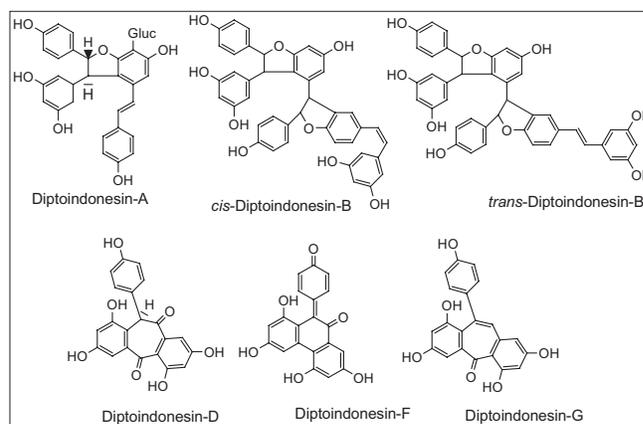
OP-46

Botanical and chemical biodiversity of aromatic plants (genus *Lippia*, Verbenaceae family) grown in Colombia, South America

E. Stashenko

Research Center for Biomolecules, CIBIMOL-CENIVAM, Industrial University of Santander, AA 678, Bucaramanga, Colombia

Separation sciences play a very important role in biodiversity assessment and in understanding the connection between chemical composition and bioactivity, since the natural fractions can be characterized down to the resolution of optical and geometric isomerism. Chemical characterization of essential oils



aromatic plants (*Lippia alba*, *Lippia citriodora*, *Lippia dulcis*, *Lippia micromera*, and *Lippia origanoides*, Verbenaceae family), grown in different Colombian regions showed the existence of several chemotypes. Principal component analysis of the compositional data permitted the distinction of three *L. origanoides* and three *L. alba* chemotypes. The *L. alba* chemotypes were classified according to the main constituents as: (I) Limonene-Carvone, (II) Citral, and (III) Carvone-Citral (previously unreported). The *L. origanoides* chemotypes were labeled as: (I) Thymol; (II) Carvacrol, and (III) α - and β -Phellandrenes. Essential oils from *L. dulcis* and *L. micromera* grown in Colombia showed high phenylpropanoid contents, and no chemotypes. Very high antioxidant activity was measured for two *L. origanoides* chemotypes and *L. micromera*, due to their high thymol or carvacrol content.

OP-47

Medicinal plants used for the treatment of jaundice and hepatitis based on socio-economic documentation

A.M. Abbasi, M.A. Khan, M. Ahmad, M. Zafar, S. Sultana

Department of Plant Sciences, Quaid-i-Azam University, Islamabad Pakistan

There is no unique treatment for jaundice and hepatitis in modern allopathic and homeopathic medicine. To our knowledge no systematic investigation on antiviral application of medicinal plants against jaundice and hepatitis based on socio-economic conditions of respondent communities has been made.

Questionnaires, semi-structured interviews and discussions were applied during field work. A total of 30 plant species belonging to 24 families were reported for the treatment of jaundice and hepatitis. For each plant species botanical name, family, local name, parts used, chemical constituents, preparation and application are provided. The most important plant species are *Adiantum capillus*, *Boerhaavia procumbens*, *Equisetum debile*, *Carissa opaca*, *Cucumis sativus*, *Hordeum vulgare*, *Justicia adhatoda*, *Morus alba*, *Morus nigra*, *Phyllanthus emblica*, *Phyllanthus niruri*, *Plantago ovata*, *Prunus domestica*, *Punica granatum*, *Raphanus sativus*, *Rhus chinensis*, *Saccharum officinarum* and *Tamarandus indica*.

The majority of the reported species are wild and rare. These demand an urgent attention to conserve such vital resources so as to optimize their use in the primary health care system. Now a day, conservation of traditional knowledge is greatly menaced by a lot of factors related to dernization of the region and lack of interest in traditional healers, in transferring it to next

generation. It is, therefore, urgent to save the cultural heritage of the natives, by confirming the therapeutically used plants with scientific criteria. In this context, screening for active substances and testing their activities against jaundice and hepatitis causing organisms form an interesting subject for the feature studies.

OP-48

The potential of ethnobotany in Iğdır province (eastern anatolia/Turkey)

E. Altundağ¹, N. Özhatay²

¹Department of Biology, Faculty of Arts and Sciences, Düzce University, 81620, Düzce, ²Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, 34116, Istanbul, Turkey

Iğdır province is located in the Eastern Anatolia Region of Turkey. The province is one of the most interesting cities in Turkey because of having different altitudes and consisting of mountain, plain and halophilic habitats and also having borders with Persia, Armenia, and Naxçivan. An ethnobotanical survey was carried out in Iğdır province between May 2007 and October 2008, when plants were in flowering and fruiting periods. During this research 78 villages, belong to the four districts (Central, Tuzluca, Aralık, Karakoyunlu) have been visited and 1170 plant specimens were collected with the help of the informants. The information for these plants, such as local names, their usages, methods of preparation, administration dosage and duration of treatments have been recorded. As a result of identification of the collected plant specimens, 292 taxa belonging to 44 families have been determined which are used by local people. Among them 162 taxa are used as medical purposes, 143 taxa as food, 25 taxa as spice or tea, 82 taxa as fodder and animal diseases, 22 taxa as fuel. Additionally 70 taxa have been designated for different usages. The collected plant material was stored in ISTE (The Herbarium of the Istanbul University, Faculty of Pharmacy).

OP-49

Healthcare through ethnomedicine: An appraisal of kashmir himalayan ethnic communities

M.A.A. Siddique¹, H. Ashraf², R.C. Gupta³, M.A. Bhat⁴

¹Division of Floriculture, Medicinal, and Aromatic plants, SKUAST-K Shalimar, ²Division of Environmental Science, SKUAST-K Shalimar, ³Department of Botany Punjabi University, Patiala, ⁴Division of Plant Breeding and Genetics SKUAST-K Shalimar, India

Himalayan ecosystem represents one of the world hot spot biodiversity centers and a mosaic of cultural, ethnic and floristic complexities. These ethnic communities have inherited highly efficacious techniques of curing human ailments using the “herbs” available with them in their natural environments. This indigenous traditional knowledge provides useful leads for the development of new medicines not only for the dreadful diseases but also for some of the ailments like coughs and colds, fevers, diarrhoea, urinary infections, nervous problems, loss of memory power and appetite, respiratory tract ailments, excessive/irregular menstrual cycles, leucorrhoea, jaundice, general weakness and orthopedic disorders so common among the hill communities. Tribals of Kashmir Himalayas have been using about 88 different plant species for the treatment of these ailments. Among these, 24% constitute root drugs; 12.5% leaf drugs; 11.45% whole plant drugs; 7.29% each as aerial plant parts and flower drugs; 2.08% each as corm and stem; 1.04% each as bark, rhizome, stem, branches and stigma drugs. These people, thus employ

mostly seeds and roots for curing various ailments. The plants are either used as single drug preparations or compound drug formulations while as their administration varies from external application (as poultice or paste) to internal usages through infusions, decoctions and powders etc with a wide variation in dosage and periodicity (from few grams to several tablespoons and nearly 200 ml to 500 ml day⁻¹). A detailed appraisal of species like *Achillea millefolium*, *Althaea officinalis*, *Fumaria indica*, *Hypericum perforatum*, *Malva sylvestris*, *Picrorhiza kurrooa*, *Portulacca oleracea*, *Rheum australe*, *Trapa nutans*, *Tribulus terrestris*, employed against urinary tract infections; *Valeriana wallichii* and *Coriandrum sativum* used against cardiac troubles; *Acorus calamus*, *Adiantum capillus – veneris*, *Arnebia benthamii*, *Cydonia oblonga*, *Lavendula officinalis*, *Nepeta cataria*, *Urtica dioica*, *Viola odorata* and *Zizyphus vulgaris* used against respiratory tract ailments has been provided. The joint pains are being alleviated by *Colchicum luteum*, *Cotula anthemoides* and *Taraxacum officinale*. These claims, though highly efficacious against various human ailments, need a systematic and scientific study in order to authenticate the claims to give a boost to modern drug development scenario. Pertinently, the hill people have been employing some of the endangered medicinal plants non-judiciously. This practice has brought many herbs like *Aconitum heterophyllum*, *Picrorhiza kurrooa*, *Arnebia benthamii*, *Saussurea costus*, *Rheum australe* etc. to the brink of extinction and, therefore, warrant immediate conservation through *in vitro* active gene banks and *in vitro* base gene banks.

OP-50

Plant-based ethnoveterinary practices against gastrointestinal disorders in the salt range areas-punjab, pakistan

M. Zafar, M. Ahmad

Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan

An ethnomedicinal survey was carried out in the Salt range areas Punjab, Pakistan during August 2007 to April 2009. A total of 45 plant species belonging to 34 families were documented. Local inhabitants use 52 phytotherapies to cure gastrointestinal disorders in live stock. *Azadirachta indica*, *Aesculus indica*, *Allium cepa*, *Brassica campestris*, *Canabis sativa*, *Erigeron conyzanthus*, *Eruca sativa*, *Foeniculum vulgare*, *Melia azedarach*, *Trachyspermum copticum*, and *Trigonella foenum-graecum* show high degree of ethnoveterinary uses. This study was the need to document ethnoveterinary knowledge and standardization of doses which leads to further clinical studies for the discovery of some new, safer and cost effective medicines of global interest.

OP-51

Endemic plant diversity as a source of new drugs and their possibility in modern therapy

S. Redzic^{1,2}

¹Department of Botany, Faculty of Science, University of Sarajevo, ²Department of Natural and Mathematical Sciences of the Academy of Sciences and Arts of Bosnia-Herzegovina, 71 000 Sarajevo, Bosnia-Herzegovina

It grows daily need for finding of new drugs. Especially feel the lack of safe drugs that can be used in the treatment of both standard and well-known and new diseases. Herbal preparations on the pharmacological again dominate the market as Europe and around the world. This requires the need for finding new materials for the production of new and more efficient drugs in

contemporary prevention, therapy and dietetics. Endemic plants are a significant source. Most of them are not pharmacologically investigated. One of the areas that is very rich in endemic plants, the Western Balkans.

Main aims of this work is to found of richness of medicinal wild flora and to determinate their phylogenetic-biochemical relationship with common medicinal plants as well as to evaluate biodiversity of the potential medicinal flora and its possibility in terms of new chemical compound's discoveries and modes of use in contemporary phytotherapy and dietetics.

In order to aims, it has been applied adequate methodology: intensive field research on different vertical profiles, including ethno botanical interviews, followed at the end by comparative taxonomic-biochemical method.

Among plants that could be potentially significant in terms of the pharmacology and pharmacy it was detected app. 700 endemic species of W Balkan. The most significant new resources are contained within endemic genera: *Polytrichum* and *Marchantia* (Bryophyta), *Asplenium* and *Ceterach* (Pteridophyta) *Pinus* and *Picea* (Pinaceae), *Drypis*, *Dianthus*, *Minuartia*, *Silene* (Caryophyllaceae), *Aquilegia* and *Helleborus* (Ranunculaceae), *Aubretia*, *Malkolmia*, *Alyssum*, *Cardamine* (Cruciferae), *Potentilla*, *Sibireja*, *Geum* (Rosaceae), *Astragalus*, *Genista*, *Oxytropis*, *Trifolium* (Fabaceae) *Athamanta*, *Pancicia*, *Peucedanum*, *Seseli*, *Bunium* (Apiaceae), *Acinos*, *Micromeria*, *Salvia*, *Satureja*, *Stachys*, *Teucrium*, *Thymus* (Labiatae), *Euphrasia*, *Pedicularis*, *Scrophularia* (Scrophulariaceae), *Veronica*, *Plantago* (Plantaginaceae), *Achillea*, *Amphoricarpus*, *Centaurea*, *Crepis*, *Leucathemum*, *Senecio*, *Doronicum* (Asteraceae), *Lilium*, *Chonardia*, *Allium* (Liliaceae), *Iris* (Iridaceae), *Anthoxanthum*, *Phleum*, *Festuca* (Poaceae) and others.

Those plants are potential sources of new metabolites, such as alkaloids, heterosides, saponins, essential oils, tannins, carbohydrates, proteins as well as other secondary and primary metabolites. The most of them have significant possibility in new therapy and generating of pharmaco-economical profit.

OP-52

Studies on nutritional requirement of *Coleus forskohlii* briq. Under canal irrigated condition

A. B. Mastiholi¹, S. M. Hiremath²

¹Department of Agronomy, KRC College of Horticulture (University of Horticultural Sciences, Bagalkot), Arabhavi-591 310, Gokak (Tq), Belgaum (Dist), ²Department of Agronomy, College of Agriculture, University of Agricultural Sciences, Dharwad-580 008, Karnataka, India

The *Coleus forskohlii* Briq. belongs to the family Lamiaceae and contain an alkaloid known as 'forskolin'-a diterpenoid in its tuberous roots. Forskolin is effective against hypertension, congestive heart failure, glaucoma and certain type of cancers. The farmers of South Indian states (Karnataka, Tamilnadu, Andrapradesh etc.) are cultivating *C. forskohlii* as a commercial medicinal crop. The demand for suitable NPK dose, by the farmers, for its profitable cultivation was felt. Hence, a field study was conducted under canal irrigated condition of Karnataka, India for two consecutive years (2004 and 2005). Nitrogen application @ 50 kg/ha was sufficient for higher dry tuber (1.65 t/ha) and forskolin (13.48 kg/ha) yield, and net returns (Rs. 55 243/ha) on pooled basis. Crop responded to the application of higher level of P (100 kg P₂O₅/ha) in terms of tuber (1.45 t/ha) and forskolin yield (11.20 kg/ha) and net returns (Rs. 42 194/ha). Potassium application @ 50 and 100 kg K₂O/ha recorded

significantly higher tuber (1.49 and 1.36 t/ha, respectively) and forskolin yield (11.01 and 10.52 kg/ha, respectively) and net returns (Rs. 45 899 and 39 119/ha, respectively). However, NPK levels did not influence the forskolin content. Based on the interaction effects, application of nutrients in the combination of 50 kg N+50 kg P₂O₅+50 kg K₂O/ha was found optimum for getting higher tuber (1.80 t/ha) and forskolin (197.1 mg/plant and 16.06 kg/ha) yield and also net returns (Rs.64 535/ha).

OP-53

Genetic relatedness among medicinal plant *Achillea fragrantissima* populations using amplified fragment length polymorphism (AFLP) markers in Jordan

I.M. Rawashdeh¹, N.I. Haddad², A. Amri³

¹Director of Biotechnology Unit, National Center for Agricultural Research and Extension (NCARE), P.O. Box 639, Baq'a, ²Horticulture and Crop Science Department, Faculty of Agriculture, University of Jordan, Amman, Jordan, ³Head of Plant Genetic Resource Section, International Center for Agriculture Research in the Dry Areas (ICARDA), Aleppo, Syria

Amplified fragment length polymorphism (AFLP) analysis was used to study the genetic variation among and within populations of *Achillea fragrantissima* species collected from five regions in Jordan. Five primer combinations were used which produced about 3297 amplified fragments, out of which 1137 are polymorphic, showing high genetic variability among populations (regions) and within populations. The dendrogram and matrix of similarity obtained by the Unweighted Pair-Group Method with arithmetic average (UPGMA) allowed the discrimination of individual entries into eleven clusters with accessions from the same region most often included in the same cluster. For example, the eighteen entries of Shoubak populations formed a separated group. High similarity (48%) among all tested entries was observed between the two samples of Shoubak and the two samples of Mwaqqar. AFLP is confirmed as a powerful tool for studying the genetic diversity of *A. fragrantissima* and could help in collection and conservation of this species.

OP-54

Assessment of genetic variability among yarrow (*Achillea millefolium* L.) Populations based on inter simple sequence repeat

S. Gharibi¹, M. Rahimmalek¹, A. Mirlohi¹, M. M. Majidi¹, B. E. S. Tabatabaei², M. Khorrami³

¹Department of Agronomy and Plant Breeding, ²Department of Agro-biotechnology, ³Department of Animal science, College of Agriculture, Isfahan University of Technology, Isfahan 84156 83111, Iran

Yarrow (*Achillea millefolium* L.) is an important medicinal plant with different medicinal and ornamental uses. ISSR markers were used for the first time for the detection of genetic polymorphism in this medicinal herb from populations collected from various geographical regions of Iran and North Hemisphere. Fourteen primers were used to amplify 221 bands out of which 199 (90.4%) were polymorphic. Simple-matching (Sm) similarity indices were subjected to UPGMA cluster analysis. The dendrogram revealed three major groups. The groups 1 included the Iranian genotypes (SW Asia). The group 2 was composed of European and American genotypes and the group 3 belonged to Japanese (E Asia) accession. Iranian group showed three major sub-groups based on their geographic distribution. The populations from West and North-West have been classified in the same group,

while Central-West populations were more similar to Northern populations. Totally, more genetic variation observed in North and Central-West and less genetic variation was found in West and North-West populations. In this study, different subspecies of the *Achillea millefolium* from North Hemisphere were re-grouped with Iranian populations. The results showed that E Asian genotypes had more genetic distance from European genotypes and less genetic distance from American populations. Among European genotypes Spain accession was more similar to American one in comparison with others. Cluster and PCoA analyses showed that most of the genotypes were grouped based on their altitude and geographical divergence and these criteria were more important in comparison with subspecies classification.

OP-55

Essential and potentially toxic trace elements in medicinal and aromatic plants - another approach to quality

D. Radanović¹, S. Antić-Mladenović²

¹Institute for Medicinal Plant Research "Dr J. Pančić", T. Košička 1, 11000 Belgrade, ²University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

This paper reviews the factors affecting uptake, accumulation and distribution of essential and potentially toxic trace elements (Fe, Zn, Cu, Mn, Co, Cd, Pb, Cr, Ni) in medicinal and aromatic plants (MAPs). Worldwide, those plants are cultivated on rather small scale and on vary different soils. Spontaneous MAPs vegetation mostly cover mountain soils often developed over ore deposits or metal-carrier minerals (such as serpentinite) as parent material. During the last decades, under an increasing anthropogenic influence, some soils have been additionally loaded with heavy metals as well as with some other potentially harmful substances. Beside heavy metal origin and source, other important processes and factors affecting metal mobility and availability in soils are: adsorption/desorption processes, salinity, sulphur, carbonates, soil pH and plant growth.

More over, MAPs represent a multitude of different species that can affect the metal mobility in soils by oxidizing their rhizosphere, taking up metals, excreting exudates and stimulating the activity of microbial symbionts in the rhizosphere. Combined effects of different factors result in wide range of trace elements concentrations within the same, as well as in different species. As MAPs have an increasing role in human diet, their composition regarding heavy metals must be taken into consideration.

As most heavy metals, after uptake, tend to accumulate in root tissue, their translocation in herb is significant part of quality aspect. Uptake, accumulation and translocation of metals can be controlled by combining site characteristics and plant organ for further utilization.

Another aspect, which has to be considered, is an extractability of metals and their transfer from raw material to final product to be consumed. Available data imply that there are significant differences in extraction efficiency between common extractants: hot water, alcohol or propylene glycol. Hot water is been identified as less effective heavy metals extractant. Distilled essential oils, as well as teas, are usually free or low in heavy metals, even produced from plants with high heavy metals content.

OP-56

The effect of different silver nitrat concentrations on anther cultures of *Anemone coronaria* var. *Coccinea*

E. Arı¹, S. Büyükalaca²

¹Batı Akdeniz Agricultural Research Institute, 07100, ²Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Turkey

Anemone coronaria L. (Ranunculaceae), known also as an old medicinal plant due to its various phytochemical compounds, is cultivated generally for ornamental purpose. Because of high heterozygosity in the species, at least 8-10 years are required to reach to homozygous lines. For this reason, the use of anther culture technique has been seen as an important advantage in the breeding studies. The effect of different AgNO₃ concentrations on the anthers of *Anemone coronaria* var. *coccinea* were investigated in this study for haploid embryo production. In the total 40 treatments, two basic tissue culture media (NN and B5) were tested with 3 different auxin-cytokinin combinations and 4 different AgNO₃ concentrations.

According to the results, pollen embryogenesis occurred in 25 AgNO₃ treatments in spite of low percentages. The highest rate of pollen embryogenesis was achieved from both NN+15 mg/l AgNO₃+1 mg/l IAA+0.1 mg/l BAP medium and B5+10 mg/l AgNO₃+1 mg/l IAA+0.1 mg/l BAP medium. An important other point must be stressed that formation of pollen embryogenesis in some AgNO₃ treatments without hormone was found significant in terms of the stimulator effect of AgNO₃ even in the absence of hormone. Additionally, organogenesis and callus formations were obtained apart from direct pollen embryogenesis in this study.

Acknowledgement

This study was carried out under the FBE 2002 - D200 project supported by Cukurova University.

OP-57

In vitro antioxidant and anticholesterol activity of oolong tea (*Camelia sinensis* L.) Extract

W. Widowati¹, T. Herlina², H. Ratnawati³

^{1,3}Medical Research Centre, Faculty of Medicine, Maranatha Christian University, Bandung, ²Sciences and Mathematic Faculty, Padjadjaran University, Sumedang, Indonesia

Epidemiologic studies have demonstrated an association between increased intake antioxidant and reduced cardiovascular disease. This association has been explained that atherogenesis is initiated by lipid peroxidation. The research was carried out to evaluate the free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and anticholesterol activity of methanol extract of Oolong tea (*Camelia sinensis* L.). To know the antioxidant activity of oolong tea extract were compared with (-)-epigallocatechine 3-gallate (EGCG) and to evaluate the anticholesterol of oolong tea extract were compared with simvastatin. The DPPH free radical scavenging and anticholesterol activity were carried out at 6 concentrations level (500 µg/mL; 250; 125; 62.5; 31.25 and 15.625 µg/mL). The results demonstrated that all concentrations of oolong tea extract had high antioxidant activity between 89.478 % and 92.923 % similar with EGCG, all concentrations of oolong tea extract had high anticholesterol activity between

91.813 % and 94.087 % were lower than simvastatin.

OP-58

Chemopreventive effect of γ -irradiated caraway essential oils on 1,2-dimethylhydrazine-induced colon carcinogenesis

A. Dadkhah^{1,2}, A. Allameh², H. Khalafi¹, F. Fatemi^{1,2}, J. Ashrafi-Helan³, M. Seyhoon¹

¹Radiation Application Research School, Nuclear Science and Technology Research Institute, ²Department of Clinical Biochemistry, Faculty of Medical Science, Tarbiat Modares University, Tehran, ³Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Caraway [*Carum Carvi* L., Umbelliferae] is a well known traditional herbal drug believed to contain essential oils with pharmacological properties. Although, irradiation is an important method of processing herbal drugs, our understanding about the effects of γ -irradiation on pharmacological properties of seed products such as caraway essential oils is very limited. In this study, caraway seeds were irradiated in a ⁶⁰Co facility at dose levels of 0, 10 and 25 kGy. Then the chemopreventive efficacy of caraway essential oils on the formation of aberrant crypt foci (ACF) has been investigated by measuring the levels of hepatic oxidative stress and xenobiotic metabolizing enzymes involved in 1,2-dimethylhydrazine (DMH)-induced colon cancer in rats. Male Wistar rats were divided into 6 groups. Group 1 served as the untreated control, and groups 2&3 received only powdered commercial pellet diet containing 0.01 and 0.1 % of caraway oils. Group 4 received only DMH (20 mg/kg b.w) injection (s.c) once a week for a period of 5 weeks and the rats in group 5 -10 received diet containing fresh and irradiated essential oils concomitant with the DMH injections until the end of whole experimental period of 16 weeks. Our results indicate that the chemopreventive activity of extracted essential oils was not affected due to γ -irradiation. Essential oils extracted from irradiated caraway seeds as well as fresh caraway seeds could prevent ACF formation by modulating the activities of hepatic detoxification enzymes, glutathione s-transferase (GST) and

cytochrome P450 1A1 (CYP1A1) whereas antioxidant statuses are not affected. It is obvious that one of the mechanisms of chemoprevention of colon tumorigenesis by caraway essential oils may be the enhancement of carcinogen detoxification but not antioxidant system in the liver which is maintained by essential oils prepared from γ -irradiated caraway seeds.

OP-59

Antimicrobial screening of some mosses collected from anatolia

E.M. Altuner¹, B. Çetin², C. Çökmüş²

¹Kastamonu University, Faculty of Arts and Sciences, Department of Biology, TR-37100, Kastamonu, ²Ankara University, Faculty of Science, Department of Biology, TR-06100, Ankara, Turkey

This study aims to determine the antimicrobial activity of *Anomodon attenuatus* (Hedw.) Hüb., *Dicranum bonjeanii* De Not, *Fontinalis hypnoides* Hartm., *Laecobryum glaucum* (Hedw.) Schimp, *Mnium spinosum* (Voit.) Schwaegr and *Pterigynandrum filiforme* Hedw. samples which were collected from several locations in Anatolia. Eight different extraction solvents, namely benzene, chloroform, diethyl ether, ethanol, ethyl acetate, methanol, sdH₂O and 0.5M Tris-HCl buffer, pH=8.0 were used in the study. All the extracts were investigated for *in vitro* antimicrobial activity against *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 95071, *Escherichia coli* ATCC 11220, *Escherichia coli* O157:H7, *Listeria monocytogenes* ATCC 7644, *Salmonella enteritidis* ATCC 13076, *Staphylococcus aureus* ATCC 25923, *Trichophyton rubrum* (clinical isolate) and *Yersinia enterocolitica* O3 by using the disc diffusion method. As a result it was observed that methanol and sdH₂O extracts of *Anomodon attenuatus* have activity against *B. subtilis*, ethyl acetate extract of *Dicranum bonjeanii* has activity against *C. albicans*, buffer extract of *Fontinalis hypnoides* has activity against *T. rubrum*, buffer and sdH₂O extracts of *Laecobryum glaucum* have activity against *T. rubrum*, buffer, chloroform, diethyl ether and sdH₂O extracts of *Mnium spinosum* have activity against *T. rubrum* and benzene extract of *Pterigynandrum filiforme* has activity against *B. subtilis*.