

**WOCMAP ABSTRACTS**

*Afr. J. Traditional,
Complementary and
Alternative Medicines*
www.africanethnomedicines.net

ISSN 0189-6016©2009

ABSTRACTS OF THE WORLD CONGRESS ON MEDICINAL AND AROMATIC PLANTS,
CAPE TOWN NOVEMBER 2008

In a meeting of the Secretariat of the International Union of Biological Sciences (IUBS) in Paris, nine international organizations decided to establish an international non-governmental body entitled: International Council for Medicinal and Aromatic Plants (ICMAP) [www.icmap.org] with the general objective of promoting international understanding and cooperation between national and international organizations on the role of medicinal and aromatic plants in science, medicine and industry, and to improve the exchange of information between them.

One of the functions of ICMAP is to arrange a world conference on medicinal and aromatic plants [WOCMAP] every five years on different continents. The first was held in Europe [Maastricht, Netherlands 1992], the second in South America [Mendoza, Argentina 1997], and the third in Asia [Chiang Mai, Thailand, 2003].

ICMAP Bureau invited Prof J. N. Eloff, the Leader of the Phytomedicine Programme at the University of Pretoria to organize WOCMAP IV in South Africa in Cape Town in November 2008. With just more than 400 attendees WOCMAP was much smaller than previous WOCMAPs possibly due to the international financial crisis. A Business Forum and Trade Fair on medicinal and aromatic plants was also organized at the same time in the same venue to bring all the different role players in this field together. The scientific conference and Trade Fair was generously supported by several Private Companies and also by the South African Department of Science and Technology and the National Research Foundation of South Africa. As in previous WOCMAPs, The International Society for Horticultural Science has again agreed to publish the Proceedings in *Acta Horticulturae*. The presentations of the delegates who have submitted their manuscripts will eventually be available on their website.

WOCMAP IV was privileged that some of the leading international figures accepted the invitation to deliver the plenary lectures. The rest of the oral and poster presentations were organized in different themes as presented below.

We thank, the Editor, Prof Clement Adewunmi for agreeing to publish the abstracts in the African Journal for Traditional, Complementary and Alternative Medicines.

Kobus Eloff
Chairman Organizing Committee WOCMAP IV

PLENARY LECTURES

AN AFRICAN HERBAL PHARMACOPEIA COULD UNDERPIN THE MEDICINAL PLANT INDUSTRY ON THE AFRICAN CONTINENT

A. Gurib-Fakim

University of Mauritius, Reduit, Mauritius
fakima@uom.ac.mu

Paper prepared and presented on behalf of the 'Association of African Medicinal Plants Standards – AAMPS'

Africa is a continent endowed with enormous wealth of plant resources. Over 5000 distinct species are known to occur in the forest regions alone and most of them have been used for several centuries in traditional medicine for the prevention and treatment of diseases. African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. The biological and cultural diversity of Africa is reflected in the marked regional differences in healing practices and unfortunately the various systems are poorly recorded and remain so to this day. Yet ancient kingdoms and empires of Africa had extensively codified healing practices.

African *Materia Medica* consists of a mixture of many elements and includes many potent herbs. Few of these are, however, yet recognised in modern pharmacopoeias. Exceptions are *Strophanthus*, *Kola*, *Catharanthus* and *Harpagophytum* which have been included in several western pharmacopoeias. Although there are several important research publications on medicinal plants from Africa, the study of African medicinal plants has remained fragmented and neglected as compared to India and China. To date Africa has only contributed 83 of the world's 1100 leading commercial medicinal plants despite the fact that the Sub-Saharan region as well as the Indian Ocean Islands, contain approximately 60.000 plant species – roughly a quarter of the world's total.

The potential that this represents for the business and agricultural sectors is enormous but Africa will lag behind unless it prepares internationally recognised medicinal plant standards. The Medicinal Plants Forum for Commonwealth Africa held in Cape Town in 2000, highlighted the lack of suitable technical specifications and quality control standards as a major constraint to the African herbal industry. Moreover the lack of official recognition from governments generally has been the major handicap to what could become an important income and foreign exchange earner for the continent. A follow up meeting in 2005 witnessed the creation of the **Association of African Medicinal Plants Standards (AAMPS)** a non profit making company registered in Mauritius set up to implement recommendations outlined in the Centurion Declaration which advocated the preparation of an **African Herbal Pharmacopoeia**. Over 50 standards have since then been prepared by a team of internationally renowned experts covering most of the most important African medicinal plants. The Association hopes that the publication of these monographs will inter alia, increase Africa's capacity to build a viable herbal industry incorporating Good Agricultural, Good Manufacturing and Good Laboratory Practices and above all increase the visibility of African Medicinal plants as a source of income generation.

Since 2005, AAMPS has established itself as the leading organisation working on medicinal plant standards on the continent. A Scientific Advisory Committee has been established and a number of long term projects have been launched to enable AAMPS to become a self-sustaining entity. These include AAMPS Publishing, which will produce books, manuals and some simple merchandising products including possibly a photo-library service specialising in African medicinal plants and herbal products. AAMPS aims at providing also a

laboratory and technical network and a Consultancy and Management Service. In collaboration with other international bodies such as the **International Foundation for Sciences** (IFS), AAMPS will today launch a joint Fellowship programme and award an annual AAMPS/IFS prize for lifetime achievements in the promotion of African medicinal and aromatic plants. More details on the services to be provided for AAMPS can be obtained from the website: <http://www.aamps.org>

EVALUATION OF THE PHARMACOLOGICAL POTENTIAL OF CHINESE HERBAL MEDICINE

R. Bauer

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

Traditional Chinese medicine is based on thousands of years of experience and is based on a specific theory with a holistic approach for disease and health management. In order to evaluate the full pharmacological potential of Chinese herbal medicine it is necessary to consider the classical concepts of diagnosis and medication. The approach of systems biology is certainly best suited since it allows the broadest analysis of a metabolic status and of the effects of treatments. For the identification of the active principles metabolic transformation as well as synergistic effects must be considered. Also the rationale for the application of mixtures of herbs needs to be evaluated.

Some recent examples of our research on Chinese herbs with anti-inflammatory and anti-cancer activity will be presented: From *Centipeda minima* (L.) A. Braun & Asch. (Asteraceae) we have isolated the sesquiterpene lactones 6-O-methylacrylplenolin, 6-O-angeloylplenolin and 6-O-tigloyl-plenolin, which exhibited strong inhibitory properties on inducible nitric oxide synthase (iNOS) in RAW 264.7 macrophages after stimulation by interferon-gamma and LPS [1].

From the fruits of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae), we have isolated the quinolinone alkaloids 1-methyl-2-nonyl-4(1H)-quinolinone, 1-methyl-2-(6Z)-6-undecenyl-4(1H)-quinolinone, 1-methyl-2-(4Z,7Z)-4,7-tridecadienyl-4(1H)-quinolinone, evocarpine and 1-methyl-2-(6Z,9Z)-6,9-pentadecadienyl-4(1H)-quinolinone, which showed strong inhibitory activity on leukotriene biosynthesis in human polymorphonuclear granulocytes [2]. Moreover they were very effective against mycobacteria [3]. They showed no cytotoxic activity and might bind to the lipid binding site of 5-LOX [4]. Extracts from Chinese herbs have been also screened for inhibition of expression of NF- κ B1 in THP-1 cells by a new quantitative method based on real time PCR [5]. Overexpression of this transcription factor is associated with inflammatory diseases, like rheumatoid arthritis, atherosclerosis, asthma and inflammatory bowel disease.

[1] Fischer, M., Kunert, O., Schmidt, K., Bauer, R. Sesquiterpene lactones from *Centipeda minima* with iNOS inhibitory activity. in preparation (2008).

[2] Adams, M., Mahringer, A., Kunert, O., Fricker, G., Efferth, T., Bauer, R. Cytotoxicity and P-Glycoprotein Modulating Effects of Quinolones and Indoloquinazolines from the Chinese Herb *Evodia rutaecarpa*. *Planta Med.* 73, 1554-1557 (2007)

[3] Adams, M., Kunert, O., Haslinger, E., Bauer, R. Inhibition of leukotriene biosynthesis by quinolinone alkaloids from the fruits of *Evodia rutaecarpa*. *Planta Med.* 70, 904 - 908 (2004).

[4] Adams, M., Abebe Wubea, A., Kunerth, O., Haslinger, E., Bucar, F., Bauer, R. Quinolone alkaloids from *Evodia rutaecarpa*: A potent new group of antimycobacterial compounds. *Int. J. Antimicrob. Agents* 26(3), 262 -264 (2005).

[5] Gusenleitner, S., Bauer, R.: Screening of Chinese herbal drugs for inhibition of NF-kB1 expression. *Planta Med.* 73, 844 (2007).

Acknowledgements: Part of the work has been supported by Zukunftsfonds Styria ("TCM Research Center Graz").

HERBS AND HERBAL PRODUCTS IN ANIMAL NUTRITION AND VETERINARY MEDICINE

Ch Franz

Institute for Applied Botany and Pharmacognosy, University of Veterinary Medicine,
Veterinärplatz 1, 1210 Vienna, Austria

Following the trends in human health care towards herbal medicinal products and plant derived dietary supplements also in Veterinary medicine and livestock production an increasing use of herbs, essential oils and plant extracts can be observed. This is not only due to an ethnoveterinarian revival but also based on the fact that

- a) antibiotic growth promoters are banned in more and more countries all over the world due to the risk of (cross) resistances
- b) in organic livestock production the use of synthetic drugs is very restricted, and
- c) many pet animal and horse owners prefer natural products and "soft medicine"

Microbial induced diarrhoea remains one of the crucial problems especially in large scale farm animal production. As alternatives to in-feed antibiotics several herbal mixtures and essential oils are promising, showing significant antimicrobial, antioxidant and growth promoting effects. Porcine proliferative enteropathy could be controlled by e.g. carvacrol-rich oregano oil, necrotic enteritis of chicks was successfully prevented by several essential oil compounds. In ruminants some herbal preparations are effective in reduction of methane and ammonia production, which is of high environmental relevance [1].

A number of plants containing bioactive secondary products are used also in health care of horses and pet animals, as e.g. fennel, anis and caraway against bloat and flatulence, tea-tree oil to treat dermatological problems or milk thistle extracts to prevent liver damages.

The increasing use of herbs and herbal products is not reduced to Europe and North America, but has an enormous significance and potential all over the world especially where ethnoveterinary knowledge still exists [2]. Phytochemical as well as *in-vivo* and *in-vitro* studies will help to exploit these resources for the benefit of humans and animals.

[1] Franz, Ch.: Functional Plant Products in Veterinary Medicine and Animal Nutrition. *Planta med.* 73, 799 (2007)

[2] ICS-UNIDO: Medicinal and Aromatic Plants in Veterinary Health Care. Expert Group Meeting Trieste, Italy (2007)

CONSERVATION, SUSTAINABLE USE, TRADE AND INDUSTRY

A. B. Cunningham

People and Plants International, 84 Watkins St, Fremantle, 6162, Australia

This paper places the African traditional medicines trade into context through a comparison with Asia, suggesting that learning from Asia comes at an opportune time. The recent inter-Ministerial meeting in Libreville, Gabon (August 2008) on environment and health and the draft South African policy on African Traditional Medicine support this view. In Asia, particularly China, India, Pakistan and Vietnam, government support for the development and modernization of traditional medical systems is likely to increase harvest levels from wild stocks. Sustainable harvest of wild stocks is crucial as they continue to provide the main supply sources for 75-95% of species in trade in Asia and Africa. The resource base of the herbal medicines trade is being affected, however, by multiple factors simultaneously, at different spatial and time scales. The most serious of these are habitat loss and fragmentation, global climate change, species-specific over-exploitation and invasive species. Commercial trade, often driven by rapid urbanization combined with cultural values placed on traditional medicines, is a challenge facing conservation of medicinal plants in many parts of Asia, Africa and in some Latin American countries. In addition, some native medicinal plants are considered powerful and effective in treating common and chronic diseases and have been widely commoditized in China and India. Many of the same high-altitude genera or species harvested from the Himalayas for trade to large cities affect wild stocks in neighbouring countries. A massive traditional medicines trade also occurs in West Africa and in South Africa, similarly affecting favoured plant species in neighbouring countries.

NEW ANALYTICAL TECHNIQUES FOR THE IDENTIFICATION OF PLANT CONSTITUENTS AND FOR THE QUALITY ASSESSMENT OF HERBAL MEDICINES

K. Hostettmann

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

Plants represent an extraordinary reservoir of novel molecules and there is currently a resurgence of interest in the vegetable kingdom as a possible source of new lead compounds for introduction into screening programs. Plant constituents of interest are usually isolated following a bioactivity-guided fractionation procedure. In order to render this approach more rapid and efficient, the dereplication of crude plant extracts with LC-hyphenated techniques represents a strategic element to avoid finding known constituents and to target the isolation of new bioactive compounds

In order to obtain direct spectroscopic information on-line, from crude plant extracts, hyphenated techniques such as HPLC coupled to UV photodiode array detection (LC-DAD/UV), to mass spectrometry (LC/MS) and to nuclear magnetic resonance (LC/NMR) have been investigated. In our laboratory, these techniques have been fully integrated into the isolation process and are used for the chemical screening of crude plant extracts. As a complement to this approach, capillary NMR (CapNMR) can be performed. Since the volume of the CapNMR probe is 5 μ l, very small samples can be analysed.

When combined with bioassays performed after LC/microfractionation of extracts, on-line identification of bioactive compounds is possible. Examples of rapid localisation of bioactive compounds will be given, together with the potential of hyphenated techniques for the quality control of herbal medicines.

PLANTS AS SOURCES OF HUMAN HEALTH PRODUCTS

Peter Houghton

Kings College London, United Kingdom, peter.houghton@kcl.ac.uk

Plants continue to provide novel molecules for therapeutic use but also there is increasing interest in the acceptance of extracts or mixtures of plants in Western medicine. Examples of molecules achieving clinical importance in recent years are galantamine, huperzine A and artemisinin whilst other natural products are valuable as lead compounds. Many clinical trials are now being carried out on herbal products e.g. ginkgo, black cohosh, Job's tears and some encouraging results are being obtained which substantiate their acceptance in mainstream Western medicine as well as in their traditional cultural environments. Regulatory systems are becoming more sympathetic but also recognising the need for good quality and adequate safety testing. Novel analytical and drug discovery approaches are consolidating the scientific appraisal of plants as sources of new medicines.

CONSERVATION OF SOUTH AFRICAN MEDICINAL PLANTS: TOOLS FOR THE FUTURE

J. van Staden and M. E. Light

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

The extensive use of medicinal plants worldwide is increasing and there has been much emphasis on the potential for discovering novel drugs from these natural resources. Along with this interest in the development of traditional medicine is the need for propagation and improved cultivation methods for heavily utilized medicinal plants. This is particularly important for those species which are over-exploited and have become severely depleted in the wild. In South Africa, it is estimated that as much as 70 000 tonnes of plant material is consumed per year [1]. Thus, the provision of plants for re-introduction into natural areas and as alternative plant sources for the production of medicinal plant material for sale through informal markets and larger commercial concerns is an important strategy for the conservation of medicinal plants. Several studies related to aspects of seed germination, *in vitro* propagation and cultivation of medicinal plants for small-scale farming have been conducted in our Research Centre. This presentation will highlight some examples of our research in this field and discuss some of the problems facing the cultivation and conservation of medicinal plants in South Africa.

Acknowledgements: National Research Foundation (NRF, Pretoria), University of KwaZulu-Natal.

1. Mander, M. & Le Breton, G. (2006) Overview of the medicinal plants industry in southern Africa. In Diederichs, N. (Ed.) Commercialising Medicinal Plants: A Southern African Guide. Sun Press, Stellenbosch, South Africa. pp. 1-8

BIOPROSPECTING: EXPLORING OUR ONLY RENEWABLE NATURAL RESOURCE

R. Verpoorte

Department of Pharmacognosy, Section Metabolomics, Institute of Biology Leiden,
PO Box 9502, 2300RA Leiden, The Netherlands,
Email: VERPOORT@LACDR.LeidenUniv.NL

Biodiversity is our only renewable natural resource. Long time ago our ancestors found all kinds of uses for plants, microorganisms, etc. But as we are so used to our daily life, most people do not realize that it is based on these natural resources. We all know that milk comes from a factory! But in fact we use plants and other organisms for the production of e.g. food, medicines, dyes, flavours, fragrances, agrochemicals, clothing, paper, as well as for construction, fuel and many other applications. Much of the economic activities in the world are based on this. So we should be very grateful with the heritage of our ancestors. Still there is a lot of this traditional knowledge that has not yet been really explored, e.g. there is an estimated number of medicinal plants of 40,000-70,000 species, most of which have never been studied in detail. Using this knowledge in combination with our powerful scientific tools there are many possibilities for developing novel products and concepts from biodiversity. Biodiversity as source for developing novel medicines will be discussed in some more detail.

SOUTH AFRICA'S MEDICINAL AND AROMATIC FLORA - A TREASURE CHEST OF OPPORTUNITIES FOR SCIENCE AND INDUSTRY

Viljoen A.

Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680,
Pretoria, 0001

The pharmaceutical, fragrance and flavour industries remain challenged and under immense consumer pressure to produce innovative and efficacious products for this lucrative industry. In a quest to satisfy consumer expectations and to produce products with some competitive edge, these industries most often turn to nature for guidance, inspiration and as a source of novel compounds for commercial development.

South Africa represents a global epicenter of medicinal and aromatic plants. Despite this rich botanical diversity (matched by chemical diversity) it is surprising that many of the indigenous assets remain latent and are not systematically studied which is an obvious pre-requisite for these unique botanical assets to be transformed into consumer products. South Africa has offered the world two indigenous aromatic plants which have both been developed into a commercial success. Geranium oil (obtained from various cultivars of *Pelargonium graveolens*) and Buchu oil (from *Agathosma betulina*) are only two species of the over 1 000 aromatic plants indigenous to South Africa. Despite the extensive use of plants by the various ethnic groups in South Africa in the form of traditional medicines, South Africa (at this stage) cannot boast many international success stories derived from its indigenous medicinal plants.

An extensive exploratory phase and efficient researching infrastructure underpins any commercial development. The paper is a brief reflection of past and present research and will

unequivocally confirm the value, unique opportunities and constraints in exploring one of the most biodiverse areas in the world.

RECENT DEVELOPMENTS IN REGULATORY MATTERS ON HERBAL MEDICINAL PRODUCTS (HMPS)

Arnold J. Vlietinck

University of Antwerp, B-2610 Antwerp, Belgium

Within the group of herbal or botanical products there is a large variation with regard to the properties and the legal status worldwide of these products. Some herbal products come closer to or are medicines, while others are close or even identical to foods such as dietary supplements, functional foods, novel foods etc. And still others are considered as cosmetics or medical devices.

It is therefore not surprising that recently appropriate regulatory actions have been undertaken to regulate and harmonize the legal status of these various groups of plant preparations throughout the different Western countries. The European Union has recently considered herbal products in several legislative texts. Medicinal use has been harmonized through the Traditional Medicinal Products Directive (Directive 2004/27/EC amending Directive 2001/83/EC as regards THMPs). Use of herbal preparations in unit dose form under law is covered in the Food Supplements Directive (FSD) 2002/46/EC. Regulations on nutritional and health claims and the addition of vitamins and minerals and certain other substances to foods were adopted on October 12, 2006 (Directive 2006/80E/02 and 03). Nevertheless, the distinction between traditional herbal medicinal products and food supplements containing herbal products without nutritional value but having physiological effects remain vague and controversial.

In this lecture the implementation of the current European regulations at the level of the EU Member State authorities and manufacturers in terms of quality, safety and efficacy of these herbal products will be discussed. A comparison will be made with other existing concepts worldwide, taking into account not only the abovementioned properties, but also aspects such as access to the market, cost prize and prospects for innovation of herbal products.

CLINICAL EVALUATION OF AFRICAN HERBAL MEDICINES

Charles Wambebe

International Biomedical Research in Africa, Garki Post Office Box 3359, Abuja, Nigeria,
wambebe@yahoo.com

The criteria for clinical evaluation of African herbal medicines are exactly the same as those for assessing orthodox medicines (i.e. quality, safety and efficacy). The African herbal medicine may be a standardized freeze dried extract formulated into suitable dosage forms. The quality of the herbal medicine is determined by various factors which may influence the chemistry of the plant, pharmacology, toxicology and pharmaceutical formulation indicating the significance of establishing phytochemical and biological fingerprints. Generation of robust pre-clinical data on safety and efficacy based on standard pharmacological and toxicological methodologies using the principles of WHO. Good Laboratory Practice is a prerequisite to

consideration of clinical trials. The design of the protocol for the clinical evaluation of herbal medicine should take cognizance of the ethnomedicinal use, clinical observational study data, national drug regulatory authority guidelines, ICH and WHO Good Clinical Practice principles. The principles indicated above were applied in the clinical evaluation of a herbal medicine called NIPRISAN for the management of Sickle Cell Disorder (SCD). Patenting of the process technology and potential therapeutic use of NIPRISAN was undertaken.

In Africa where about 70% of SCD patients reside, the prevalence is about 2% (SS genes) and 25% (AS genes) among the general population while infant mortality is about 8%. Furthermore, survival rate in rural areas of SCD children by age 5 years is about 20%. Since there is no standard therapy in Africa for SCD patients, most patients use traditional herbal medicines.

NIPRISAN is a standardized extract from four medicinal/food plants: *Piper guineenses* seed, *Pterocarpus osun* stem, *Eugenia caryophyllum* fruit and *Sorghum bicolor* leaves. Pre-clinical data of NIPRISAN using both *in vitro* and *in vivo* methodologies indicated profound efficacy and safety profiles. SCD (Hb SS) patients confirmed by haemoglobin electrophoresis in alkaline/acid media with moderate-to-severe recurrent episodes who had experienced at least 3 painful or vaso-occlusive crises in the previous year were recruited for the study. Double-blind, placebo-controlled, randomized cross-over clinical trial of NIPRISAN was undertaken at the National Institute for Pharmaceutical Research and Development (NIPRD) clinic, Abuja between April 1997 and August 1998. Health diaries were used by all study participants. Clinical monitors (nurses) visited patients at home every week. 100 patients were recruited but 82 satisfied inclusion criteria at 3 months pre-trial period. NIPRISAN has been licensed to XECHEM Inc (an American company) and it is being commercially produced at Abuja for the global market.

THEME 1

BIODIVERSITY PROSPECTING AND ETHNOPHARMACOLOGY

ANTIFUNGAL ACTIVITY OF SOUTH AFRICAN *PODOCARPUS* SPECIES USED IN TRADITIONAL MEDICINE

Abdillah H., Finnie J., Van Staden J.

University of KwaZulu-Natal, South Africa, 206526388@ukzn.ac.za
University of KwaZulu-Natal, South Africa, Finnie@ukzn.ac.za, University of KwaZulu-Natal,
South Africa, rcpgd@ukzn.ac.za

Species of *Podocarpus* are utilized traditionally in treating all manner of ailments in various parts of the world. In South Africa, four species of *Podocarpus* (*P. elongatus*, *P. falcatus*, *P. henkelii* and *P. latifolius*) are used as herbal remedies to treat both human and livestock diseases. In this study the antifungal activity of these species against *Candida albicans* was investigated. Petroleum ether, hexane, dichloromethane, acetone, and 80% ethanol were used in the extraction of plant material. Microdilution and M27-P-broth dilution assays were used to assess antifungal activity. All extracts exhibited significant antifungal activity with minimum inhibitory concentration (MIC) values of less than 1 mg/ml. For the M27-P-broth dilution method, extracts showed MIC values ranging from 0.13 mg/ml to 6.25 mg/ml while in the microdilution assay, extracts showed lower MIC values ranging from 0.03 to 2.34 mg/ml after 48 h. *P. latifolius*

leaves inhibited growth of *C. albicans* at the lowest concentration of 0.02 mg/ml after 24 h and 0.03 mg/ml after 48 h for dichloromethane extracts and 0.02 mg/ml after 24 h and 0.04 mg/ml after 48 h for acetone extracts. At concentrations higher than 1.25 mg/ml the inhibition was fungicidal while at lower concentrations the inhibition was fungistatic for all extracts tested. In general, all four species exhibited good inhibition against *C. albicans*. The results obtained from our screening confirm the therapeutic potency of these four species and thus provide a rationale for their use in traditional medicine.

THE GASTROPROTECTIVE AND ANTIOXIDANT EFFECT OF *BELLIS PERENNIS* L. (ASTERACEAE) EXTRACTS

Leyla Acik, İlhan Gurbuz, Hulya Demir, Fatih Demirci

Gazi university, science and art faculty, department of biol, turkey, leylaacik@gmail.com
Gazi University, Faculty of Pharmacy, Department of Pharmaco, Turkey, igurbuz@gazi.edu.tr
Gazi University, Ataturk Vocational Training School, Ankara, Turkey, hdemir40@gmail.com
Anadolu University, Faculty of Pharmacy, Department of Pharm, Turkey, demircif@gmail.com

Bellis perennis is used in Turkish folk medicine against various diseases including peptic ulcer [1-2]. In order to confirm the claimed activity on peptic ulcer, aqueous and methanol extracts were prepared from the aerial parts of the material. Both extracts were tested for their anti-ulcerogenic potential on the ethanol-induced gastric ulcer model using rats. The aqueous and methanol extracts were administered orally at 1100 and 600 mg/kg doses, respectively, where both extracts demonstrated significant anti-ulcerogenic activity statistically ($p < 0.001$). Additionally, the antioxidant potential of the extracts was evaluated by DPPH free radical scavenging and total antioxidant capacity methods. The results showed that *B. perennis* extracts can be used as a good potential source of natural antioxidants. Chromatographic fingerprints and constituents of these extracts were also documented using LC and LC-MS techniques. Further studies are needed in order to isolate and define the active constituent(s).

EFFECT OF *OLEA EUROPAEA AFRICANA* L. (OLEACEAE) LEAF AQUEOUS EXTRACT ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

Amabeoku G., Bamuamba K.

University of the Western Cape, South Africa, gamabeoku@uwc.ac.za
University of the Western Cape, South Africa, kbamuamba@yahoo.com

The leaf aqueous extract of *O. europaea* ssp. *africana* L. was tested for antidiarrhoeal activity against castor oil-induced diarrhoea in mice. Acute toxicity studies and phytochemical analysis of *O. europaea africana* were also carried out. *O. europaea* ssp. *africana* significantly reduced the number of diarrhoeal episodes induced by castor oil. *O. europaea* ssp. *africana* also significantly decreased the stool mass produced as a result of castor oil-induced diarrhoea and significantly prolonged the onset of the diarrhoea. The number of animals exhibiting diarrhoea over a five hour period was significantly decreased by *O. europaea* ssp. *africana*. Similarly, loperamide completely abolished the diarrhoeal episodes and stool mass, and protected the animals against castor oil-induced diarrhoea. The results obtained suggest that the aqueous

extract of *O. europaea* ssp. *africana* has antidiarrhoeal properties. The relatively high LD50 of 3475 mg/kg (p.o.) obtained with the aqueous extract indicates that the plant species may be safe and/or nontoxic in mice. The phytochemical analysis done on the plant species showed the presence of saponins, tannins, flavonoids, triterpene steroids and reducing sugars.

PHARMACOLOGICAL ACTIVITIES OF *BURCHELLIA BUBALINE*

Amoo S., Ndhala A., Finnie J., Van Staden J.

University of KwaZulu-Natal, South Africa, 205527320@ukzn.ac.za
University of KwaZulu-Natal, South Africa, 207526994@ukzn.ac.za University of KwaZulu-Natal,
South Africa, Finnie@ukzn.ac.za
University of KwaZulu-Natal, South Africa, rcpgd@ukzn.ac.za

Burchellia bubalina is an endemic, monotypic plant which is extensively used in traditional medicine. Extracts obtained from stems and leaves of *Burchellia bubalina* were evaluated for antibacterial, antifungal and anti-inflammatory (COX-1 and COX-2) activities in order to detect potential new sources of antimicrobial and anti-inflammatory agents. Antibacterial and antifungal activities were evaluated using micro-dilution assays against two Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*) and the fungus *Candida albicans*. The stem petroleum ether extract showed the lowest minimum inhibitory concentration (MIC) of 0.39 mg/ml against both Gram-positive bacteria. The dichloromethane (DCM) extract of the leaf and stem showed MIC of 0.15 mg/ml and 0.39 mg/ml respectively against the fungus. The dichloromethane extract of the leaf had the best fungicidal activity at a concentration of 0.20 mg/ml. The petroleum ether and DCM extracts of both the leaf and stem exhibited good inhibition (> 70%) of prostaglandin synthesis in both COX-1 and COX-2 assays. The presence of comparable antibacterial, antifungal and anti-inflammatory activities in both the stem and leaf extracts of this plant is in line with the idea of substituting bark, root and stems with leaves. Leaves can be harvested sustainably while using this plant for medicinal purposes without the inherent survival threat associated with the over-harvesting of parts like roots and stems.

SPICATIC ACID: A 4-CAROXYGENTISIC ACID FROM A *GENTIANA SPICATA* EXTRACT WITH POTENTIAL HEPATOPROTECTIVE ACTIVITY

Ayoub N.

Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, ayoub.n@link.net

Investigation of the protective mechanisms of the aqueous alcoholic extract of *Gentiana spicata* (Gentianaceae) in carbon tetrachloride intoxicated rats was undertaken. Rats were treated with carbon tetrachloride at a dose of 1 ml/kg body weight intraperitoneally once every 24 hrs for 14 days. The hepatoprotective activity of the extract of *Gentiana spicata* was evaluated by measuring levels of serum marker enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Administration of the extract (250 mg/kg, b.wt.) significantly ($p < 0.05$)

prevented CCl₄-induced elevation of levels of serum AST and ALT. Treatment of rats with CCl₄ led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with a significant reduction of the hepatic antioxidant system e.g. reduced glutathione (GSH). These biochemical alterations resulting from CCl₄ administration were significantly ($p < 0.05$) inhibited by pretreatment with the extract of *Gentiana spicata*. These results suggest that the aqueous alcoholic extract of *Gentiana spicata* may act as a hepatoprotective and antioxidant agent. Phytochemical studies demonstrated a high phenolic content and led to the isolation and identification of the new carboxygentisic acid, 1,4-dicarboxy 2,5-dihydroxybenzene, for which we suggest the name spicatic acid, together with the two known flavonoids, quercetin 3-O-[(2,3,4-triacetyl-a-rhamnopyranosyl)-1"6"]-3-acetyl-galactopyranoside and quercetin 3-O-[(2,3,4-triacetyl-a-rhamnopyranosyl)-1"6"]-4-acetyl-galactopyranoside. All structures were elucidated on the basis of conventional analytical methods and confirmed by high resolution FTMS, 1D- and 2D-NMR data. The new phenolic carboxylic acid, spicatic acid, is of special interest as it represents the first phenolic acid which bears two carboxyl functions in one aromatic ring.

ON THE MECHANISM OF ACTION OF *CRINUM MACOWANII* BULB EXTRACT (CAEB) EFFECT ON THE HAEMODYNAMICS OF THE WISTAR RAT

Burger A., Persson K., Mugabo P., Green I.

Medical Biosciences, University of the Western Cape, South Africa, aburger@uwc.ac.za
Medical Biosciences, University of the Western Cape, South Africa, kikkipersson@yahoo.com,
Pharmacology, University of the Western Cape, South Africa, pmugabo@uwc.ac.za, Organic
Chemistry, University of the Western Cape, South Africa, igreen@uwc.ac.za

Crinum macowanii is used in traditional medicine. The mechanism of action has to be investigated to improve efficacy and safety. The effects of the CAEB (0.05 to 3 mg/kg, i.p.) on the haemodynamics (HR, Psys, and Pdiast) of anaesthetized normotensive Wistar rats as well as the general method are reported on separately. The toxic dose (80%) as determined from dose response curves for Atropine, Atenolol, Prazosin, Reserpine and Verapamil was administered in combination with CAEB to elucidate the mechanism of action in the *in vivo* anaesthetized normotensive rat model. Atropine (1.2 mg/kg i.v.), Atenolol (6.0 mg/kg i.v.), Prazosin (400 ug/kg i.v.), and Reserpine (0.6 mg/kg i.v.) in combination with CAEB did not significantly ($P > 0.01$) affect any of the measured parameters when compared to CAEB on its own. The inability of these drugs (shown in brackets below) to alter the response to CAEB at the above doses indicate that CAEB does not exert its effects through muscarinic receptors (Atropine), adrenoceptors (Atenolol), 1-adrenergic receptors (Prazosin) or the postganglionic sympathetic nerve terminals (Reserpine). When rats were pre-treated with the Ca-channel blocker Verapamil (4,8 mg/kg) significant ($P < 0.01$) differences in the HR, Psys and Pdiast were observed when compared to CAEB on its own. It could be interpreted that the Ca-channels are involved in the cardiovascular effect of CAEB, or that Verapamil caused a general decrease in the parameters (analogous to a baseline shift). Correcting for this baseline shift indicates that the CAEB effect is not mediated by a mechanism involving Ca-channels.

EXPERIMENTAL EVALUATION OF ANTICONVULSANT POTENTIALS OF *OCIMUM GRATISSIMUM* L. LEAF EXTRACTS

Adaobi Ezike, Charles Okoli, Oge Agwagah

University of Nigeria Nsukka, Nigeria, adaobiezike@yahoo.ca
University of Nigeria Nsukka, Nigeria, drcharlesokoli@yahoo.com, University of Nigeria Nsukka,
Nigeria,
A.C. Ezike, C. O. Okoli and O. C Agwagah Dept of Pharmacology and Toxicology, Faculty of
Pharmaceutical Sciences; University of Nigeria, Nsukka 410001, Enugu State, Nigeria

Leaves of *Ocimum gratissimum* L. (Lamiaceae) are used in the traditional medicine of South-East Nigeria to manage seizures. This study was designed to evaluate the anticonvulsant and central depressant properties of the leaves. Dried powdered leaves of *O. gratissimum* were successively extracted using petroleum ether (40-60°C) and methanol in a Soxhlet to afford PF and MF respectively, which were assessed for acute toxicity and lethality (LD50) (1) and phytochemical constituents (2,3). The anti-seizure properties of the extracts (200 and 400 mg/kg) were evaluated using pentylenetetrazol-induced seizure in mice. Also the central nervous system depressant activity was evaluated by assessing locomotor and anxiety activities using the open field model in mice. Data collected were analyzed using one way ANOVA and further subjected to LSD Post Hoc tests. The extracts significantly ($P < 0.05$) increased the time of onset of tonic and tonic-clonic seizures, and the time of death compared to control. In the open field study, the extract and fractions reduced the rate of grooming, rearing against a wall, centre square entries and line crossing. Oral LD50 was estimated to be greater than 5000 mg/kg. MF tested positive for alkaloids, while PF gave positive reactions to alkaloids, steroids, terpenes, saponins, flavonoids and others. The results suggest that the extracts of *O. gratissimum* offer protection against seizures and also depress the central nervous system; these attributes may contribute to the effectiveness of the plant in the management of seizures.

ANTIMICROBIAL ACTIVITIES OF TWELVE MEDICINAL PLANTS USED FOR THE TREATMENT OF GASTROENTERITIS IN SOUTH AFRICA

Fawole O., Finnie J.F., Van Staden J.

University of KwaZulu-Natal, South Africa, 207527001@ukzn.ac.za
Research Centre for Plant Growth and Development, School of Biological and Conservation
Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209,
South Africa

This study focused on the *in vitro* antimicrobial evaluation of South African traditional medicinal plants that are used for the treatment of gastroenteritis. Different parts of twelve plant species were extracted using petroleum ether, dichloromethane, ethanol (70%) and water. Crude extracts were tested using microdilution assays against Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*) bacteria as well as *Candida albicans*. Water extracts showed poor antimicrobial activity. Most solvent extracts exhibited some antimicrobial activity. The ethanol extracts of *Becium obovatum* leaves

showed the best antibacterial activity with a MIC value of 0.074 mg/ml against *B. subtilis*. The petroleum ether extracts of leaves of *Cucumis hirsutus*, *Haworthia limifolia*, and *Protea simplex* showed good antibacterial activity with MIC values ranging between 0.910 to 0.098 mg/ml against all the test bacteria. *Agapanthus campanulatus*, *Dissotis princeps*, *Gladiolus dalenii* and *P. simplex* showed activity against *C. albicans* with MIC values ranging between 0.014 to 0.39 mg/ml. The results obtained in this study show that these plants are indeed effective for the traditional treatment of gastroenteritis problems of bacterial and fungal origins.

Acknowledgement: National Research Foundation and University of KwaZulu-Natal.

ETHNOBOTANY: INTEGRATING PEOPLE, NATURE, AND BUSINESS WHILE MAINTAINING BOTANICAL, CULTURAL AND ECONOMIC INTEGRITY

Flaster T.

Botanical Liaisons, LLC, USA, tflastersprint@earthlink.net

Bioprospecting can be called the black sheep of ethnobotany, but done well it can reduce poverty, train botanical practitioners in communities and of course offer tools to medical practitioners in any ailing nation. Lately there was a re-surgence in this discipline of botany to identify new products based on traditional knowledge. Finding the right plant that can be used as a natural product is not an easy lab task, but what about the other issues? How does bioprospecting support sustainability? This short presentation discusses one system that seems to be working even though the long-term results are still not evident.

A NOVEL, HERBAL REMEDY FROM *SIPHONCHILUS AETHIOPICUS* FOR THE TREATMENT OF ASTHMA

R.M. Horak, E. Wadiwala, P. Meoni, G. Fouche, S. van Rooyen, N. Harding and V.J. Maharaj

CSIR, Biosciences, P.O. Box 395, Pretoria, 0001, South Africa, gfouche@csir.co.za

Scientific research on traditional medicines can lead to new pharmaceutical products, typically herbal formulations or new chemical entities. The Bioprospecting group of CSIR Biosciences focuses on the scientific validation of traditional medicines leading to new therapeutically active ingredients to be developed as herbal medicines or prescription drugs. South Africa's rich plant biodiversity is estimated to consist of approximately 24 000 indigenous plant species, representing about 10% of all higher plants on earth. CSIR's bioprospecting scientists work with traditional healers to gather indigenous knowledge and through a collaboration programme, *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt, was identified for the treatment of asthma. Literature studies on this species mainly provided anecdotal information and little scientifically evaluated biological data for the treatment of asthma.

Fractionation of the organic extract of the rhizomes led to the isolation of a major compound that was chemically characterized as a previously reported furanoterpenoid [1]. The structural assignment of the compound was based on a detailed study of the high field 1D and 2D NMR. Biological assaying of extracts of the plant and the furanoterpenoid showed effects in *in vitro* glucocorticoid receptor binding, 5-lipoxygenase, phosphodiesterase 4 and nuclear factor-

κB assays pointing at a possible anti-inflammatory and bronchodilation effect of the plant material. The extract was also tested for inhibition of bronchoconstriction in rodent models possibly supporting the traditional use of the plant which has been chemically profiled and standardized using HPLC mass spectrometry.

Reference

[1] Cedric W. Holzapfel, Ben-ErikVan Wyk, (2002), *Phytochemistry*, 59, 405–407

ASSESSMENT OF MEDICINAL PLANTS POTENTIAL OF THE KORUP FOREST THAT ARE USED BY TRADITIONAL HEALERS FOR THE CURE OF TUBERCULOSIS, MALARIA AND OTHER HIV AND AID RELATED DISEASES. MUNDEMBA, S.W. CAMEROON

Fuashi N.A., Moua I.S.

Uuiversity of Buea, Cameroon, afnkwatch@yahoo.com

Uuiversity of Buea, Cameroon, delmosua@yahoo.com

The tropical region and Africa in particular is endowed with an enormous wealth of plant resources. The aim of this study was to assess the medicinal plants potentials of Korup National Park that are used by traditional healers of the Oroko, Korup, and the Ejagham tribes for the cure of tuberculosis, malaria and other HIV and AIDS related diseases. This has complemented the existing Biological data of the Park for the elaboration of participatory management strategies that has promoted the conservation of the Korup National Park amongst stakeholders. For this study to achieve its aim, it elaborated a series of objectives and sub objectives that were considered at the field level as activities and sub-activities. The study made use of existing Biological data of the Korup Forest Dynamic Plot (KFDP). This was complemented by the use of Participatory Rural Appraisal (PRA) tools, questionnaires, visual assessment and direct observation to gather both quantitative and qualitative data for analysis. This was carried out over a one-year period and over two seasons for the collection of meaningful data that were analyzed using appropriate statistical package. The results thereof showed that 13 plant species and 47 plant parts consisting of leaves, roots, sap, fruits, flowers, nuts, oil, backs, and fibres combined in various forms for the cure of tuberculosis, malaria and other HIV and AIDS related diseases. Chemical extracts and the identification of chemical compounds these plant parts are still being awaited.

DNA AND ANTIMICROBIAL FINGERPRINTING OF MEDICINAL ALOE SPECIES FROM THE MASCARENE ISLANDS

J. Govinden-Soulange, S. Khoyratty, M. Ranghoo-Sanmukhiya, N. Makunga, H. Kodja

Department of Agricultural and Food Sciences, University of Mauritius, Reduit, Mauritius
Institute of Plant Biotechnology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602,
Republic of South Africa

Department of Plant Physiology; Faculty of Science, Université de la Reunion, Reunion Island

Antimicrobial and molecular studies of two endemic medicinal plants from Mauritius *L. tomentorii* and *L. purpureum* and *Lomatophyllum macrum* from Reunion Island was done to differentiate them from *Aloe vera* which belongs to the same family Asphodelaceae. These endemic Aloes are used in the Mauritian and Reunion pharmacopoeia to treat bacterial

infections and as antispasmodic.³ Phytochemical screening of the crude extracts revealed the common presence of alkaloids, anthraquinones, coumarins, phenols, saponins and tannins. Anthraquinones and saponins had also been reported as bioactive compounds present in Aloioideae and in *Aloe vera*^{1,4}. Antimicrobial activity was tested against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* using the microplate dilution method. Extracts of *Lomatophyllum* species showed more potent antimicrobial activity than *Aloe vera* with MIC ranging 0.003-0.006mg/L as compared to 0.012-0.028mg/L for *Aloe vera*. The antimicrobial properties of *L. tormentorii* and *L. purpureum* were attributed to the presence of alkaloids, coumarins, and saponins which are known to possess antimicrobial attributes. In this study we also make use of a modified RAPD-PCR technique to elucidate the genetic closeness of *Lomatophyllum* to *Aloe*. Genomic DNA was extracted using a modification of the method used by Govinden-Soulange *et al* (2007)². RAPD-PCR technique has revealed that *Lomatophyllum* species and *Aloe vera* share some genetic similarities. Consequently we report the genetic diversity of the *Lomatophyllum* species by measuring the genetic distance, which they have with *Aloe vera*. Moreover some biologically active compounds within the *Lomatophyllum* are established and their possible similarities with *Aloe vera* are unveiled.

Key Words: Biological activities, *Lomatophyllum*, Aloes, genetic distance

References:

1. Atherton, P. (1999). *Aloe vera*: myth or medicine? Positive Health, 20 June/July.
2. Govinden-Soulange J, Ranghoo-Sanmukhiya M, Seeburrun SD. (2007). Tissue culture and RAPD analysis of *Cinnamomum camphora* and *Cinnamomum verum*. Biotechnology. 6(2):239-244
3. Gurib-Fakim, A. Gueho, J., Sewraj, M. and Dulloo, E. (1994). Plantes Medicinales de l'Ile Rodrigues. Editions de l'Ocean Indien, Maurice.
4. Mark, W.C., Anette, Y.D., Anthony, V.C., Gail, R., Paula, J.R. Margaret, A.T. and Lui, E.E. (2000). Phylogenetics of Asphodelaceae (Asparagales): Analysis of Plastid rbcL and trn-L-F DNA Sequences. Annals of Botany, 86: 935-951.

SPECIES DIVERSITY OF UNDERGROWTH MEDICINAL PLANTS IN PHU POE COMMUNITY FOREST, KALASIN PROVINCE, THAILAND

Homchuen S., Pichaensoonthon C.

Department of Environmental Science, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand, 40002, samang@kku.ac.th

Department of Pharmaceutical Botany and Pharmacognosy Faculty of Pharmaceutical Sciences Khon Kaen University, Khon Kaen, Thailand, 40002

Phu Poe Community Forest, a young recovery forest in Tumbon Naboan, Ampur Kammuang, Kalasin Province, Thailand consists of a compound dry dipterocarp forest with mixed deciduous forest. The main objective of this study is to compile a checklist of undergrowth plants in this forest and explore their ethnobotanical information. The belt transect method, 10 meters wide with 1,200 meters long from the ground level to the top of the mountain (253-426 msl), was applied for the plant collection. There were altogether 16 belt transects with a distance of an interval about 250 meters each. The plant specimens were collected from July to October 2003. The general morphology and habitat of the plants collected with their uses (if any) were documented. A total of 101 species (72 genera in 49 families) were enumerated with one

species each of non-seeded plant and the gymnosperm, 20 species (11 genera in 7 families) of the monocotyledons and 79 species (59 genera in 40 families) of the dicotyledons. From all the specimens collected, the number of the family Zingiberaceae was the greatest (10 species) used for medicinal plants. Though this forest has just been discovered, the species richness was so high and from the study one rare species (*Stahlianthus campanulatus*) was found. Acknowledgements: Khon Kaen University, Thailand provided funding and Naboan Villagers provided the information.

COMPOSITIONAL CHARACTERISTICS OF ESSENTIAL OILS OF WILD POPULATIONS OF *HYPERICUM PERFORATUM* (ST. JOHN'S WORT) FROM LITHUANIA AND FRANCE

Asta Judzentiene, Jurga Budiene, Isabelle Laffont-Schwob and Jean-Marie Bessièr

Institute of Chemistry, A. Gostauto 9, LT – 01108, Vilnius, Lithuania

fax: + 370 5 2649774, e-mail:judzent@ktl.mii.lt

University of Provence, Institut Méditerranéen d'Ecologie et de Paléoécologie, UMR-CNRS 6116

IMEP, Case 17, 3 place Victor Hugo, 13331 Marseille Cedex 3, France

Ecole Nationale Supérieure de Chimie de Montpellier, 8 rue Ecole Normale, 34296 Montpellier, France

Hypericum is a genus of many species that occurs in all temperate parts of the world. Five species of the genus grow wild in Lithuania whereas twenty-eight species of the same genus occur in France. *H. perforatum* is the most abundant and frequently used as a medical plant and is very significant in pharmacology. Many studies confirmed its antidepressant, antimicrobial, antiviral, anticancer and other activities. Numerous classes of bioactive chemical constituents have been investigated in St. John's wort, but number of studies on volatile oils is limited.

The aim of this study was to evaluate characteristics of volatile oils in various wild populations of *H. perforatum* from Lithuania and France.

Essential oil qualitative and quantitative analyses were performed on aerial parts of *H. perforatum* collected in different places from Lithuania (2002-2007) and France (2000-2003). The oils were analysed by gas chromatography (FID and capillary columns HP-FFAP and CP Sil) and gas chromatography-mass spectrometry GC-MS (nonpolar cap. column DB-5). Qualitative analysis was based on a comparison of retention times, indices and mass spectra with the corresponding data in the literature and computer mass spectra libraries.

A great variability was pointed out between the compositions of the essential oils from the various localities of collection. Even if trans-caryophyllene and its oxide, germacrene D, spathulenol and several aliphatic compounds were the major compounds in all the oils, those from Lithuanian plants were rich in α -pinene, while this compound was almost not present in the oils from France.

Acknowledgements: Lithuanian Science and Studies Foundation, project "BIOMARKS" (V-08/2008, reg. No. V-08033) in the programme "Gilibert".

ANTIBACTERIAL ACTIVITY OF ELEVEN SOUTH AFRICAN PLANTS USE IN TREATMENT OF DIARRHOEA IN FOLKLORIC MEDICINE

Adamu Ahmad Kaikabo, Mohammed Musa Suleiman, Babatunde B Samuel, J N Eloff

Phytomedicine Programme, University of Pretoria, South Africa, South Africa,

Correspondence: karkafa@yahoo.co.uk, kobus.eloff@up.ac.za

Antibacterial activity of eleven South African plants use in treatment of diarrhoea in folkloric medicine were examined. The acetone extracts of eleven South African plants belonging to nine families, were screened for their antibacterial properties by determination of their minimum inhibitory concentrations (MIC) against 4 nosocomial bacterial pathogens. MIC screening was conducted at concentrations of 0.02 to 2.5 mg/ml using a two-fold serial microdilution method¹ against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. All the extracts had activity against at least one of the test organisms over a period of 1, 2 or 24 hours. The extracts of *Macaranga capensis*, *Garcinia livingstonei*, *Diospyros rotundifolia* and *Dichrostachys cinerea* had good antibacterial activity with minimum MIC values of 0.03, 0.04, 0.06 and 0.08 mg/ml, respectively. The average MIC values of the plant extracts against the tested pathogens ranged from 0.23-1.77 mg/ml. *S. aureus* was the most susceptible bacterial pathogen with average MIC of 0.36, the extract of *Diospyros rotundifolia* was the most active with activity against all the organisms and had a lowest average MIC of 0.23 mg/ml when compared with other extracts. The extracts of *Buxus natalensis*, *Dracaena mannii*, and *Pittosporum viridiflorum*, *Acacia sibeiriana*, *Erythrina lattissima*, *Cassine papillosa* and *Pavetta schumanniana* had low antibacterial activity at the concentrations used.

References:

1. Eloff JN, 1988 *Planta Medica* 64, 711-714.

WEST AUSTRALIAN DESERT PLANTS: AUSTRALIAN ABORIGINAL MEDICINES WITH ANTICANCER POTENTIAL?

Johns N., Kerr P.

Charles Sturt University, Australia, njohns17@hotmail.com
Charles Sturt University, Australia, pkerr@csu.edu.au

The semi-arid Australian native plants *Scaevola spinescens* (Goodeniaceae) and *Codonocarpus cotinifolius* (Gyrostemonaceae) have been reported as having been used by Western Australian Aboriginal people in combination as a cancer cure (1). Very little phytochemical research is published on either of these species. A monograph in an Aboriginal Pharmacopoeia (2) and brief notes by Collins et al. (3), record the constituents of the leaf volatile oil and biological activity of *C. cotinifolius* respectively. Anecdotal reports of the medicinal use of these plants by Aboriginal people and the authors interest in Australian native plants, prompted samples of *S. spinescens* (whole plant) and *C. cotinifolius* (leaves, stem-wood and root-bark) to be extracted (Soxhlet - methanol). The resulting extracts were fractionated (non-polar to polar solvents) and tested for their anti-tumour activity using the crown gall tumour assay with doxorubicin, a well-known antineoplastic drug, as a positive control. Results (Students t-test, $p < 0.05$) indicated that a number of fractions possessed > 20% inhibition of tumour formation and are therefore considered worthy of further investigation (4). GC/MS analysis was performed to identify major constituents. Our findings support the anecdotal, traditional medicinal uses of these plants.

References:

- 1 Reid, EJ & Betts, TJ 1979, *Planta Med.*, 36, 164-173.
- 2 Aboriginal Communities of the Northern Territory. 1993, *Traditional Aboriginal Medicines in the Northern Territory of Australia*. CC of the NT of Australia, Darwin.
- 3 Collins, DJ, Culvenor, et al. 1990, *Plants for Medicines*. CSIRO Publications, Melbourne.
- 4 Ferrigni, NR, Putnam, et al. 1982, *J. Nat. Prod.*, 45(6), 679-686.

ETHNOBOTANICAL STUDIES IN SOUTH WEST BURKINA FASO: TRADITIONAL USES OF PLANT SPECIES FROM GALLERY FORESTS IN SENOUFO ETHNIC AREA, ESPECIALLY BY TRADITIONAL HEALERS AND DOZOS HUNTERS

Marc O., Paulin O., Sita G.

SAMA BIOCONSULT, France, oliviersama@yahoo.fr
Institut des Sciences de la Vie et de la Nature (ISNV) Univer, Burkina Faso,
ouobapaulin@hotmail.com
Laboratoire de Botanique et Ecologie Végétale UFR /SVT Uni, Burkina Faso, sguinko@univ-ouaga.bf

In the South West part of Burkina Faso, both provinces of Comoé and Léraba are characterized by an accented relief, a humid climate and many places hard to reach. Many permanent rivers show developed gallery forests, which have not been well-studied both from botanical and ethnobotanical points of view. We present results concerning ethnobotanical studies carried out between April 2006 and December 2007 on traditional uses of plants from gallery forests, especially those plants used by people belonging to Senoufo ethnic community, which are in a large majority in study area. We mainly worked with traditional healers and Dozos hunters, a local secret confraternity, but also with old people, especially women. Our survey led us to collect data on about 61 plant species, representing 51 genera and 34 families. Plants used for nutrition, medicine (malaria, hypertension, diabetes, sickle – cell anaemia, etc.), cosmetics, native craft industry, hunting, and animist rituals were registered with vernacular names and methods of use. Many species typical of the Guinean phytogeographical area were found (*Uapaca togoensis* Pax., *Voacanga africana* Stapf.), even though Burkina Faso flora belongs mainly to Soudanian and Sahelian areas. We observed that due to agricultural practices, gallery forests are threatened in the studied area. Discussing with women, we could identify two species with an economic potential, *Carapa procera* DC. and *Pentadesma butyracea* Sab, (traditional production of vegetal oil and butter used in food and cosmetics). We conclude that helping women to sell their products could encourage populations to protect gallery forest biodiversity.

EVALUATING PLANT SPECIES USED IN VENDA (RSA) TO ADDRESS HIV/AIDS SIDE EFFECTS

Masevhe N, Eloff J. N.

University of Venda, South Africa, masevhen@univen.ac.za
University of Pretoria, South Africa, kobus.eloff@up.ac.za

A total of 15 indigenous plants used by the local traditional medical practitioners for the treatment of candidiasis were collected. Hexane, acetone and methanol leaf extracts were evaluated for antifungal activity against *Candida albicans* using both microdilution¹ and bioautography methods. Hexane leaf extracts of four indigenous plants were active against *Candida albicans*. *Clerodendrum glabrum* E. Mey had MIC value of 0.04 mg/ml, *Faurea saligna*,

Schotia brachypetala and *Strychnos potatorum* all had MIC values of 0.08 mg/ml. *Faurea saligna* is the only plant that had substantial antioxidant activity. Bioautography revealed antifungal activity ranging from three to four components. The results support the use of these plants in folk medicine to treat candidiasis. Further work is in progress to conduct anti-inflammatory activity and bioassay-guided fractionation of the active extracts in order to isolate the active components and structure elucidation.

References:

1. Eloff JN, 1988 *Planta Medica* 64, 711-714.

FACTORS INFLUENCING THE PHARMACEUTICALLY IMPORTANT CHARACTERISTICS OF *LOBELIA INFLATA* L.

Ákos Máthé, Éva Szöke, László Kursinszki, Mária Takácsné Hájos

University of West Hungary, Faculty of Agriculture and Food Sciences, Hungary,
amathe@mtk.nyme.hu

Semmelweis University, Department of Pharmacognosy, Budapest, Hungary

Semmelweis University, Department of Pharmacognosy, Budapest, Hungary

Tessedik Sámuel College Faculty, Institute of Agriculture, Hungary

Lobelia inflata L. (Lobeliaceae) contains pharmacologically important secondary metabolites, which consist of more than 20 piperidine alkaloids. Lobeline with its stimulatory effect on the respiratory centre is used in anti-smoking preparations. The pharmaceutical importance could be improved by the polyacetylenes (lobetyol, lobetyolin, lobetyolinin). The main goal of the experiments: to introduce the species into field production in Hungary and to identify the factors influencing its pharmaceutically important characteristics. Plantlets were propagated and raised both *in vitro* and *in vivo*. After acclimatization in a glass-house they were transplanted to irrigated open field conditions at 4 Hungarian locations. *In vitro* organized cultures produced intensive growth and considerable secondary metabolites. In contrast to lobetyolin (polyacetylene), the alkaloid (lobeline) content decreased in the open field. The effect of nutrient supply was also considered. Phytochemical analyses revealed differences in the total alkaloid content, both in view of the geographic region of cultures and the propagation technology. The highest alkaloid and polyacetylene contents were detected in the population grown at Mosonmagyaróvár. Lobetyolin content exceeded in all samples the content of lobetyolinin. No similar differences were observed with lobeline. Field grown plants contained sufficient amounts of active principles. The nearly 1 t/ha biomass yield and active substance production could be increased by higher plant density and fertilization. N-fertilization increased the above ground phytomass, due to the increased number of side-branches. Dry mass was highest under the influence of MgSO₄ (2%) foliar spraying. A similarly positive influence was detected both in the total alkaloid production and the lobeline content. As a contrast, a decrease in the total alkaloid content was observed with the doubling of both polyacetylenes.

Acknowledgements: GVOP 3.1.1.-0309/3

SCREENING OF SOUTH AFRICAN PLANTS FOR ANTIBACTERIAL AND ANTIMYCOBACTERIAL ACTIVITY

Katlego Mayekiso, J. N. Eloff, Lyndy McGaw

University of Pretoria, South Africa, katlivm@yahoo.com
University of Pretoria, South Africa, kobus.eloff@up.ac.za, University of Pretoria, South Africa,
lyndy.mcgaw@up.ac.za

The resistance of microorganisms such as multidrug-resistant (MDR) *Mycobacterium tuberculosis*, to available drugs has led to plants being investigated as alternative sources of drugs. Ten plant species from South Africa were tested for antibacterial and antimycobacterial activities after showing good activity in a preliminary screening. The biological activities were determined using a serial microdilution method¹ and bioautography with four bacterial species, *Staphylococcus aureus*, *Enterococcus coli*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* and two mycobacterial species, *Mycobacterium smegmatis* and *M. fortuitum*. All the plant leaf acetone extracts of *Ziziphus mucronata*, *Prunus africana*, *Ricinus communis*, *Rhus lancea*, *Antidesma venosum*, *Ptaeroxylon obliquum*, *Markhamia zanzibarica*, *Tecomaria capensis*, *Melianthus major* and *Lantana camara* had good antibacterial and antimycobacterial activities with minimum inhibitory concentration (MIC) values reaching as low as 0.02 mg/ml. One plant species, *Ptaeroxylon obliquum*, was chosen for further investigation as bioautography revealed the presence of three antimycobacterial compounds, and the MIC of the crude acetone extract was 0.16 mg/ml. Isolation of bioactive compounds from *P. obliquum* is underway.

References:

1. Eloff JN, 1988 *Planta Medica* 64, 711-714.

ARTEMISIA: ARTEMISININ METABOLISM AND METABOLOMICS

Meyer M., Hussein A., Muranaka T., Seki, H., vd Kooy F., Verpoorte R.

Department of Plant Science, University of Pretoria, 0002 Pretoria, South Africa,
marion.meyer@up.ac.za

Kihara Institute for Biological Research, Yokohama City University, Japan
Division of Pharmacognosy, Section of Metabolomics, Institute of Biology, Leiden University, PO
Box 9502, 2300 RA Leiden, The Netherlands

Artemisia annua has a long history of use in traditional Chinese medicine. We determined that *A. afra*, a well-known indigenous South African medicinal plant does not synthesise artemisinin. It does however produce several other terpenoids including sesquiterpenes. We performed comparative molecular genomics and metabolomics studies between *A. annua* and *A. afra* and succeeded to profile their terpenes and the genes involved in their biosyntheses. Two enzymes 1) amorpha-4,11-diene synthase (ADS), catalysing the first regulating step leading to artemisinin, and 2) CYP71AV1, oxidising amorpha-4,11-diene to produce artemisinic acid (one of biosynthetic intermediates for artemisinin), have previously been shown to be involved in the biosynthesis of artemisinin in *A. annua*. We determined that the gene for CYP71AV1 is expressed but that the ADS gene is not, in *A. afra*. A transgenic

system was established for *A. afra* and transgenic plants have been created, we will report on these results. Our goal in this project was to create an artemisinin-producing *A. afra* for a sustainable supply of the drug. We will also report on the results of a metabolomic quality control analysis of *A. afra*, *A. annua* and tablets of *A. afra* used in the prevention of malaria.

EFFECT OF PLANT GROWTH REGULATORS ON MICROPROPAGATION OF ENDEMIC *IRIS STEONPHYLLA HAUSSKN & SIEHE EX BAKER SUBSP. ALLISONII* B. MATHEW IN TURKEY

Miric S.I., Nasrclar A.G.

Education Faculty, Turkey, semramirici@akdeniz.edu.tr
Education Faculty, Turkey, nasircilar@akdeniz.edu.tr

Most geophytes are widely used for pharmaceutical and perfume industry as well as ornamental flowers. Most of them are collected from natural habitats. However, *Iris stenophylla* Hausskn & Siehe Ex Baker subsp. *allisonii* B. Mathew is a critically endangered geophyte. Therefore *in vitro* micropropagation is an important alternative method for the protection and production of this species. We have cultured bulb scale, leaf, skap and immature embryo explants of *Iris stenophylla* on Murashige-Skoog (MS) media supplemented with various concentrations of plant growth regulators. Immature embryos produced a higher number of bulblets than others. The most bulblets were produced from immature embryos on MS medium supplemented with 1 mg/l BA and 0.25 mg/l NAA. Effective surface sterilization of explants to a level suitable for micropropagation is an important stage to overcome infection. We report a series of experiments with *Iris stenophylla* bulbs that involved various concentrations of sodium hypochlorite and HgCl₂ treatments of whole bulbs. The best results were obtained from 80% commercial bleach for 20 min.

Acknowledgement: This study was supported by the Scientific and Technical Research Council (Project no: TOVAG-105 0 246) of Turkey

CHOLESTEROL, TRIGLYCERIDE AND LOW DENSITY LIPOPROTEIN LOWERING EFFECTS OF SEED POWDERS OF *MANGIFERA INDICA*, *SYGYZIUM CUMINI* AND *TAMARINDUS INDICA* IN RATS

**Mohammad Rahamat Ali, Salahuddin Mohammad Sumon, Md. Saiful Islam and
Mohammed Rahmatullah**

Faculty of Life Sciences ,University of Development Alternative,Dhanmondi, Dhaka,Bangladesh

Seed powders from *Mangifera indica* (MSP), *Syzygium cumini* (SSP) and *Tamarindus indica* (TSP) are considered to have hypoglycemic potential in the traditional medicinal system of Bangladesh. We examined serum glucose, total cholesterol (TC), triglyceride (TG) and low density lipoprotein (LDL) lowering ability in Wistar rats when the above seed powders were administered in diet, alone or in combination. Rats were divided into 11 groups. Group 1 was fed a high fat-high sucrose diet. The other groups received respectively (w/w diet), 0.1 and 1% MSP (Groups 2, 3), 0.1 and 1% TSP (Groups 4, 5), 0.1 and 1% SSP (Groups 6, 7), 0.1% SSP + 0.1% TSP (Group 8), 0.1% SSP + 1% TSP (Group 9), 1% SSP + 0.1% TSP (Group 10), and 1% SSP

+ 1% TSP (Group 11). All groups received diet *ad libitum* for 30 days. Significant reduction of serum glucose was observed in Groups 4, 5 and 8. Significant reduction of serum TC was observed in Groups 8-11. Significant reduction of serum TG was observed in Groups 3-5. Significant reductions in serum LDL was observed in Groups 6-11. Taken together, the results validate the use of TSP in traditional medicine to lower glucose and further suggest that the above seed powders, alone or in combination, can be used for lowering of serum TC, TG and LDL. The rats did not manifest any signs of toxicity, when given the above seed powders.

ANTIMICROBIAL PROPERTIES OF A ZULU HERBAL MIXTURE 'IMBIZA EPHUZWATO'

Ndhlala A., Stafford G., Finnie J., Van Staden J.

University of KwaZulu-Natal, South Africa, 207526994@ukzn.ac.za
 University of KwaZulu-Natal, South Africa, 972150566@ukzn.ac.ukzn, University of KwaZulu-Natal, South Africa, Finnie@ukz.ac.za, University of KwaZulu-Natal, South Africa, rcpgd@ukzn.ac.za

Imbiza ephuzwato is a Zulu herbal preparation consisting of roots, bulbs, rhizomes and leaves of several medicinal plants extracted with boiling water. The herbal mixture, sold in muthi shops around KwaZulu-Natal is used to treat microbial and fungal infections, boost energy and relieve general body pains. Imbiza ephuzwato was tested against two Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria and *Candida albicans* using the microdilution assay to evaluate its antimicrobial effects. The mixture showed antibacterial and antifungal activity with a minimum inhibitory concentration (MIC) of 0.78 mg/ml against the microbes tested. Six of the plant constituents (*Corchorus aspleniifolius* root, *Cyrtanthus obliquus* bulb, *Drimys robusta* bulb, *Gunnera perpensa* rhizome, *Hypericum aethiopicum* leaf and *Zanthoxylum capense* root) of Imbiza ephuzwato were extracted with petroleum ether, 80% ethanol and water. The extracts were subjected to similar tests to investigate the source of activity of the mixture. The aqueous extracts of *G. perpensa* rhizome showed MIC values of 0.78 mg/ml against the bacteria and fungus. The petroleum ether extract of *C. aspleniifolius* root showed MIC values of 0.195 mg/ml against all the bacteria and 0.39 mg/ml against *C. albicans*. The rest of the plants had MIC values ranging from 0.39 to 12.5 mg/ml against both the bacteria and fungus used. The results suggest *G. perpensa* rhizome could be the source of the activity detected in the product. *G. perpensa* rhizome contains several compounds with antibacterial activity. Isolation of active components will be attempted from the other active extracts.

PHYTOCHEMICAL PROFILING ON *HYPERICUM* SPECIES

J. Radušienė, V. Janulis, L. Ivanauskas and C. Çırak

Institute of Botany, Zaliuju ezeru 49, Vilnius, LT-0840 Lithuania. (jolita.r@botanika.lt)
 Kaunas University of Medicine, Mickeviciaus 9, LT-44307 Lithuania.
 University of Ondokuz Mayıs, Kurupelit, Samsun, Turkey.

Increased market demand for *Hyperici herba* has led to intensive studies on the chemistry and biological activities of different *Hypericum* species. The investigations were made on thirteen spontaneous *Hypericum* species from Turkey.

HPLC method with photodiode array detection (PAD) was performed for chemical analyses of secondary metabolites. Eleven compounds with pharmacological interest, namely hyperforin, hypericin, pseudohypericin and phenolics such as chlorogenic acid, rutin, hyperoside, apigenin-7-O-glucoside, quercitrin, quercetin, kaempferol and amentoflavone, were separated and quantified in ethanolic extracts from stems, leaves and flowers. Among different plant parts, flowers were found to be principle organ for hypericin, pseudohypericin, hyperforine, chlorogenic acid, apigenin-7-O-glucoside, kaempferol, quercetin and amentoflavone accumulations, while hyperoside, rutin and quercitrin were accumulated mainly in leaves.

Results from the present study indicated that *H. adenotrichum*, *H. aviculariifolium*, *H. bupleuroides*, *H. hyssopifolium*, *H. montbretii*, *H. organiifolium*, *H. perfoliatum*, and *H. triquetrifolium* accumulated higher concentrations of hypericin, pseudohypericin, quercitrin, hyperoside, amentoflavone, apigenin-7-O-glucoside or chlorogenic acid, when compared to *H. perforatum*, a well known commercial source of examined compounds.

The occurrence of dark secretory glands in an organ is regarded as a reliable indicator of the presence of hypericins in *Hypericum* species [1]. The morphological observations of *Hypericum* species in the present study confirmed the corresponding phenomenon. We found a close relationship between dark gland density of leaves and hypericin content in several *Hypericum* species such as *H. aviculariifolium*, *H. perforatum*, *H. montbretii*, *H. pruinatum* and *H. lydiium*.

The research indicated *Hypericum* species of interest for further evaluation and new chemotaxonomical data of corresponding genus.

[1] Robson, NBK: 1977. Studies in the genus *Hypericum* L. (Guttiferae). 2. Characters of the genus. Bull. Br. Mus. (Nat. Hist.) Bot. 8, 55-226.

ANTIVIRAL ACTIVITY OF *EUPHORBIA MICROSCIADIA* EXTRACT

Ramezan M, Behravan J., Arab M.

Mashhad University of Medical Sciences, Iran, ramez_ani@hotmail.com
Behravanj@mums.ac.ir, arabm@mums.ac.ir

In traditional medicine, the extracts of different species of *Euphorbia* have been successfully used for the treatment of skin diseases. Therefore, the antiviral effects of *Euphorbia microsciadia* extracts were investigated using a plaque reduction assay. Plant material was collected, dried and ground and extracted either with methanol using a Soxhlet apparatus or by maceration in methanol. After applying several enriching stages of phage CP51, phage titration was performed to determine the phage concentration in phage lysate for specifying the dilution factor of the phage to be used as negative control for the next working stages. Then IC50 of trifluridine, as a positive control, for phage CP51 was determined. The MIC of the extracts for *Bacillus cereus* was determined as 1.25 and 0.5 mg/ml for Soxhlet and maceration extracts, respectively. To determine whether the extracts have the ability to inhibit the adsorption of virus to host cell, it was pre-incubated with phage CP51 for 30 min at 25°C. The growth and reproduction of phage was inhibited by more than 50% at concentration of 1 and 0.25 mg/ml, respectively. In order to test the effects of extract on the transcription process, *Bacillus cereus*, phage CP51 and extract were incubated together. The growth and reproduction of phage was inhibited by more than 50% at concentration of 0.75 and 0.125 mg/ml for Soxhlet and macerated extracts, respectively. These results indicated that both extracts of *E. microsciadia* have considerable antiviral activity.

THE PATTERNS OF ETHNOBOTANY OF WILD MEDICAL PLANTS IN WESTERN BALKAN (SE EUROPE)

Sulejman Redzic

Dep. of Biology, Faculty of Science University of Sarajevo, Bosnia and Herzegovina,
sredzic@pmf.unsa.ba

Results of ethnobotanical research provide answers to forms of ethnogenesis of certain ethnical groups, as well as applicable forms of anthropogenesis [1]. The Balkan Peninsula area was for years a stage where cultures and civilizations of East and West collided [2]. Along with floristic richness, this area developed different forms of use of wild plants in traditional medicine [3]. Using method of ethnobotanical interview (455 adults, age between 50 – 93) it was determined that about 700 species of plants were used in traditional medicine in this territory, which is almost 10 % of total flora of this area. About 150 species are used in Mediterranean areas for preparation of traditional mixtures (tinctures in grape brandy or wine), aromatic alcoholic beverages and lotions based on rosin used in treatment of stomach, respiratory, heart and skin diseases. About 50 plant species growing in difficult mountain conditions are used in preparation of infusions, tinctures in plum brandy, and different lotions used in treatment of chronic stomach, respiratory, heart, skin and other diseases, and in treatment of child diseases. The level of use of wild flora in traditional medicine of people in western Balkan is in correlation with floristic richness, climate, social – economic circumstances, isolation of ethnic groups and difficult natural conditions. Certain differences in preparation of medicines are also determined in different ethnic groups.

IN VITRO AND IN VIVO ANTIMALARIAL ACTIVITY OF DCM EXTRACT OF *AGATHOSMA BETULINA***Sekhoacha M., Campbell W., Smith P.**

Division of Pharmacology, Department of Medicine, University of Cape Town, Observatory 7705,
South Africa, Mamello.Sekhoacha@uct.ac.za

The problem of drug resistance of *Plasmodium* species has emphasised the need for more effective alternative treatment for malaria. The study investigates the antimalarial potential of a South African medicinal plant, *Agathosma betulina*. The aerial parts of *A. betulina* were obtained from Grassroots Cape Botanicals in Stellenbosch. The *in vitro* antiplasmodial activity was assessed in a bioassay of cultured blood stage parasites of *P. falciparum* using the pLDH assay. Cytotoxicity was tested on CHO mammalian cell line using MTT assay. The *in vivo* antimalarial activity was investigated in Plasmodium ANKA mouse model. The DCM extract was fractionated using Solid Phase Extraction and HPLC. Active compounds were identified by GC-MS. The DCM extract showed *in vitro* antiplasmodial activity (IC₅₀ = 14.4 µgml⁻¹) and low cytotoxicity (IC₅₀ = 170 µgml⁻¹). Three fractions showed enhanced *in vitro* antiplasmodial activity with IC₅₀ values in the range of 1.6-2.8 µgml⁻¹, and low cytotoxicity (IC₅₀ > 100 µgml⁻¹). These fractions combined showed no toxicity effects *in vivo* when administered at 500mg/kg daily for 7 consecutive days, and showed good antimalarial activity when given at 250mg/kg

daily for 4 days. The fractions exhibited parasite inhibitory activity and good prophylactic effect with parasitaemia suppression averages of 60% and 75% respectively. The active components were identified as hepatodenoic acid, hexadenoic acid, phosphoric acid, and 9,-12-octanedenoic acid.

MEDICINAL RESEARCH ON BRITISH PLANTS

Monique S.J. Simmonds

Royal Botanic Gardens, Kew Richmond TW9 3AB

Many British plants are used in herbal medicines and some provided leads for pharmaceuticals. However, in the last 40-50 years natural-product researchers in Britain has had a tendency to work on more exotic species from Africa, Asia and South America.

Since the late 1990s researchers at Kew are involved in two key projects that have an emphasis on medicinal properties of British plants. The first project involves investigating the traditional uses of the plants and will provide chemical data that that could be valuable in the quality control of plant material being used. The other is a project called Ethnomedica or "remembered remedies" that concentrates on collating information about the medicinal uses of plants in UK between 1900 and 1948. This is just before the start of the National Health Service. Both projects are very collaborative. The Ethnomedica project involves herbalists, the Natural History Museum, Eden Project, Chelsea Physic Garden and students from different universities, including, Kent and Edinburgh that have been helping to gather information.

The talk will give a summary of both projects especially those with wound healing, anti-microbial and anti-oxidant activity and those that contain compounds that have potential for use in studying Alzheimer, cancer and cardiovascular diseases. It will also provide an overview of the links between the traditional use data gathered via the Ethnomedica project and research into the chemistry of the different species.

DETECTION OF ANTIMICROBIAL COMPOUNDS BY DIRECT BIOAUTOGRAPHY IN SOME SOUTH AFRICAN PLANTS

M.M. Suleiman, L.J. McGaw, J. Picard, V. Naidoo and J.N. Eloff.

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

The hexane, acetone, dichloromethane and methanol extract of *Commiphora harveyi* (Engl.) Engl. (Burseraceae), *Combretum vendae* (A.E. van Wyk) (Combretaceae), *Khaya anthotheca* (Welm.) (Meliaceae), *Kirkia wilmsii* (Engl.) (Kirkiaceae), *Loxostylis alata* (A.Spreng. ex Rchb) (Anacardiaceae), *Ochna natalitia* (Meisn.) (Walp.) (Ochnaceae) and *Protorus longifolia* (Bernh.) Engl. (Anacardiaceae) were screened for their antimicrobial activity against

bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*), and fungi (*Aspergillus fumigatus*, *Sporothrix schenckii*, *Microsporum canis*, *Cryptococcus neoformans* and *Candida albicans*) using simple bioautographic procedure on thin layer chromatographic (TLC) plates developed in solvents of varying polarities. All the extracts showed varied levels of antimicrobial activity against at least one of the tested pathogen. This activity was denoted by white spots against a red-purple background on the TLC plates. *L. alata* is the most active plant; it showed the highest inhibition zones against bacteria and fungi. Of the bacteria tested, *S. aureus* is the most susceptible organism having been inhibited by most of the compounds contained in the plants. Similarly, *C. neoformans* depicted the highest susceptibility among fungal organisms. This study clearly shows that the South African flora offers great potential in the search for natural compounds with antimicrobial activity. Isolation and characterisation of active compounds from extracts that showed good antimicrobial activity is presently in progress in our laboratory. Moreover, we are also carrying out toxicological and pharmacological evaluations on the active plant extracts.

ETHNOPHARMACOLOGICAL IMPORTANCE OF *MORINDA CITRIFOLIA* L.

Padmavathy S., Jeyachandran R., Cindrella L.

Nirmala College for Women, Coimbatore- 641018, INDIA, rajpadmah@yahoo.com
St. Joseph's College, Tiruchirappalli-620002, INDIA, jeyachandranrobert@gmail.com, National
Institute of Technology, Tiruchirappalli-620015, INDIA, cind@nitt.edu

Morinda citrifolia L. (commonly known as Noni) has been used in folk remedies over the years and has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects. The effect of oral administration of the bioactive principles isolated from *Morinda citrifolia* L. was studied in streptozotocin induced diabetic nephropathy rats to assess the anti-diabetic nephropathy activity in terms of glucose, protein, urea, uric acid, creatinine in serum, potassium, sodium, alkaline phosphatase and urinary creatinine. Streptozotocin induced animals showed high level of protein compared to control group. The increased serum protein level is due to increased renal failure with progressive atrophy of the nephrons. Treatment of diabetic nephropathy rats with *Morinda citrifolia* fruit extract has been found to decrease the damage of kidney and has restored the level of glucose, protein, urea, uric acid, creatinine, potassium, sodium and kidney alkaline phosphatase to that of normal level. Noni is rich in proxeronine, which combines with enzymes in the body to form an essential substance known as xeronine. It activates the immune system at cellular level thereby repairing and protecting kidney from damage. The extract of noni is found to possess nephroprotective effects as revealed by the histopathological studies. The fruit extract of the plant has anti-diabetic nephropathy effects, and possesses both high antioxidant properties and immunomodulatory properties. *Morinda* is believed to fortify and maintain cell structures. This can be accomplished by acting as an adaptogen that can aid "sick" cells in repairing themselves.

ETHNOBOTANY OF AQUEOUS PLANTS AT NONG HAN KUMPHAWAPI WETLAND, UDORNTHANI, THAILAND: SAVING TRADITIONAL KNOWLEDGE THROUGH LOCAL RESOURCE-BASED ECONOMY

Pagdee A., Homchuen S., Pornpipat A.

Dept. of Environmental Science, Faculty of Science, Khon Kae, Thailand, adcpag@kku.ac.th
Dept. of Environmental Science, Faculty of Science, Khon Kae, Thailand, samang@kku.ac.th
Dept. of Environmental Science, Faculty of Science, Khon Kae, Thailand

An ethnobotanic study of aquatic plants at Nong Han Kumphawapi—a wetland of international importance, Udonrthani, Thailand was conducted from June to August, 2006. Data were collected by in-depth interviews of seven senior villagers and traditional healers, coupled with field and local market surveys. In total, 62 species (33 families) of aquatic plants were reported to be used for subsistence, including food, medicines, household tools, animal feed and community rituals. Edible and medicinal plants are most frequently harvested, accounted for 45% and 26% of all identified species respectively. The family most frequently used is Cyperaceae i.e. *Scirpus grossus* L.f. and *Cyperus corymbosus* Hook.f. Key informants reported that ethnobotanic knowledge was passed along through storytellers rather than written documentation. Currently, due to technology development, socio-cultural transformation, access to market goods and nature of knowledge transfer, ethnobotany especially use of medicinal plants is fading away. Hence, loss of potential usefulness is likely even before these species can be studied. Wetland resource management, including detailed assessment of ecosystem status, biodiversity quantities, productivity potential, and sustainable use methods is necessary. Furthermore, local communities need to recognize the importance of ethnobotany and participate in the management. Day-to-day use and value addition of these species need to be promoted, so ethnobotany becomes part of a community economy. This can be seen in Thailand under the national program called “OTOP—One Tambon (an products made from local resources and selects one to generate its economy. administrative level equivalent to sub-district) One Product.” Every Tambon develops its most unique

ETHNOPHARMACOLOGICAL SURVEY OF MEDICINAL HERBS USED BY RURAL AND TRIBAL COMMUNITY IN BETUL DISTRICT OF MADHYA PRADESH, INDIA**Patil U.K., Bhargava C.S., Yadav S.K.**

Department of Pharmacognosy, VNS Institute of Pharmacy, Bhopal (M.P.) 462044 India,
umeshpatil29@yahoo.com

The study of local knowledge about traditional herbal medicine is becoming increasingly important in defining strategies and actions for conservation of medicinal plants. This study therefore considered it worthwhile to collect information from local rural and tribal population living in Betul district of Madhya Pradesh (India) concerning the use of medicinal plants; identify the most important species used; determine the relative importance of the species surveyed and calculate the informant consensus factor (ICF) in relation to medicinal plant use. Data collection relied predominantly on qualitative tools to record the interviewee's personal information and topics related to the medicinal use of specific plants. The present study revealed that 119 plant species grown in the study region are in use by rural and tribal community in traditional medicine for the treatment of various diseases. Most of the locals interviewed dealt with well-known safe medicinal plants, such as *Allium sativum*, *Acacia arabica*, *Embllica officinalis*, *Momordica*

charantia and *Ocimum sanctum* with use values of 0.62, 0.54, 0.52, 0.51 and 0.50 respectively. Dental, inflammation-pain and female problems scored the highest ICF of 0.85, 0.78 and 0.77 respectively. The literature from different Indian traditional systems of medicine evidenced some concordance with the solicited plant uses mentioned by the rural and tribal informants.

Acknowledgements: CDACR, Bhopal for financial assistance.

THE ANTIPLASMODIAL ACTIVITY AND TOXICITY PROFILE OF TEN SOUTH AFRICAN *COMMIPHORA* SPECIES

van Zyl R.L. and Viljoen A.M.

Department of Pharmacy and Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, 2193, South Africa. E-mail: Robyn.vanZyl@wits.ac.za
Department of Pharmaceutical Chemistry, Tshwane University of Technology, Private Bag X680, Pretoria, 0001, South Africa

The emergence of drug-resistant strains of *Plasmodium falciparum* has resulted in an urgent need to develop new antimalarial chemotherapeutic agents. Traditionally, *Commiphora* (Burseraceae) species, commonly known as 'corkwood or kanniedood', have been used in the treatment of fever, infections and malaria. In this first report on the antiplasmodial activity of South African *Commiphora* species, ten species were investigated along with their toxicity profiles. Aerial parts were collected from natural populations in the Limpopo Province (South Africa) and the leaves and stems extracted with chloroform:methanol (1:1). Antimalarial activity was determined against chloroquine-resistant *Plasmodium falciparum* using the tritiated hypoxanthine incorporation assay. The haemolytic properties of the extracts were assessed to differentiate between the direct antimalarial activity and the lytic nature of the extracts which would indirectly inhibit the growth of the intra-erythrocytic parasite. Of the twenty extracts, *C. viminea* (stem) and *C. marlothii* (leaves and stems) were more potent than *C. glandulosa* (leaves), *C. tenuipetiolata* (stems) and *C. edulis* (leaves). At 500µg/ml, *C. mollis* (stem) and *C. neglecta* (leaves) were haemolytic; however there was a favourable safety index with *C. viminea* (stem) and *C. marlothii* (leaves and stems). There was no linear relationship between the haemolytic nature of the extracts and their antimalarial activity, indicating that haemolysis does not contribute to the efficacy of the more active extracts. These findings validate the traditional use of South African *Commiphora* species as potential antimalarials.

ANTIOXIDANT ACTIVITY OF CURCUMIN: A COMPARISON WITH RESVERATROL IN A HEME-ENHANCED OXIDATION REACTION

Vieira A.

Simon Fraser University, Canada, avvieira@sfu.ca

Preparations of the turmeric (*Curcuma longa* Linn.; family: Zingiberaceae) rhizome have been used medicinally and as spices for millennia in several Asian countries. Some of the medicinal properties are attributed to curcumin (diferuloylmethane), a major polyphenolic component (~5-10% of dry weight) of the rhizomes. Biomedical investigations of curcumin (and curcuminoids) have provided evidence for a wide range of molecular and cellular activities, most

related to redox reactions and signal transduction. The main goal of the present study was to compare antioxidant activities of curcumin with those of resveratrol (3,5,4'-trihydroxystilbene), a polyphenol present in many non-dietary plants (e.g. *Polygonium* genus) and a few dietary ones such as grapes and peanuts. Combinations of the two were also examined for potential synergism. The methods involved a heme-enhanced N,N,N',N'-tetramethyl p-phenylenediamine-based oxidation reaction. Polyphenols were tested at 10 microM (5 microM each for combinations) over time periods of 1-5 min. Results: Curcumin exhibited a statistically significant antioxidant effect at all time points, e.g., $30.5 \pm 11.9\%$ (s.e.m.) relative to controls without phytochemicals ($p < 0.01$) at 3 min, a time chosen for comparisons. Resveratrol was significantly less potent ($p < 0.05$), about half of curcumin's activity. Curcumin and resveratrol together resulted in a synergistic antioxidant effect: $15.5 \pm 1.7\%$ greater than an average of individual activities. This synergy was significantly greater ($p < 0.05$; about 4-fold) than that of curcumin with the flavonol quercetin. In conclusion, curcumin is a potent antioxidant in a reaction that may be relevant to in vivo toxicity. In relation to two other well-known antioxidants, curcumin shows significantly greater synergism with resveratrol than with quercetin.

DRUG ABSORPTION ENHANCING PROPERTIES OF ALOE VERA ACROSS INTESTINAL EPITHELIUM

Weiyang Chen, Josias Hamman, Alvaro Vlijoen

Tshwane University of Technology, South Africa, ChenW@tut.ac.za
Tshwane University of Technology, South Africa, HammanJH@tut.ac.za
Tshwane University of Technology, South Africa, VlijoenAM@tut.ac.za

Oral drug delivery is considered as the preferred route of administration, but the bioavailability of orally administered hydrophilic, macromolecular drugs is usually poor because of the hostile gastric environment and also poor gastrointestinal mucosal permeability. Co-administration of absorption enhancers is one way to improve oral bioavailability of these drugs. Numerous classes of compounds have been reported to enhance the intestinal absorption of poorly absorbable drugs, but most of them have toxic effects and are not highly efficient. Therefore, development of safe and effective absorption enhancers is still a challenge. The effect of Aloe vera (L.) Burm.f. (*Aloe barbadensis* Miller) gel and whole leaf extract on the permeability of Caco-2 cell monolayers was determined. Both the A. vera gel and whole leaf extract were able to significantly reduce the transepithelial electrical resistance of the Caco-2 cell monolayers at concentrations above 0.5 % w/v and thereby showed the ability to open tight junctions between adjacent cells. This effect was fully reversible as the electrical resistance of the cell monolayers returned to the original value upon removal of the test solutions. The A. vera gel and whole leaf extract solutions significantly enhanced the transport of insulin across the Caco-2 cell monolayers compared to the control. The results suggest that these plant products have a high potential to be used as absorption enhancers in novel dosage forms for drugs with poor bioavailabilities when administered orally.

THE USE OF HORSERADISH IN FOOD AND MEDICINE IN THE NORDIC COUNTRIES

K. Wedelsbäck Bladh, K. Olsson, Å. Asdal, A. Koldinska Brantestam

Swedish Biodiversity Centre PO Box 57 230 53 Alnarp, Sweden

Swedish Agricultural University PO Box 101 230 53 Alnarp, Sweden
 Norwegian Genetic Resource Centre BioforskÖst Landvik 4886 Grimstad, Norway
 NordGen PO Box 41 230 53 Alnarp, Sweden

Horseradish, *Armoracia rusticana* P. Gaertn., B. Mey. & Scherb. has a long history as a plant used by man and it was already mentioned in "De material medica libri quinque" written in 100 A. D.¹ The plant, which has its origin in southeast Europe, was brought to the Nordic countries by monks during the thirteenth century. In the Nordic countries, horseradish was used both as a bitter spice and as a medicinal plant. The plant contains high levels of sulfur-containing glycosides, so called glucosinolates. The most dominant is sinigrin, which causes a pungent taste². Some glucosinolates also possess anti-microbial characters, and horseradish has traditionally been used as a component in preserving. The root also has a high content of vitamin C and was taken to prevent scurvy on long sailing tours. Horseradish was also used as a medicinal plant for headaches, digestive disorders, high blood pressure and gout. Earlier it was also believed that the plant had magical powers and could prevent snakebites if you had rubbed your hands with it. The production of horseradish has decreased in the Nordic countries due to high production costs, changes in traditional cooking manners and a decrease in homemade preserving. NordGen, the Gene Bank for the Nordic countries, has a collection of horseradish consisting of 170 accessions. The roots were collected from old gardens or as refugees from gardens in Denmark, Finland, Norway and Sweden. The accessions have been evaluated for contents of glucosinolates and vitamin C and a large variation in bioactive substances has been found³.

A RAPID IN VITRO METHOD FOR THE EVALUATION OF CANDIDATE REPELLENTS
 AGAINST *LEPTOTROMBIDIUM CHIGGERS*

Wuttikorn Rodkvamtook A. Prasartvit, J. Gaywee and C. Jatisatienr

Department of Biology, Faculty of Science, Chiang Mai Univer, Thailand, Itwutti@hotmail.com
 Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
 Department of Disease Control Ministry of Public Health, Nonthaburi 11000, Thailand. 3Armed
 Forces Research Institute of Medical Science (AFRIMS), Bangkok 10400, Thailand

Scrub typhus is an acute febrile zoonotic disease resulting from infection with the Gram-negative intracellular bacteria *Orientia* (formerly *Rickettsia*) *tsutsugamushi* (Hyachi) (Seong et al. 2001). The disease is endemic in much of south and central Asia, with approximately one million cases each year and more than a billion people at risk worldwide (Rosenberg 1997). Scrub typhus is transmitted by several species of larval trombiculid mites which are commonly known as chiggers (Tanskul et al. 1998). Repellents provide an effective method of protecting individuals from arthropods (Gupta and Rutledge 1994). In this study 6 essential oils were tested to evaluate their repellent activity against the chigger, *Leptotrombidium imphalum* Vercammen-Grandjean and Langston. A rapid and economic *in vitro* procedure which requires only 5 min and a small number of chiggers was used to determine the median effective doses. The results showed that clove oil was significantly more effective than others with ED50 of 53.2 µg followed

¹ Courter and Rhodes, Historical Notes on Horseradish, Economic Botany, Volyme 23pp.156-164, 1969

² Li and Kushad, Glucosinolate Content and Myrosinase Activity in Horseradish, J. Agric. Food Chem., Vol. 52, No. 23, 2004

³ <http://www.aces.uiuc.edu/news/stories/news3066.html>

by vetiver oil (<4.0 mg), orange oil (6.31 mg), oil of *Zingiber cassumunar* Roxb. (6.40 mg) and turmeric oil (32.6 mg) whereas oil of *Boesenbergia rotunda* (Linn.) Mansf. expressed slight efficiency only.

INHIBITORY ACTIVITY OF FIVE TROPICAL PLANTS IN MALAYSIA AGAINST MEDICALLY IMPORTANT MICROORGANISMS

Yeh Siang L., Soo P.Y., Teo S. R., Teoh Sit K.S.N.W.

UTAR, Malaysia, sitnw@mail.utar.edu.my

The development and spread of microbial resistance has necessitated a search for novel antimicrobial agents for treatment of infectious diseases. Thus, the present study was carried out to evaluate the potential antibacterial activity of five tropical plants against medically important bacteria (Gram positive: *Bacillus cereus* and *Staphylococcus aureus*; Gram negative: *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) using a colorimetric broth microdilution method with resazurin as a growth indicator. The crude ethanolic extract and its various fractions (hexane, chloroform, ethyl acetate, butanol and water) of each plant were evaluated at concentrations ranging from 5 to 0.04 mg/mL using two-fold serial dilution. All the extracts and fractions tested were found to have percent activity of 40% except the ethyl acetate fraction of *Artocarpus integer* fruits (20%), chloroform fraction and aqueous fraction of *Euphorbia heterophylla* stem (both 0%). The lowest minimal inhibitory concentration (0.04 mg/mL, average of two replicates) was given by the ethyl acetate fraction of *Manihot esculenta* leaves against *B. cereus* and *P. aeruginosa*. In contrast, the highest total activity was recorded on the crude ethanolic extract of *Citrus grandis* leaves against *P. aeruginosa* (171.9 mL/g). Among the bacteria studied, *E. coli* and *S. aureus* were the least susceptible bacteria, with the bacterial susceptibility index (BSI) of 36.7%. In addition, this study also demonstrated the limitation of using resazurin in natural product research, as auto-reduction of this indicator occurred in some of the fractions of *Spondias cytherea* fruits.

GENETIC AND PHYTOCHEMICAL VARIATION IN *PLANTAGO MAJOR*

Zubair M.

Swedish University of Agricultural Sciences, Sweden, muhammad.zubair@ltj.slu.se

Leaves of the common weed *Plantago major* (common plantain) have been used as a wound healing remedy in almost all parts of the world, and in the treatment of a number of other diseases in folk medicine. The biological activities of *P. major* leaves are wound healing, anti-inflammatory, analgesic, antioxidant, weakly antibiotic, immuno-modulating and anti-ulcerogenic. The aim of study is to develop: 1. *Plantago major*, well-known from folk medicine, into a modern medicinal herb, 2. Optimize organic cultivation procedures to produce leaf extracts for use in a superior, biologically active wound dressing. DNA analyses (RAPD) will be conducted for determining genetic variation among populations and for checking homogeneity in offspring from

selected mother plants. Chemical analyses (HPLC and mass chromatography) will be performed in order to study different polyphenolic compounds (flavonoids, phenylpropanoid glycosides and iridoids) that are likely to be involved in the wound-healing ability of *P. major*. Greenhouse cultivation experiments will be undertaken to check the effects of different levels of light supplementation, heating and fertilization.

THEME 2

CONSERVATION, CULTIVATION AND SUSTAINABLE USE

ACCUMULATION OF BIOLOGICALLY ACTIVE COMPOUNDS IN ELEUTHERO (*ELEUTHEROCOCCUS SENTICOSUS* /RUPR. ET MAXIM. / MAXIM) GROWN IN POLAND

Baczek K., Weglarz Z., Przyby J.I.

Department of Vegetable and Medicinal Plants, Warsaw University, Poland,
katarzyna_baczek@sggw.pl

Eleuthero (Siberian ginseng) is a slender shrub, usually 2-3 m high. It is native to northeastern Asia. Underground parts of this plant (rhizomes with roots) are used as a medicinal raw material and are classified as a drug with adaptogenic activity. Eleutherosides are regarded as main active compounds of eleuthero. Taking into consideration the interest of the Polish herbal industry in standardized eleuthero raw material, in the Department of Vegetable and Medicinal Plants of Warsaw University of Life Sciences – SGGW the studies on cultivation and raw material quality of eleuthero were undertaken. In the experiment, two-, three- and four-year-old plants grown under reduced light accessibility were investigated. In late autumn rhizomes with roots and bark from woody stems were harvested. The content of eleutherosides B and E and phenolic acids in the raw materials were determined by HPLC. The total content of eleutherosides B and E in the dried rhizomes with roots exceeded considerably the content of these compounds required by the British Pharmacopoeia (80 mg/100 g). In the raw material of two-, three- and four-year-old plants it amounted to 104.7, 167.4 and 292.4 mg/100 g, respectively. The content of eleutherosides in the bark of woody stems was almost twice as high as the content of these compounds in the rhizomes with roots. Both rhizomes with roots and bark of woody stems were characterised by high content of phenolic acids, especially chlorogenic acid (849.8 and 421.0 mg/100g, respectively).

THE EFFECT OF FERTILIZERS ON GROWTH, CONCENTRATION OF NAPHTHOQUINONES AND ANTIMYCOBACTERIAL ACTIVITY OF *EUCLEA NATALENSIS* (EBENACEAE)

Johanna Bapela, Marion Meyer, Elsa Du Toit, Namrita Lall

University of Pretoria, South Africa, s22320688@tuks.co.za

The effect of Nitrogen: Phosphorus: Potassium (NPK) (2:1:2 (44)) fertilizers on growth, content of bioactive naphthoquinones (shinanolone, 7-methyljuglone, diospyrin, isodiospyrin and neodiospyrin) and antimycobacterial activity was investigated in seedlings of *Euclea natalensis* grown under shade and in field conditions. Seedlings were subjected to three differential fertilization regimes (Treatment 1 at 10 g/l, Treatment 2 at 20 g/l and Treatment 3 at 40 g/l). Naphthoquinones were quantified using High Performance Liquid Chromatography (HPLC). The bioactivity of root extracts from seedlings was tested against *Mycobacterium smegmatis* and *M. tuberculosis*. Treatment 2 enhanced the vegetative performance of seedlings grown under shadehouse conditions ($P < 0.001$). A significant correlation was found between the concentration of shinanolone ($P < 0.01$), isodiospyrin ($P < 0.5$) and neodiospyrin ($P < 0.5$) with fertilization in seedlings grown under field conditions. Treatment 2 increased the content of neodiospyrin ($P < 0.5$) in seedlings under shadehouse conditions. Extracts acquired from field-grown seedlings were more active against strains of *M. smegmatis* with a lowest MIC value of 0.78 mg/ml. Extracts from the Control group and Treatment 1, which had less applied fertilizers, were more active against strains of *M. tuberculosis* with MIC value of 10 µg/ml. The study indicated that subjecting seedlings to controlled environments coupled with higher levels of fertilizers could have a negative impact on the antimycobacterial activity of *E. natalensis*.

MEDICINAL PLANTS OF COLD ARID TRANS-HIMALAYA AS SOURCES OF HEALTH PRODUCTS AND ITS SUSTAINABLE UTILIZATION

Chaurasia O.P., Singh S.B., Ballabh B., Kumar P.

Field Research Laboratory, India, opchaurasia@indiatimes.com

Plants have always been a source of food, medicine and other necessities of life since time immemorial. Ladakh and Lahaul-Spiti regions of Trans Himalayas are endowed with highly peculiar climate and topographical conditions which have provided a wide range of medicinal, aromatic and other important plants. Even today medicinal plants represent the only source of natural drugs for majority of world's population. The people of Ladakh and Lahaul-Spiti have their own medical system called "Amchi system of medicine" (Tibetan Medicine) and the practitioners are called Amchis (Superior of all). The widely used medicinal plants from High altitude regions are *Aconitum heterophyllum*, *Dactylorhiza hatagirea*, *Ephedra gerardiana*, *Hippophae rhamnoides*, *Inula racemosa*, *Podophyllum hexandrum*, *Rheum webbianum*, *Rhodiola imbricata*, etc. Field Research Laboratory has carried out extensive ethnobotanical survey of the regions and it was observed that medicinal plants were quite useful against high altitude maladies faced by lowlanders viz. stress, fatigue, loss of appetite, loss of memory, UV radiation, sleep disturbance, frostbite, etc. FRL has developed certain herbal health products using high altitude medicinal plants to improve the working efficiency of lowlanders deployed in the high altitude cold desert region of India. A Multivitamins Herbal Beverage and Herbal tea have been formulated using high altitude medicinal plants and carried out its biochemical profile, patented and commercialized. The cultivation practices of selected medicinal plants have been standardized for their commercial cultivation, conservation and sustainable utilization.

ENVIRONMENTAL AND PHYSIOLOGICAL FACTORS AFFECTING *IN VITRO* BULBLET FORMATION OF *EUCOMIS ZAMBESICA*

Cheesman L., Finnie J., Van Staden J.

University of KwaZulu-Natal, South Africa, 203502173@ukzn.ac.za
University of KwaZulu-Natal, South Africa, finnie@nu.ac.za, University of KwaZulu-Natal, South Africa, rcpgd@ukzn.ac.za

Eucomis L Hér. is a genus of 11 species that fall within the Hyacinthaceae family. *Eucomis* species are used in southern African traditional medicine for the treatment of various ailments, in particular, pain and inflammation. *Eucomis zambesiaca* Baker is a related species to *Eucomis autumnalis* which is widely used in South Africa. It is a summer-blooming bulbous geophyte occurring from northern South Africa to Malawi. Micropropagation is a useful technique for rapid clonal multiplication of plant material which could potentially yield useful secondary metabolites as well as alleviate the pressure on the wild plant populations. Experiments were carried out to determine optimal growth conditions for bulblet production. Different carbohydrates such as fructose, sucrose and glucose were tested at concentrations of 3, 6, 9 and 12%. Plant growth regulators (GA3, IAA, IBA, NAA, BA, zeatin, iP and others) were tested at concentrations of 1, 2 and 5 mgL⁻¹. Maximum number of bulblets per explant was obtained at 20°C, with an average of three bulbs per leaf explant. The average bulblet mass was 52 mg, which was significantly higher than bulblets formed at other tested temperatures. Explants at 25°C grew more rapidly but fewer bulblets formed. A protocol for mass bulblet production has been established and plants were successfully acclimatized.

MOLECULAR BASIS OF CANAVANINE SYNTHESIS IN ORGAN CULTURES OF *LESSERTIA FRUTESCENS***Colling J., Jens Kossmann J., Makunga N.**

Stellenbosch University, South Africa
jcolling@sun.ac.za

Lessertia frutescens, formerly known as *Sutherlandia frutescens*, has a long-standing history of use as an ethnomedicinal plant for various ailments and diseases in the Western Cape (South Africa). Phytochemical analysis indicates the presence of a complex mixture of compounds including triterpenoid glycosides, flavonoids, amino acids and small amounts of saponins. L-canavanine, asparagine, GABA, L-arginine and pinitol are key components of many legumes including *Lessertia*. L-Canavanine is thought to largely contribute to the health promoting effects of this plant. In the Western Cape, this plant is reputed to be useful in retarding cancerous growths and is thus locally known as 'kankerbos' (cancer-bush). The efficacy of *Lessertia* as a chemotherapeutic agent remains largely inconclusive. Even so, a biotechnological approach using organ cultures has been a useful tool to study the molecular basis of canavanine synthesis in our laboratories. Fifteen transgenic hairy root clones were generated through *Agrobacterium* transformation using hypocotyls explants and these established readily in both liquid and solid culture. However, four putative transformed clones exhibited typical hairy root characteristics. Transformation was examined via PCR amplification with a 600 bp fragment confirming presence of rol C as the transgene. Concomitant to this, a vigorous plantlet regeneration system using nodal explants with rooting occurring spontaneously has been

developed. Metabolic profiling and molecular biological analysis of hairy root and in vitro shoot cultures has indicated that canavanine synthesis may be manipulated by adjusting components of the culture micro-environment.

THE EFFECTS OF BUD SIZES AND BENZYLADENINE ON IN VITRO PROPAGATION OF *CURCUMA AERUGINOSA* ROXB.H.

Srisulak Dheeranupattana, Jiraporn Palee, Araya Jatisatienr

Faculty of Science, Chiang Mai University, Thailand scisdhrn@chiangmai.ac.th

Curcuma aeruginosa Roxb.H is one of the most valuable oriental herbs. Its rhizome extract has been used as antifungal agent and reported to have anticarcinogenic activity in animals. Natural propagation by rhizome is very poor and can not be done in winter because of its dormancy. The objective of this study was to investigate the effects of bud sizes and benzyladenine (BA) on *in vitro* propagation of *C. aeruginosa*. Buds in various sizes were cultured on Murashige and Skoog (MS) media under 1,500 lux of light intensity at 25°C for 4 weeks. It was found that 3.0 – 4.0 cm buds provided 66.67% survival, produced 100% shoots and gave the highest numbers of 2 shoots per explant. To determine the effect of BA, 3.0 – 4.0 cm buds, was also cultured on MS media supplemented with 0, 1, 2 and 3 mg/l of BA under the same condition for 4 weeks. The MS medium with 1 mg/l BA was found to induce 100% shoot formation with an average of 2.27 shoots per explant and induced the highest root formation of 72.73 %. Acknowledgements: This research was supported by Faculty of Science, Chiang Mai University, Thailand.

SUSTAINABLE WILD COLLECTION: REQUIREMENTS FOR RESOURCE MANAGEMENT AND RESOURCE USE FOR NATURAL INGREDIENTS

Duerbeck K.

Klaus Duerbeck Consulting, Germany, k.duerbeck@duerbeck.de

Following-up on the implementation of the WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants (WHO 2003), organic certification of wild collection and the International Standard for Sustainable Collection of Medicinal and Aromatic Plants (ISSC-MAP) focus on ecological aspects of GACP and the need for thorough but cost-effective resource assessments and the determination of sustainable yields. Social Accountability and Fair Trade have become important indicators to select business partners in a global market place. There are a number of approaches and label schemes available; however, they are not applicable for most wild collection products in a global market. A newly developed FairWild Standard (www.fairwild.org), which was set up by SIPPO and FairWild Foundation, allows collectors, workers and companies in the wild collection trade to jointly work on truly sustainable production and to receive a fair price for their goods. A small premium price shall be paid for certified products, which allows for social community projects in the collection areas. For all these processes, Standard Operating Procedures (SOPs) are developed. These SOPs for botanical identification, identification of collection areas and access rights, and resource

assessments are complemented with SOPs in the form of a Collectors' Manual and Plant Monographs, which are to provide guidance during the collection, followed by an SOP for Post Harvest dealing with the procedures in the collection stations. The final goal of the process documentation is to prepare for proper documentation for GMP, GACP and the above mentioned standards.

TISSUE CULTURE OF SOME IMPORTANT MEDICINAL PLANTS IN THE SUDAN

Sayadat El-Tigani

Botany Department, Faculty of Science, University of Khartoum, ,Khartoum,Sudan

Callus cultures of *Calotropis procera*, *Solenostemma argel*, *Senna alata* L. and *Hibiscus sabdariffa* were initiated on Murashige and Skoog media. The media were supplemented with indole butyric acid, naphthalene acetic acid and 2,4-D and [6-benzylaminopurine (BAP) or kinetin]. Explants from cotyledonary leaves and hypocotyl tissues were used. *C.procera* showed a high potentiality for callus initiation when 10 mg/l IBA and 12.5 mg/l BAP were used. The callus developed a large number of roots. No shoot primordials were observed. 2,4-D at 2 mg/l combined with 0.5 mg/l kinetin enhanced callus initiation while 10 mg/l NAA with 12.5 mg/l BAP had slowed callus initiation. The callus initiated from *S.argel* explants showed a lower growth rate when compared with that of *C.procera* tissues grown on MS basal media supplemented with 10 mg/l IBA and 12.5 mg/l BAP. *S.alata* has shown a high potentiality for callus cultures in vitro. The pigmentation of the callus changed with age. Ovule cultures of *Hibiscus sabdariffa* were initiated on MS media supplemented with 10 mg/l IBA and 12.5 mg/l BA. Initially the haploid callus showed a very slow growth rate. After establishment, the callus has doubled its size in 6 weeks period. Highly organized root-like structures and extensively lignified tracheids were observed in *C.procera* and *S.argel* callus tissues. Less organization was observed in *S.alata* callus tissues. Most of the extracted tissues seem to produce and accumulate the compounds present in the parent plants. Alkaloids, cardinolides and flavonoids were detected in *C.procera* and *S.argel* callus tissues. *S.alata* callus cultures contained high levels of anthraquinones.

EFFECT OF DIFFERENT PLANT DENSITY ON QUANTITY YIELD OF CHICORY (*CICHORIUM INTYBUS* L.) IN DIFFERENT WATER CONDITIONS

Hossein Aliabadi Farahani, Alireza Valadabadi and Mahdi Taheri Asghari

Islamic Azad University of Takestan branch, 10 km Quzwin-Zanjan highway, Azad University of Takestan, Faculty of Agriculture, No. 1 Iran

This experiment was conducted at Iran in 2006. Field experiment was carried out by a split plot design with 4 replications. The factors which studied were according irrigation with 75, 150 and 225 mm water evaporation from evaporation pan and plant density with 6, 9, 12 and 15 plant m⁻². The results showed that irrigation according significant effect on biological yield, number of lateral branch, number of leaf, number of pod in main stem, number of pod in plant, number of pod in lateral branch and weight dry matter of leaf and stem ($\alpha=1\%$), root length ($\alpha=5\%$) and root diameter and highest plant were not significantly affected due to water deficit. Means compression showed that highest biological yield, number of lateral branch, highest plant, number of leaf, root length and diameter, number of pod in main stem, number of pod in

plant and weight dry matter of leaf and stem were achieved under irrigation according of 75 mm water evaporation. Also plant density significant effect on all chicory characteristics ($\alpha=1\%$) and means comparison showed that highest upon characteristics were achieved under 15 plant m^{-2} and highest root length, number of pod in lateral branch, number of lateral branch, number of pod in main stem, number of leaf and number of pod in plant were achieved under 6 plant m^{-2} . The results this experiment showed that drought stress decreased quantity yield and morphological characteristics of chicory sorely.

Key words: chicory, plant density, biological yield and different water conditions.

EFFECT OF PLANT DENSITY ON OIL YIELD OF CASTOR (*RICINUS COMMUNIS* L) IN APPLICATION OF NITROGEN LEVELS CONDITIONS

Hossein Aliabadi Farahani and Behrooz Aref

Islamic Azad University of Takestan branch, 10 km Quzwin-Zanjan highway, Azad University of Takestan, Faculty of Agriculture, No. 1 Iran

This experiment was conducted at Qazvin, Iran in 2005. Field experiment was carried out by a split plot design with 4 replications. The factors which studied were nitrogen levels 0, 40, 80 and 120 $kg\ ha^{-1}$ and plant density with 2.5, 4 and 5.5 plant m^{-2} . The results showed that nitrogen application significant effect on seed yield, oil yield and biological yield ($\alpha=1\%$), seed thousand weight and oil percent ($\alpha=5\%$). Means comparison showed that highest seed yield, biological yield and oil yield were achieved under application of 80 $kg\ ha^{-1}$ nitrogen application, highest seed thousand weight was achieved under 120 $kg\ ha^{-1}$ nitrogen application and highest oil percent was achieved under 40 $kg\ ha^{-1}$ nitrogen application. Also plant density significant effect on seed yield, oil yield and biological yield ($\alpha=1\%$), seed thousand weight and oil percent ($\alpha=5\%$). Means comparison showed that highest seed yield, biological yield and oil yield were achieved under 5.5 plant m^{-2} , highest seed thousand was achieved weight under 4 plant m^{-2} and highest oil percent was achieved under 2.5 plant m^{-2} . Interaction of nitrogen application and plant density significant effects on oil yield and oil percent ($\alpha=1\%$), seed yield, seed thousand weight and biological yield ($\alpha=5\%$). The results of this experiment showed that oil percent decreased under increase of nitrogen application and plant density but oil yield increased in conditions that this can have very important for achieve to maximum oil yield of castor.

Key words: Castor, nitrogen, plant density and oil yield

EFFECTS OF NITROGEN ON OIL YIELD AND ITS COMPONENT OF CALENDULA (*CALENDULA OFFICINALIS* L) IN DROUGHT STRESS CONDITIONS

Hossein Aliabadi Farahani, Alireza Valadabadi and Navid Rahmani

Islamic Azad University of Takestan branch, 10 km Quzwin-Zanjan highway, Azad University of Takestan, Faculty of Agriculture, No. 1 Iran

This experiment was conducted at Iran Research Institute of Forest and Rangelands in 2006. Field experiment was carried out by a split plot design with 4 replications. The factors which studied were irrigation according with 50, 100 and 150 mm water evaporation from evaporation pan and 0, 35, 70 and 105 $kg\ ha^{-1}$ nitrogen. The results showed that drought stress significant effect on oil yield, seed yield, oil percent, seed thousand weight and seeds of head

($\alpha=1\%$). Means comparison showed that highest oil yield, seeds of head, seed thousand weight and seed yield with 505.3 kg ha^{-1} , 25.67 mm, 31 seeds, 15.18 g and 3044 kg ha^{-1} were achieved under irrigation according 50 mm evaporation and highest oil percent with 24.15 percent was achieved under irrigation according 150 mm evaporation. Also nitrogen significant effect on oil yield, seed yield, oil percent, seed thousand weight and seeds of head ($\alpha=1\%$). Means comparison showed that highest oil yield, seed thousand weight, seed yield and seeds of head with 377.4 kg ha^{-1} , 12.66 g, 1998 kg ha^{-1} , 23.96 mm and 29.25 seeds were achieved under application of 105 kg ha^{-1} nitrogen and highest oil percent with 22.16 percent was achieved under application of 70 kg ha^{-1} nitrogen. Drought stress and application of 60 kg ha^{-1} nitrogen were increased oil percent but oil yield of calendula decreased in these conditions.

Key words: Calendula, drought stress, nitrogen and oil yield.

EX SITU STUDY ON CHEMICAL VARIABILITY OF WORMWOOD (*ARTEMISIA ABSINTHIUM* L.)

Geszprych A., Weglarz Z., Kosakowska O.

Department of Vegetable and Medicinal Plants, Warsaw Univers, Poland,

anna_geszprych@sggw.pl

Wormwood (*Artemisia absinthium* L.) is a perennial growing wild in Poland. The herb of this plant is used both in the phytopharmaceutical and food industry as well as in organic agriculture. The application of wormwood extracts depends on their chemical composition, especially the content of bitter and volatile compounds, including thujones regarded as neurotoxins. There are reports indicating intraspecific variability of this species. In our study fourteen populations of wormwood from natural sites in Poland and from four European botanic gardens were compared in respect of the content of biologically active compounds in herb. Plants were grown at the experimental field of the Department of Vegetable and Medicinal Plants of Warsaw University of Life Sciences – SGGW. Herb was harvested in the second year of plant vegetation at the beginning of blooming period. Content of essential oil in the raw material was determined by hydrodistillation method and composition of essential oil by GC. Content of flavonoids and phenolic acids as well as bitterness value were also determined, according to Polish Pharmacopoeia VI (2002). The populations differed considerably in the content of all investigated compounds: essential oil (0.21-0.85%), flavonoids (0.08-0.27%), phenolic acids (0.12-0.25%), and in bitterness value (34,000-83,000). Regarding essential oil composition, the populations represented different chemotypes, with sabinyl acetate, chrysanthenyl acetate, (Z)-myroxide or beta-thujone as a dominant constituent. Only two populations were characterised by a distinct content of thujones in essential oil.

IMPACT OF SYSTEMATIC RESEARCHES ON AROMATIC GRASSES ON THE ECONOMIC UPLIFTMENT OF RURAL MASSES IN THE GANGETIC TRACT OF WEST BENGAL, INDIA

Gosh M.

Bejoy Narayan Mahaviadyalaya, India, mohan49gh@yahoo.com

The aromatic grasses have provided diversification of crop pattern in agricultural sector and are gaining acceptance to the farmers and traders. There is ample scope for its research and development to make the plants as more profitable cash crops. This study attempts to introduce and cultivate three economically important aromatic grasses viz: lemon grass (*Cymbopogon flexuosus*), citronella (*Cymbopogon winterianus*) and palmarosa (*Cymbopogon martini*) in the rich soil of the gangetic tract of West Bengal, India in the context of exploiting them as non-traditional cash crops for reclamation of waste land and upliftment of rural economy. The plants were subjected to various experimental conditions in the area to investigate the role of different agronomical, physical and chemical factors in the growth and development of the plants and synthesis of essential oils. In another attempt, the author showed the feasibility of cultivating the plants for commercial purpose analyzing the economics of cultivation. The study clearly revealed the significant role of different external factors viz. agronomical, physical and chemical as well as growth and developmental physiology in the quantity and quality of essential oils. The study also established the morphological and biochemical indexing for the synthesis of essential oils. The plant lemon grass is best suited in the area under reference. The author tried to disseminate the fruits of research to the local farmers by organizing training, seminars and workshops sponsored by the University Grants Commission, Government of India to cultivate the plants in their fallow land for the economic upliftment of rural masses.

THE RESPONSE OF QUALITATIVE AND QUANTITATIVE CHARACTERISTICS OF GERMAN CHAMOMILE (*MATRICARIA RECUTITA L*) TO THE FERTILIZING IMPACTS OF VERMICOMPOST

Parastoo Hoseinzadeh, Abdollah Hatamzadeh, Ershad Tavakol

Department of Agriculture, Guilan University, Rasht, Guilan, Iran, p.hoseinzadeh@gmail.com

Department of Agriculture, Guilan University, Rasht, Guilan, Iran, Hatamzadeh@guilan.ac.ir

Department of Agriculture, Tehran University, Tehran, Iran, E.Tavakol@gmail.com

Chamomile (*Matricaria recutita L.*) is an important medicinal plant and its multi-therapeutic, cosmetic and nutritional values have been established through years of traditional and scientific application. In order to examine the impact of organic agriculture on the plant's qualitative and quantitative characteristics along with producing a healthy plant as a source of human health products, while considering preservation of natural resources, this experiment was designed to determine the influence of vermicompost derived from cattle manure and crop residues without the application of chemical fertilizers on the essential oil and phenolic compounds of chamomile in temperate climate condition. The results represented that different vermicompost levels (0, 4, 8, 12 & 16 t ha⁻¹) improved and enhanced plant growth, plant height, number of shoots and flower accumulative dry matter through increasing fertilization levels. As a result, the implementation of 16 t ha⁻¹ had the most significant effect on the mentioned characteristics. Oil yield was increased by comparing to the control (non-fertilized). Analysis of chamomile volatile constituents by GC and GC-MS, identified fifty-one components, which represented 97.5% of the oil. In addition, the highest contents of main constituents, including bisabolol oxide A (35.5%) and B (8.6%), β -farnesene (4.1%), and chamazulene (17.5%), were achieved from the implementation of 16 t ha⁻¹ treatment.

HPLC analysis of chamomile extract confirmed apigenin 7-O-glucoside as the major oil constituent in all treatments. Although the 16 t ha⁻¹ treatment had the highest impact on evaluated characteristics, there were no significant differences between 12 t ha⁻¹ and 16 t ha⁻¹ treatments.

[1]Mann, C. and E.J. Staba. 1986. The chemistry, pharmacology, and commercial formulations of chamomile, p. 235-280. In: L.E. Craker and J.E. Simon (eds). Herbs, Spices, and Medicinal Plants. Recent Advances in Botany, Horticulture, and Pharmacology. Food Products Press. 1, p: 235-280.

DIOSPYROS LOTUS: MICROPROPAGATION AND ANTIOXIDANT ACTIVITY

Hwang S., Ock Boo H., Suk Na M., Pak S.

Department of Biology, College of Natural Sciences, Chonnam, South Korea, jimhwang@jnu.ac.kr, Department of Biology, College of Natural Sciences, Chosun U, South Korea, Department of Beauty Science, Kwangju Womens' University, Gw, South Korea, School of Biomedical Sciences, Charles Sturt University, Bat, Australia

Diospyros lotus is a deciduous tree that is native to the Middle East and south Asia, especially China, Korea, and Japan. It bears edible small fruits in autumn and leaves are used widely in folk medicine for their medicinal properties. A protocol for the date plum (*Diospyros lotus*) was developed. For shoot proliferation, *in vitro* nodal and apical explants were cultured on various media containing 0.5-2.0 mg/l BA, kinetin, or TDZ. WPM medium supplemented with 0.5 mg/l BA displayed the highest proliferation rate (4-7 shoots per explant). The rooting was optimized using MS medium supplemented with 0.5 mg/l IBA with 3-5 roots after 2 months of culture. The rooted plantlets were successfully acclimatized and transferred to potting mix with 92% survival. Antioxidant properties of *in vivo* grown tissues were evaluated using various assays. The levels of total phenolics and flavonoids of the leaves extracts were also determined. The alcohol extract of date plum leaves had good phenolic (445.6 mg/g) and flavonoid (260.9 mg/g) contents, and showed the strong antioxidant and free-radical scavenging activity (scavenged 92.1% of DPPH at 250 µg/ml). Leaf extracts had higher activities of anti-oxidant-related enzymes including superoxide dismutase (SOD; 20.5 U/mg protein), catalase (CAT; 4.9 U/mg protein), and ascorbate peroxidase (APX; 105.8 U/mg protein) than mature seed extracts (SOD, 2.1 U/mg protein; CAT, 74.5 U/mg protein; APX, 3.7 U/mg protein). These results establish the antioxidant potential of date plum, which could be used as natural antioxidant source.

SCREENING OF TOTAL FLAVONOID CONTENTS IN LOCAL ACCESSIONS OF *CENTELLA ASIATICA* AS MEDICINAL HERB

S. S. Moghaddam, H. Jaafar and R. Ibrahim

Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Centella asiatica is a small herbaceous annual plant²Agrotechnology & Biosciences Division, Malaysian Nuclear Agency, Bangi 43000 Kajang, Selangor, Malaysia of the family *Apiaceae*, locally known as Pegaga in Malaysia. It is one of the most common medicinal plants that have been widely used as health food and in cosmetic industries. Flavonoid compounds, especially quercetin have anti-tumor activity and it is clear that *Centella asiatica* has higher flavonols (quercetin) and flavanols (naringin) content compared with vegetables and fruits. A study was conducted to analyse the total flavonoid contents (TFC) in 8 local accessions. Significant differences were observed both in their morphological characters and TFC, detected

at a range of 760.36µg/ml to 1512.27µg/ml dry weight of whole plant. Highest TFC was recorded in A2 (1512.27µg/ml) and lowest in A3 (760.36µg/ml). Analysis on different parts of the plant revealed that leaf tissue contains the highest flavonoid, followed by petiole and root. Total flavonoid content in the leaf was highest in A2 (1309µg/ml) and lowest in A3 (519µg/ml). However, A3 also recorded the highest TFC in the petiole (194µg/ml). Time to harvest was observed to be an important factor for accumulation of TFC in the plant and S3 (6 weeks after planting) was recorded to be the best for all accessions. Information on morphological, chemical and growth characteristics of these accessions can be further applied in micropropagation and mutation breeding programmes to select for desirable traits of fast growth, high biomass and flavonoid content for commercialisation.

SEGREGATION PATTERNS OF CDNA-BASED MICROSATELLITE AND RAPD-PCR MARKERS IN F2 INDIVIDUALS OF *CAPSICUM ANNUUM* L.

Ayşe Gul Ince, Mehmet Karaca, A. Naci Onus

Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, aince@akdeniz.edu.tr

Recently in our laboratories we obtained surprising results in which our analyses indicated that some of the cDNA-based microsatellite primer pairs amplified polymorphic markers within different tissue, organ and development stage specific cDNA libraries of *Capsicum annuum* L. Demre Sivrisi (DS) variety. Based on these findings, we hypothesized whether tissue organ specific microsatellite could be used in mapping studies. Forty F2 individual progenies were generated from a cross between DS and *Capsicum annuum* L. PM687 lines with aims to compare segregation patterns and polymorphism information contents of DNA markers using random amplified polymorphic DNA (RAPD-PCR), as a control, and cDNA based microsatellite polymorphism. A total of 30 expressed sequence tags (EST) based primer pairs and 30 RAPD-PCR primers were used in the amplification of genomic DNAs of the parental lines and the individual F2 progenies of DS X PM687. Segregation patterns, inheritance of the loci [dominant (3:1) or co-dominant (1:2:1)] were determined using the chi-square analyses. Analyses indicated that 6 RAPD-PCR and 4 EST microsatellite markers were polymorphic between the parental lines and among the F2 individuals. Polymorphism information contents of cDNA-based microsatellite markers were higher than RAPD-PCR markers. One of the 6 RAPD-PCR primers produced co-dominant loci while cDNA-based microsatellite primer pairs produced 3 co-dominant loci. Results indicated that cDNA-based microsatellite markers do segregate according to Mendelian law as that of the conventional genomic DNA based microsatellites indicating that cDNA-based microsatellites can be safely used in genetic mapping and diversity studies.

AN *IN SILICO* ANALYSIS OF GINGER EXPRESSED SEQUENCE TAGS FOR MICROSATELLITES

Ayşe Gul Ince, Mehmet Karaca, Ahmet Naci Onus

Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, aince@akdeniz.edu.tr

Ginger (*Zingiber officinale* Roscoe) is a perennial plant in the family Zingiberaceae and rhizomes of ginger have been used in folk medicine and cooking. However, ginger production is seriously affected by several diseases. DNA based technologies can be applied for the identification of wild germplasm or commercially important plants with fungal and bacterial

resistance genes. There exists limited research on the characterization of ginger germplasm. Microsatellite or simple sequence repeat (SSR) is very powerful marker for germplasm identification and plant genetic mapping studies. In this paper we used a total of 138,139 expressed sequence tags (ESTs) constructed from tissues representing various tissue types (leaves, roots, rhizomes) to identify new microsatellite primer pairs and investigate the distribution and frequency of microsatellites in *Zingiber officinale* Roscoe ESTs obtained from leaf, root and rhizome libraries. Based on the obtained results we concluded that different tissues of *Zingiber officinale* Roscoe ESTs possessed different amounts of microsatellites and microsatellite compositions indicating the distribution of microsatellites among the tissues or organs was not random differing from plant and animal repeats found in genomic microsatellites. Microsatellite compositions of the root ESTs were quite different from those leaf and rhizomes. In this study we also designed several dozens microsatellite primer pairs which could be used to detect the marker-QTL or comparative mapping and homologous gene cloning for ginger breeding studies. Publicly available *Zingiber officinale* Roscoe ESTs are valuable for in silico gene expression studies including for the development of microsatellite and single nucleotide polymorphism (SNP) PCR-based molecular markers.

DAMD-PCR: A MINISATELLITE DIRECTED POLYMERASE CHAIN REACTION FOR SIDERITIS GENOTYPING STUDIES

Ayşe Gul Ince, Mehmet Karaca, Kenan Turgut, Ahmet Naci Onus

Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, aince@akdeniz.edu.tr

The genus *Sideritis* comprises approximately 150 species distributed chiefly in the Mediterranean region. *Sideritis* is represented by 53 taxa and 39 of which endemic to Turkey. Sixteen of these species naturally grow in Antalya, Turkey and six of them are endemic to this region in which this study was conducted. Some species of *Sideritis* are used as tea, flavouring agents and for medicinal purposes in many countries. The use of DNA markers will enhance *Sideritis* genetic studies for sustainable cultivation, preservation and germplasm analysis. DNA markers are valuable for plant breeders and taxonomist since they offer an effective way to identify genotypes. Repetitive DNA sequences such as microsatellites, minisatellites, midisatellites and satellites can be of great value in assessing a high level of polymorphism as they distributed throughout the genomes analyzed so far. In the present study a polymerase chain reaction (PCR) method, known as the directed amplification of minisatellite region DNA (DAMD-PCR), an inexpensive, PCR-based method was evaluated to amplify minisatellite DNA regions of six different species of *Sideritis* using 12 minisatellite core specific primers which obtained from different organisms. Analyses indicated that the use of minisatellite core specific primers showed higher polymorphism information content values ranging from 55.6% to 91.4%. Higher level of polymorphism information content values and repeatability of the PCR banding patterns among the *Sideritis* species used in the present study clearly showed that *Sideritis* species contain a wide range of genetic diversity and DAMD-PCR technique is a powerful technique for *Sideritis* genotyping studies.

COMPARISON OF STS-PCR, RAPD-PCR, DAMD-PCR FOR *PHOLOMIS*, *MELISSA*, *ORIGANUM*, *ROSMARINUS* AND *SIDERITIS* SPECIES

Ayşe Gul Ince, Mehmet Karaca, Ahmet Naci Onus, Ibrahim Baktir

Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, aince@akdeniz.edu.tr
Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, mkaraca@akdeniz.edu.tr,
Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, onus@akdeniz.edu.tr,
Akdeniz University, Faculty of Agriculture, 07058-Antalya, Turkey, ibaktir@akdeniz.edu.tr

A large number of medicinal and aromatic plant species naturally grown in the Mediterranean basin of Turkey are endemic to this region. Genetic studies of these species; however, fall behind the other cultivated species. The last three decades witnessed the development of tremendous number of molecular approaches such as DNA techniques to be used in germplasm evaluation and plant genetic analysis. DNA markers have been used in plant molecular ecology, taxonomy and plant breeding studies. The use of these DNA techniques are extremely important for those difficult plant species with limited reliable taxonomic characters. Random amplified polymorphic DNA (RAPD-PCR), directed amplification of minisatellite region DNA (DAMD-PCR) and Sequence Tagged Sites (STS-PCR) were used to determine which of these markers provide repeatable and higher level polymorphism information content values on five different genus; *Phlomis*, *Melissa*, *Origanum*, *Rosmarinus* and *Sideritis*. Genomic, chloroplast and mitochondrial DNAs of two species of *Sideritis*, *Origanum*, and one species of *Phlomis*, *Melissa*, *Rosmarinus* and *Teacrium* were extracted and analyzed. Studies clearly indicated that STS-PCR markers of mitochondria and chloroplast were less polymorphic based on their PIC values. On the other hand the PIC values of DAMD-PCR and RAPD techniques were equally similar. The numbers of amplicons were lower in DAMD-PCR than that of the RAPD-PCR. In conclusion the results of the present study indicated that DAMD-PCR and RAPD-PCR were equally effective in differentiation of the species of Lamiaceae. Although STS showed lower polymorphism, they are extremely valuable in identification of naturally occurring inter-specific hybrids

EFFECT OF DIFFERENT LEVELS OF N-FERTILIZER ON THE GROWTH, DEVELOPMENT, ESSENTIAL OIL CONTENT AND COMPOSITIONS OF *AGASTACHE FOENICULUM KUNTZE*

Maryam Kaboudani, Reza Omidbaigi

Tarbiat Modares University, Iran, maryamk110@yahoo.com
Tarbiat Modares University, Iran, romidbaigi@yahoo.com

Anise Hyssop (*Agastache foeniculum* Kuntze.) is a half-hard perennial herb native to the plains of North America, which belongs to the Lamiaceae family. It has a pleasant anise-like aroma. To study the effect of nitrogen fertilizer on growth, development, essential oil content and compositions of Anise Hyssop, this research was carried out on randomized complete blocks design with three replications for every treatment. The effect of nitrogen fertilizer in 5 levels (0, 50, 100, 150 and 200 kg/ha) were studied. The essential oil was obtained by hydro distillation from the herb at full flowering stage. The results show that different levels of Nitrogen fertilizer had significant effect on the height of plant, essential oil content and compositions of Anise Hyssop. The results show that N-fertilizers had no significant effect on the number of branches per plant. The plants received 100 and 150 kg/ha nitrogen fertilizer had the highest essential oil content (2.88 and 2.83% respectively). Methyl chavicol was its main component (95.5%) and obtained from the plants were received 200 kg/ha nitrogen fertilizer. p-cymene (3.3%), caryophyllene (0.5%) and sabinene (0.1%) was the other components. It could be concluded that the suitable nitrogen fertilizer to obtain the maximum quantity and quality of active substances of *Agastache foeniculum* is recommended 150 kg/ha.

MESSAGE IN A BOTTLE: IN VITRO UPREGULATION OF BIOACTIVES IN TWO AROMATIC MEDICINAL CAPE PLANTS

Nokwanda Makunga, Johannes van Staden

Stellenbosch University, South Africa, makunga@sun.ac.za

The aromatic plants, *Salvia africana-lutea* and *Pelargonium sidoides* are highly valued as medicinal plants in South Africa. *P. sidoides* is becoming increasingly sought after for its coumarin compounds which are reputed as being effective against bronchial infections. This particular species is now threatened by uncontrolled harvesting for both local herbal markets and international export. *Salvia africana-lutea* is endemic to coastal regions of the Cape Floristic Region which are fast becoming more urbanized. At present, there are no alternative sources of the active compounds of both *Salvia africana-lutea* and *Pelargonium sidoides*. Through biotechnological manipulation, both transgenic and non-transgenic organ cultures are available to study secondary metabolism. Metabolite profiling through GCMS, LCMS and NMR indicated interesting changes in the chemical footprints. Micropropagation does not adversely alter the complexity of essential oils produced by these plants. All *S. africana-lutea* methanol:dichloromethane (1:1 v/v) extracts were effective against *Fusarium verticillioides* (a plant pathogen) (MIC range of 20 µg/ml to 320 µg/ml). Extracts analysed via GCMS exhibited a larger subset of new compounds which were not normally present in non-transgenic cultures with an increased production of caffeic acid derivatives, fatty acids and plant sterols. Differences of rol transgene integration account for intra- and inter-clonal chemical variation in *P. sidoides* clones as each rol gene has an individual impact on the hairy root traits. Multiple copy insertions may be responsible for changes in metabolic flux. Many of the putatively identified compounds from both species are useful for the pharmaceutical and agrochemical industries

CULTIVATION OF MEDICINAL PLANTS IN ZIMBABWE: A STRATEGY FOR MEETING LOCAL TRADITIONAL MEDICINE NEEDS

Alfred Maroyi

Botany Department, Rhodes University, PO Box 94, Grahamstown 6140, South Africa

Zimbabwe is endowed with a wide variety of natural resources which include potent medicinal products. Medicinal plants are traded nationally and constitute a recognized source of local traditional medicine needs and income generating activity. A combination of participatory and formal research methods were used to collect data on the ease of cultivation, management, medicinal value and importance of *Warburgia salutaris* and *Moringa oleifera*, the two widely used medicinal plants in Zimbabwe. Local people were found to combine a variety of livelihood strategies guided by perceived cost-benefit relationships with the two species. A crucial transition has taken place in the country where destructive farming practices have been replaced by new and improved cultivation methods which raise *Warburgia* and *Moringa* without destroying natural systems on which agriculture ultimately depends. Preliminary economic analysis strongly shows that expanded *Warburgia* production, particularly in the context of small holder agriculture is clearly economically attractive and conducive to improving rural incomes and livelihoods [1].

Cultivation of *Warburgia* and *Moringa* by small- scale farmers in Zimbabwe appears to be a short-term way of providing medicinal material. Medium and long-term sustainable management of the two species should involve their cultivation as commercial crops. This would diversify farming operations, enhancing the income generation potential of the land, thus helping to provide jobs and alleviate poverty in Zimbabwe and creating new opportunities for local farmers, as both urban and rural populations in Zimbabwe are highly dependent on medicinal plants for their primary health care.

[1] Veeman, T.: Muranga returns: the economics of producing a rare medicinal species reintroduced in south-eastern Zimbabwe. In: Campbell, B.M. and Luckert, M.K. (eds.). Uncovering the hidden harvest: valuation methods for woodland and forest resources. Earthscan, London, UK, 146-148 (2002).

SCREENING OF TOTAL FLAVONOID CONTENTS IN LOCAL ACCESSIONS OF *CENTELLA ASIATICA* AS MEDICINAL HERB

Sina Moghaddam, Hawa Jaafar, Rusli Ibrahim

University Putra Malaysia, Malaysia,
Malaysian Nuclear Agency, Malaysia, sina_moghaddam2003@yahoo.com

Centella asiatica is a small herbaceous annual plant of the family Apiaceae, locally known as Pegaga in Malaysia. Common names include *Gotu Kola*, Asiatic Pennywort, *Antanan*, and *Brahmi*. It is one of the most common medicinal plants used by diverse ancient cultures and has been widely used as health food and in cosmetic industries. Flavonoid compounds, especially quercetin have anti-tumor activity and it is clear that *Centella asiatica* has higher flavonols (quercetin) and flavanols (naringin) content compared with vegetables and fruits. A study was conducted to analyse the total flavonoid contents (TFC) in 8 local accessions. Significant differences were observed both in their morphological characters and total flavonoid contents (TFC), detected at a range of 760.36µg/ml to 1512.27µg/ml dry weight of whole plant. Highest TFC was recorded in A2 (1512.27µg/ml) and lowest in A3 (760.36µg/ml). Analysis on different parts of the plant also revealed that leaf tissue contains the highest flavonoid, followed by petiole and root. Total flavonoid content in the leaf was highest in A2 (1309µg/ml) and lowest in A3 (519µg/ml). However, A3 also recorded the highest TFC in the petiole (194µg/ml). Time to harvest was observed to be an important factor for accumulation of TFC in the plant and S3 (6 weeks after planting) was recorded to be the best for all accessions. Information on morphological, chemical and growth characteristics of these accessions can be further applied in micropropagation and mutation breeding programmes to select for desirable traits of fast growth, high biomass and flavonoid content for commercialisation.

GERMINATION OF *SCLEROCARYA BIRREA* SEEDS: A TREE OF MEDICINAL IMPORTANCE

Mack Moyo, Manoj Kulkarni, Jeff Finnie, Johannes Van Staden

University of Kwazulu-Natal, South Africa, 205526161@ukzn.ac.za

Sclerocarya birrea (marula) is used in many African countries for a number of ailments. The bark decoctions are administered as enemas in treating malaria and diarrhoea. Some tribes

use bark for treating fevers, stomach complaints and ulcers, and roots for sore eyes. Fresh leaves are chewed for indigestion and infusions or decoctions are drunk for diabetes. The fruit has a delicious pulp with high vitamin C. Though some attempts are being made to domesticate this high-value indigenous plant, there is limited information on the aspects of seed germination. The objective of this study was to investigate the role of light, temperature, cold stratification and dry storage on the germination of *S. birrea*. Temperatures between 25-35°C favoured germination of opercula-removed seeds under continuous dark conditions. White light inhibited the germination of opercula-removed seeds. The inhibitory effect of light was reversed when seeds were transferred to dark conditions. This photoinhibition effect on opercula-removed seeds was lost after 12 months of storage at room temperature. Cold stratification (5°C) for 14 days significantly improved germination (66%) as compared to non-stratified seeds (32%). Seeds of *S. birrea* can be considered as orthodox as they tolerated desiccation without significant loss of viability. Both intact and opercula-removed seeds readily imbibe water suggesting physiological rather than physical dormancy. This study indicates that after-ripening and seed maturation are critical factors for germination of *S. birrea*.

EFFECTS OF TYROSINE AND PLANT GROWTH REGULATORS ON GROWTH AND DEVELOPMENT OF *GLORIOSA SUPERBA* LINN., A MEDICINAL PLANT, IN VITRO

Nooduan Muangsan

Khon Kaen University, Thailand, nooduan@kku.ac.th

The study of effects of tyrosine and plant growth regulators on rhizome cultures was carried out on modified Murashige Skoog medium. The sterile rhizomes were cut and cultured on MS medium, supplemented with 1 mg/l 2,4-D, 1 mg/l BA and various concentrations of tyrosine under light and dark condition. The experimental data showed that the highest percentage of callus formation was on the medium with 50 mg/l tyrosine, in the dark condition (100%) and in the light condition (85%). The average fresh weight of callus cultured in the dark for 16 weeks was 6.25 g. Shoot induction was performed using the combination of NAA and BA for 36 weeks. The experimental data revealed that the shoot regeneration from callus was 100% on the medium with 4 mg/l BA and 4 mg/l NAA. Comparison among various concentrations of BA and NAA showed that shoot formation was 83% and 50% on the medium containing 4 mg/l BA and 4 mg/l kinetin, respectively. The chromosome number from the root tip obtained from the tissue culture was $2n = 22$.

Acknowledgements: Dept. of Biology, Khon Kaen University provided funding and all participants in this project.

SEED GERMINATION OF *ALEPIDEA* SPECIES – HEAVILY TRADED AND THREATENED MEDICINAL PLANTS IN SOUTH AFRICA

Rofhiwa Mulaudzi, Manoj Kulkarni, Jeff Finnie, Johannes Van Staden

Research Centre for Plant Growth and Development, South Africa
207527112@ukzn.ac.za

The rhizomes of *A. amatymbica* are widely used as a remedy for cold, cough, asthma,

influenza, diarrhoea and abdominal cramp. Due to the scarcity and increasing demand for this species, the rhizomes of *Alepidea natalensis* are being uprooted as an alternative plant source. As the reproductive biology of both the species is not well defined, conditions and treatments required for optimum germination of these species were studied. Seeds were exposed to various physical factors such as temperature, light conditions, cold stratification and storage. Smoke-water, the newly identified smoke-compound butenolide (3-methyl-2H-furo [2, 3-c] pyran-2-one) and plant growth regulators were also tested. *Alepidea amatymbica* and *A. natalensis* achieved the highest seed germination (72% and 80% respectively) at 25 °C under a 16:8 h light/dark condition with a mean germination time (MGT) of 18 and 12 days respectively. A temperature of 35 °C was detrimental to both species as none of the seeds germinated. Cold stratification (5°C) significantly improved the percentage germination of both species (> 90%) compared to non-stratified (control) at 25 °C under a 16:8 h light/dark regime. Five months storage of *A. natalensis* seeds at room temperature (25±2°C) showed maximum germination (99%) with a MGT of 9 days. Smoke-water-treated seeds of *A. amatymbica* significantly enhanced germination from 72 to 91%. Plant growth regulators were less effective in increasing the germination of both the species. Findings of this study can be used to propagate these medicinal species to meet the current demand. Acknowledgements: Canon Collins Trust and NRF

COMMERCIALISATION OF TRADITIONAL HERBAL PRODUCTS IN CENTRAL PROVINCE OF KENYA: CHALLENGES IN PROVIDING SUSTAINABLE SUPPLIES

Grace N. Njoroge

Jomo Kenyatta University of Agriculture and Technology
Botany Department, P.O. Box 62000, Nairobi, Kenya
gnjoroge@fsc.jkuat.ac.ke/shallomnjeri@hotmail.com

In many African countries, medicinal bio-resources are seen as a vehicle to promote sustainable livelihoods of the communities through strategic and sustainable utilization. Globally, medicinal plants trade is estimated at US \$ 800 million and is increasing at the rate of 7% per year. As the trade grows, species involved and their conservation status remain largely undocumented. An ethnobotanical study was undertaken in rural Central Province of Kenya determine important medicinal plants. Frequency of use among respondents and number of ailments each species manages were used as indices of importance. A market study was further undertaken to reveal critically important species for the trade as well as species in trade that are scarce. In total 89 species were identified as important in rural areas while only 8 species are most traded in urban centers. These results indicate that *Aloe secundiflora*, *Senna didymobotrya* and *Vernonia lasiopus* are not only the most frequently utilized but are also the ones managing most ailments and hence form the most significant medicinal plant species for the rural users. Most traded species include: *Aloe secundiflora* Engl, *Urtica Massaica* Mildbr, *Prunus africana* (Hook.f.) Kalkm, *Melia volkensii* and *Strychnos henningsii*. This study also reveals that *Aloe secundiflora*, *Prunus Africana* and *Strychnos henningsii* are the most needed in the market but in short supply. The supply chain in the rural and urban parts include plant species already known to be rare such as *Carissa edulis* and *Synadenium compactum* among others.

Key words: medicinal plants trade, conservation, sustainable livelihoods.

GENOTOXIC EFFECTS OF AQUATIC EXTRACT OF ENDEMIC PLANT *ONOSMA STELLULATA* WALDST. & KIT. (BORAGINACEAE)

A Redzic¹, S Redzic², N. Sejdic²

¹Dep. of Biology and Human Genetics, Medical faculty University of Sarajevo, Cekalusa 90, 71 000 Sarajevo, Bosnia and Herzegovina ²Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od Bosne St., 71 000 Sarajevo, Bosnia and Herzegovina

The species of genus *Onosma* contain pyrrolizidine alkaloids [1] and use in treatment of lung cancer and other respiratory diseases in some regions of W. Balkan [2, 3].

Goal of this study is to research genotoxic effects of over ground and underground parts of species *Onosma stellulata* in vitro conditions. Floral material for this research was collected during May and June of 2006 in area of Herzegovina (W. Balkan). Floral samples were dried and exposed to double mazzeration in accordance with Ph.Yug. IV in order to receive extract that was used in making 0.05% and 0.10% solution. Evaluation of geno-toxic effect was conducted by using *Allium*-test, along with observation of chromosomes abnormalities (partition spindle, irregular phases, multi-polarity, stagnating chromosomes, C-mitosis, and others). Effects were observed after 4, 8, 12 and 24-hours treatments. Testing of differences between determined (experimental group) and expected (control group) was conducted by using X² test. Extracts of both concentrations, both over and under ground parts and root, are causing geno-toxic effects in mitosis at meristematic cells of onion. Genotoxic effect is in co-relation with length of treatment and solution concentration. Aquatic extract of over ground part showed distinguished geno-toxic effect after 4-hours treatment (mitotic index was 2, 79%, and in control was 9, 18%). Determined was also statistically significant difference for 0, 10% extract of over ground part (p≤0.05). Higher degree of geno-toxic effect was determined for root extract. Genotoxic effect is reflected at changes in structure of chromosomes (conglutination, spirality), and cito-toxic reaction and certain variations in cell cycle.

References:1. El-Shazly A et al. (2003). Biochemical Systematics and Ecology, 31(5): 477-485.
2. Redzic SS (2007) Coll. Antropol. 31: 869-890 3. Redzic S (2007) Planta Med. 73: 1013.

MODELS OF SUSTAINABLE USE OF WILD MEDICAL PLANTS IN W. BALKAN (SE EUROPE)**Sulejman Redzic, Senka Barudanovic, Hazim Basic**

Department of Biology, Faculty of Sciences Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina, sredzic@pmf.unsa.ba, Centre of Ecology & Natural Resources, Faculty of Sciences, Univ. Sa, Bosnia and Herzegovina, sbarudanovic@gmail.com, Department of Biology, Faculty of Mechanical Engineering, 6 Vilsonovo, Bosnia and Herzegovina

Due to the past extensive and random use of wild medicinal plants in this territory - including the western Balkan countries (SE Europe) - those plants are significantly endangered. Many of them even appeared on "red lists of endangered floras" [1]. In order to preserve natural gene-funds of medicinal plants, it is necessary to set methods and models that would result in sustainable models for their use. Sustainability would be achieved by exploiting the biomass from natural populations with respect of the biological and ecological characteristics of given plant species and if possible according to the market requirements. This approach includes the determination of variables that could be used as a basis for the development of mathematical and statistical models in order to reach "function of sustainability". Sustainable function implies

results of two key variables, production of biomass of given medicinal plants and amount of biomass that is used or is exploited in natural populations. The graphic model for that function is diversified and it largely depends on the plant species, the used part of the plant, the vegetation season, the ecological circumstances in which the given plant is being developed, the form of picking, the total anthropogenic pressure of cutter, the pasture, the wood cutting and the global changes. This ecological statistical approach is applied to several species of medicinal plants that are intensively exploited in the Western Balkan [2, 3]. In the Mediterranean belt, it is *Salvia officinalis*, in the Mediterranean and sub-Mediterranean belt it is the species *Helichrysum italicum*, in the Mediterranean mountain belt they are the species *Satureja montana* and *Satureja subspicata*, in the mountain belt they are the species *Gentiana lutea subsp. symphyandra* and *Arctostaphylos uva-ursi*, and in the belt of the deciduous forests, it is the species *Atropa belladonna*. Research shows that the gradient of sustainable use is different in different belts. In the Mediterranean and sub-Mediterranean belt it is about 50%, in the mountain belt it is 30 %, while in the belt of forests it is up to 70 % (per km²). That means it is necessary to leave about 50 % of units of sage in free nature, approximately the same number of units of the order *Satureja*, about 70 % of units of *Gentian* and about 30 % of biomass of the species *Atropa belladonna* in order to reach the effect of sustainability. This approach is beneficial for the protection of medicinal plants and could be used as a practical instrument in reaching organic production and certification of medicinal and aromatic wild plants.

References: 1. Redzic S (2006) Proc 1st IFOAM Intern Conf Organic Wild Production, 117-141.
2. Redzic SS (2007) Coll. Antropol. 31: 869-890.
3. Redzic SJ (2006) Ecol. Food & Nutr. 45: 189-232.

PLANT PIGMENTS IN SOME MEDICAL PLANTS OF FAMILY LAMIACEAE (BOSNIA AND HERZEGOVINA, W. BALKANS)

S. Redzic, N. Sejdic, A. Palic

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od Bosne St., 71 000 Sarajevo, Bosnia and Herzegovina

Plant pigments (chlorophyll and carotenoides) are very important group of primary metabolites. Besides their role in process of photosynthesis and plant protection from extensive radiation, they have huge appliance in pharmaceutical industry, cosmetology and dietetic. Plant pigments are also given significant role in anti-oxidant activity [1, 2]. Goal of these researches is qualitative – quantitative analysis of main and side pigments in selected medical species of wild flora in BiH, including endemic species. Plant material is gathered during different seasons. It has been transported fresh to laboratories where qualitative (paper and thin layer chromatography) and quantitative (specterphotometric) analysis was taking place. Results of researches (Table 1) showed significant presence of chlorophyll a, chlorophyll b and carotenoides.

Ratio between chlorophyll a and chlorophyll b is rarely 3:1, as stated in classical literature but rather close to 3:2 and more, which makes these species even more medical and gives them higher potential anti-oxidant capacity [3].

References: 1. Redzic, S., Hodzic, N., Tuka, M. (2005) Bosn J Basic Med Sci. 5(2): 53-8.
2. Redzic, S., Tuka, M., Pajević, A..(2006) Bosn J Basic Med Sci. 6(2): 25-31.
3. Davies, K. ed. (2004) Plant pigments and their manipulation. Annual Plant Reviews, 14, 368p., Blackwell Publishing, Oxford.

Table. 1: Contents of plant pigments in selected plants of *Lamiaceae* family

Plant species	Locality (100- 2,000 m altitude)	Contents of plant pigments mg in g fresh of leaves)		
		Chlorophyll a	Chlorophyll b	Carotenoides
<i>Origanum vulgare</i> L.	Igman Mt.	4,592	4,114	1,619
<i>Origanum heracleoticum</i> L.	Mostar	5,210	4,252	1,731
<i>Satureja subspicata</i> Bartl. ex Vis.	Velez Mt.	4,750	3,870	1,670
<i>Satureja montana</i> L.	BiH coast	6,452	4,445	1,756
<i>Micromeria thymifolia</i> (Scop.) Fritsch	Sarajevo	5,780	2,100	0,890
<i>Thymus aureopunctatus</i> (Beck) K.Maly	Konjic Bjelasnica Mt.	5,723	1,124	0,679
<i>Thymus balcanus</i> Borbas	Mt.	4,678	1,670	1,620
<i>Thymus bracteosus</i> Vis. ex Benth.	Trebinje	5,120	2,020	1,230
<i>Acinos orontius</i> (K.Maly)Silic	Konjic	4,345	1,970	0,890
<i>Nepeta pannonica</i> L.	Visoko	5,670	2,230	1,120
<i>Salvia officinalis</i> L.	Ljubuški	5,100	1,800	1,234

PATTERNS FOR SUSTAINABLE USE OF THREATENED MEDICINAL PLANTS WITH HIGH MARKET REQUIREMENTS

Sulejman Redzic, Sabina Trakic, Hazim Basic

Department of Biology, Faculty of Sciences, University Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina, redzic@pmf.unsa.ba, Centre of Ecology & Natural Resources, Faculty of Sciences, University Sarajevo, Bosnia and Herzegovina, sbarudanovic@gmail.com, Department of Biology, Faculty of Mechanical Engineering, 6 Vilsonovo, Bosnia and Herzegovina

Due to the over-exploitation from their natural habitats many medicinal and aromatic plants are extremely threatened in the Western Balkan, but also in South-Eastern Europe. Some of them are included in both, the regional and the European Red list. However, a strong need for some of them exists on the markets, while an adequate replacement for diverse pharmaceuticals and dietetics contained within them has not been found yet [1]. Such is also the case with the widely known and in ethnobotany frequently applied species *Gentiana lutea* ssp. *symphyandra* Murbeck that grows only in the high mountains of the Western Balkan [2]. This plant has been used to treat stomach and heart diseases. Its root (*Gentianae radix*) is becoming an increasingly demanded drug on the modern pharmaceutical market [3]. Therefore, gentian root is being exploited without any surveillance or control which leads to the drastic decrease in number of its natural populations in most of the investigated mountains. In some mountains this species has almost completely disappeared. Despite numerous conventions (such as CITES, CBD, Bern Convention), over 65 t of dried gentian root has been illegally exported to the European market from Bosnia and Herzegovina (which is the centre of its distribution) in the last year, which is an ongoing trend. This root reaches the European market as *Taraxaci radix*. In order to achieve a

sustainable protection for this economically and pharmacologically threatened medicinal plant, it is necessary to increase the level of responsibility of not only local exporters but also of foreign importers. Both parties have to be forced to respect international documents on traffic and trade with threatened wild species. In order to protect this and other species, artificial propagation under in situ conditions has been initiated, whereby geological foundation (dolomites) and ratio between calcium and magnesium in the ground are very important. Extraordinary results in this respect have been achieved. Propagation attempts under *ex situ* conditions have not given the expected results. Directed and planned propagation of *Gentiana lutea* in natural conditions could contribute to its efficient protection, but could also help meet the requirements of the market which has to be subdued to stricter controls.

References: 1. Redzic S (2006) Proc 1st IFOAM Intern Conf Organic Wild Production, 117-141.
2. Redzic SS (2007) Coll. Antropol. 31: 869-890.
3. Redzic S (2007) Planta Medica 73:1013.

IN VITRO MULTIPLICATION OF *BRUNSVIGIA UNDULATA* FROM TWIN SCALE EXPLANTS

Laura Rice, Jeff Finnie, Johannes Van Staden

University of KwaZulu-Natal, South Africa, 203507134@ukzn.ac.za

Brunsvigia undulata F.M.Leight. (Amaryllidaceae) is one of the many South African medicinal plants threatened by over-collection and in particular habitat loss. Micropropagation is a technique that can potentially provide a means of supplying plants for the traditional medicinal plant trade. This would decrease the pressure on wild populations and help to conserve this species. For in vitro propagation, twin scales provided a successful explant for bulblet formation. The effect of photoperiod and different combinations of plant growth regulators (e.g. naphthalene-acetic acid [NAA] and benzyladenine [BA]) on bulblet formation was investigated. Further experiments to observe the effects of photoperiod, temperature, activated charcoal, cytokinin concentrations, media type and explant size, on bulblet multiplication were also carried out. Bulblets formed readily from twin scale explants on all but one of the plant growth regulator treatments. Cytokinin concentration did not significantly effect the in vitro multiplication of half-bulblets, while whole bulblets readily multiplied to produce up to 13 bulblets from one parent bulblet.

CHAMOMILE RESEARCH, DEVELOPMENT AND PRODUCTION - PAST, NOWADAYS, FUTURE

Ivan Salamon

Department of Ecology, FHNS, Presov University, Presov, Slovakia, salamon@fhpv.unipo.sk

The flowers of Chamomile, *Matricaria recutita* L., are known in the Pharmacopeia as Flos Matricariae. A large number of ingredients give the flowers their therapeutic versatility. Here are some of those effects and the ingredients responsible. Among those anti-inflammatory properties are *l*-*l*-bisabolol and its oxides, matricine, chamazulene, apigenine. Muscle relaxants include apigenine, *l*-*l*-bisabolol and the spiroether group. Bisabolol acts against the formation of ulcers. Those with antibacterial action include *l*-*l*-bisabolol, spiroether and coumarins. Among those used to treat fungal infection are *l*-*l*-bisabolol and spiroether. Nowadays chamomile research and

development is reaching the very high level and it is carried out from different aspects in various parts of our globe. The present ranges of varieties (with the very high content of *l*-*l*-bisabolol), cultivation and processing of chamomile with high quality of essential oil have the possibility to fulfill the demand for this raw-material all over the world. The new trends of chamomile research, development and production are presented in the fields: biology & biodiversity, chemistry, ecology & environment, agriculture & mechanization, pharmacy & pharmacology and veterinary medicine. Nowadays chamomile research and development is reaching the very high level and it is carried out from different aspects in various parts of our globe. The present ranges of varieties, cultivation and processing of chamomile with high quality of essential oil have the possibility to fulfill the demand for this raw-material all over the world.

Acknowledgements: Slovak Ministry of Education: AV 4/0104/06

IN VITRO PROPAGATION OF SIDA RHOMBIFOLIA, A COMMONLY USED MEDICINAL PLANT: EFFECT OF AUXINS AND CYTOKININS

Pranajit Sarker, Md. Kamrul Hasan, Fardous Mohammad, Safiul Azam, Mohammed, Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
pranajit_bge@yahoo.com

The present investigation involves *in vitro* regeneration of *Sida rhombifolia*, a medicinal plant commonly used for the treatment of fever and as a diuretic in Bangladesh. Newly sprouted shoot tips and nodes were used as explants and cultured onto Murashige and Skoog (MS) medium (pH 5.8) supplemented with different concentrations and combinations of auxins and cytokinins. Sprouting of buds and the best proliferation frequency (100%) was observed on MS medium fortified with 6-benzylaminopurine (BAP) at 1 mg/l and the micro-shoots proliferated upto 3.97 cm within four weeks. Combination of BAP (1 mg/l) and indole-3-acetic acid (0.5 mg/l) showed relatively better effect on shoot elongation. Profuse and adventitious bud multiplication (2-3 multiple shoots) was observed as a cluster from the synergistic effect of BAP (1.5 mg/l) and kinetin (0.5 mg/l). Micro-shoots were separated from the clump and incubated for root induction in ½ MS medium containing indole-3-butyric acid (IBA). Root was induced within three weeks on ½ MS medium enriched with IBA at 1 mg/l. The *in vitro* raised plantlets were hardened for seven days through gradual exposure to sunlight. For proper acclimatization, the plantlets were transplanted into polybags containing garden soil, cow dung and sand (1:1:1). Establishment of plantlets was observed with 60% survival rate. No morphological variations were observed through the passage of this clonal propagation. This protocol developed for *in vitro* regeneration can play a role for commercial propagation and conservation of this medicinal plant.

IN VITRO REGENERATION OF COCCINIA GRANDIS (L.) VOIGT., AN INDIGENOUS MEDICINAL PLANT OF BANGLADESH

Pranajit Sarker, F.M. Safiul, Azam, Rownak, Jahan, Mohammed, Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
pranajit_bge@yahoo.com, Faculty of Life Sciences, University of Development Alternat, Bangladesh, shojibbiotech@yahoo.com, Faculty of Life Sciences, University of Development Alternat, Bangladesh, atarakeet@yahoo.com, Faculty of Life Sciences, University of Development Alternat, Bangladesh, rahamatm@hotmail.com

The present study was carried out for in vitro propagation of *Coccinia grandis* (Curcubitaceae), a widely used medicinal wild creeper herb of Bangladesh. The plant extract is known to possess anti-hyperglycemic, antioxidant and hypolipidemic properties. Juvenile shoot tips were collected as explants from field grown mature climbers. After surface sterilization with 0.1 % HgCl₂ for 7 minutes, tips and nodes were cultured onto Murashige and Skoog (MS) medium containing different hormonal concentrations. Regeneration (71%) was observed when cultured in only MS medium. Highest percentage of response (81.25%) was obtained on MS medium fortified with 6-benzylaminopurine (BAP) at 1.5 mg/l and average shoot length was 6.45 cm. Synergism was observed through the combination of BAP and kinetin (Kn). An average of 2-3 multiple shoots formed when explants were cultured in MS medium having BAP at 1.5 mg/l and Kn at 1.0 mg/l. Shoot elongation (10.14 cm) was achieved with MS medium containing BAP (1.5 mg/l) + alpha-naphthalene acetic acid (NAA) at 0.5 mg/l. Successful root initiation was observed when the shootlets were inoculated in ½ MS medium enriched with Indole-3-butyric acid (1.5 mg/l) + NAA (0.5 mg/l). The in vitro raised plantlets were established in earthen pot media after acclimatization and more than 70 % survival rate was observed after three weeks in field observations. This fruitful regeneration protocol would enable large scale and commercial cultivation of this indigenous medicinal herb and as well as conservation.

ARTEMISIA ANNUA: SELECTION FOR HIGH ARTEMISININ YIELDING LINES FOR PRODUCTION IN SUB-SAHARAN AFRICA

J.E. Simon, C. Park, Q.L. Wu, M. Diatta, B. Diouf, J. Asante-Dartey, D. Acquaye, C. Quansah, R. Akromah, C. Gibrain, K. Gbewonyo, J. F.S. Ferreira, P. Melillo de Magalhães, D. Shen, P. Langenhoven and R. Juliani

New Use Agriculture and Natural Plant Products Program, Rutgers University, New Brunswick, NJ 08901, USA ASNAPP-Senegal and AES, Dakar, Senegal, ASNAPP-Ghana, Kwame Nkrumah University of Science and Technology (KNUST), BIONEXX, Antananarivo, Madagascar, BioResources International-Ghana USDA-ARS/AFSRC, 1224 Airport Rd., Beaver, WV, USA, CPQBA-UNICAMP, C.P.6171 CEP: 13081-970 Campinas-SP Brasil, ASNAPP-Rwanda; 10ASNAPP-South Africa

Malaria continues to be a major infectious disease facing those living in tropical regions of the world. Use of artemisinin-combination treatments (ACT) as the first line treatment for multi-drug resistant *falciparum* malaria relies on derivatives of artemisinin, a natural product from *Artemisia annua*. Major commercial production regions include China, Vietnam and East Africa (Tanzania/Kenya). While several challenges face new producing regions, our work focuses on developing high yielding lines for sub-Sahara Africa for co-development with source countries. Our germplasm grown in several African countries appears to be non-photoperiodic, high yielding with artemisinin >1.% dry leaf wt. The parent populations from which the selections and progeny were derived came originally from UNICAMP. Highest yielding SPS were vegetatively cloned, and further selections made in the USA and Africa. Field trials established in Ghana, Madagascar, Senegal and South Africa identified lines adaptable to local conditions. Results showed that selection and testing for artemisinin in one location held relatively stable when the plants were evaluated in other regions. Generally, lines with the highest artemisinin remained high across the countries evaluated. Greatest environment impact was observed on biomass accumulation. Further selections conducted in Africa led to improvements in plant growth form, with some reaching heights of 2 m. Advanced lines field grown in sub-Sahara Africa contained

artemisinin concentrations >1.0% reaching as high as 1.7% (dry leaf wt) and appear promising for varietal co-development in Africa. No problems relative to collecting viable seeds were observed in any country, stability of seed progeny remain to be tested.

GERMPLASM PRESERVATION BY CRYOPRESERVATION: USE OF NEW BIOTECHNOLOGY TO MAINTAIN GENETIC DIVERSITY IN *LABIATAE SPECIES*

Angelika Senula, E.R.Joachim, Keller

Leibniz Institute of Plant Genetics and Crop Plant Research, Germany,
senula@ipk-gatersleben.de

Many Labiatae species are of potential interest as medicinal and aromatic plants. Therefore, micropropagation was established, which includes clonal maintenance. Furthermore, some species are endangered. *in vitro* culture and cryopreservation increasingly contribute to the germplasm maintenance in genebanks, especially of vegetatively propagated germplasm, like in mint (*Mentha* spp.). Various cryopreservation methods are used worldwide. The germplasm collection of the German Central Crop Collection maintains 148 accessions of various mint species *in vitro* using slow growth conditions at 2°C and 10°C. Cryopreservation is applied using droplet vitrification with the cryoprotectant PVS2. At present, the cryopreserved mint collection consists of 24 accessions. The average regeneration rate is 62%. Regeneration is depending on the genotype. Recently was found that endophytes play a major role in cryopreservation success. Antibiotics are applied to support survival and regeneration in critical phases after rewarming. Despite good success of cryopreservation in various mint species, this method is still not fully applicable to another Labiatae genus, *Orthosiphon*. Here, regeneration rate is 82% in controls contrasting to 4% in cryopreserved samples. *In vitro* maintenance of these plants is possible but labour-intensive because they are not storable at low temperatures. Various measurements of the target organs were performed to elucidate the background of these differences. Differential scanning calorimetry informs about the thermal transitions of tissue water critical for storability. Its results revealed that the pretreatment time must be longer comparing to that needed for mint.

DOMESTICATION OF SOME MEDICINAL TREES IN UGANDA

Torunn Stangeland

Norwegian University of Life Sciences, Norway, torunn.stangeland@umb.no

Most rural communities in developing countries rely on medicinal plants for their health care. Unfortunately, important medicinal trees are threatened by overexploitation and landuse changes. Important trees need to be conserved. This requires a good understanding of their seed and germination ecology. In this study we investigated the influence of temperature and light on germination and seedling growth of two locally threatened medicinal trees, *Hallea rubrostipulata* and *Sarcocephalus latifolius*, in order to facilitate their establishment. Both species needed light to germinate. *H. rubrostipulata* had a temperature optimum of 25°C with 79 % germination, while for *S. latifolius* the total germination after 28 days was close to 60 % at temperatures from 20 to 35°C. Seedlings of *S. latifolius* survived well at 35°C, while those of *H. rubrostipulata* died at this high temperature. Conversely seedling of *S. latifolius* died at the low

temperature of 15°C. We have chosen the Framework species method for reforestation. We raised seedlings of 38 different, mostly indigenous, trees in nursery for 6 to 12 months. The trees are a mixture of medicinal, firewood, fruit trees and trees useful for soil conservation. In the early rainy season in April 2008 we planted the same mixture of trees in three plots, each 40 x 40 m big. The trees were monitored two weeks after planting for height, crown width, root collar diameter and health. The plots are weeded three times during the rainy season, and growth is monitored once a month for one year.

ROOTING RESPONSE OF ROSEMARY AND LAVENDER CUTTINGS TO YARD TRIMMING COMPOST, MANURE AND HUSK AS SUBSTRATES IN NURSERY MEDIUM

Ershad Tavakol, Parastoo Hoseinzadeh

Mahabghodss, Iran, E.Tavakol@gmail.com
Yekom, Iran, P.Hoseinzadeh@Gmail.com

Propagation from cuttings is the commonest vegetative method. The rooting medium conditions can affect the rooting percentage and speed significantly. Most biological processes are speeded up when temperatures are higher and so raising compost temperature usually increases the rapidity with which cuttings root. Bottom heat systems are prepared to stimulate rooting speed and diminish the rooting period of the transplanted cuttings. Considering the energy consumption in bottom heat systems, implementation of yard trimming compost, manure and husk as the substrate of medium in order to simulate the effects of bottom heat systems and surveying its impacts on rooting of two medicinal plants (*Rosmarinus officinalis* L. and *Lavandula officinalis* L.), were evaluated. The cuttings were collected and transplanted during February 2008. Rooting percentage, rooting speed, root dry matter and leaf number were assessed. Results presented higher rooting percentage and leaf number as well as rooting speed and root dry matter in cuttings which were treated with yard trimming compost substrate compared to other treatments and control. Husk substrate had the least effect on the mentioned characteristics.

DETECTION OF SOMACLONAL VARIATIONS AMONG MICROPROPAGATED POPULATION OF SOME IMPORTANT MEDICINAL PLANTS

Sharad Tiwari, D. S. Jatav

Division of Plant Tissue Culture and Transgenics, Biotechnol, India, stiwari@lycos.com

Demand of healthy medicinal planting material in India and international drug market is increasing continually, while, many important medicinal plants have been declared endangered due to less seed viability, low germination percent and vegetative propagation also susceptible for disease. Plant tissue culture has come up as an alternative of poor natural germination of seed and vegetative propagation which provides desired number genetically uniform pest free plants in a short span of time. One of the major problems of in vitro plant cultivation is the high incidence of somaclonal variation. The RAPD analysis was carried out to assess genetic relationship among micropropagated population the basis of scoring of band exhibited by amplified products using NTSYS_pc. RAPD and ISSR marker were used to assess clonal fidelity

of medicinal plants viz., *Chlorophytum borivilianum*, *Bacopa monnieri*, *Rauwolfia serpentina* and *Oroxylum indicum*. Initial screening for somaclonal variation on the basis of morphological traits was carried out starting second month of culture. Among six to eighth month old cultures <0.3% phenotypic variation were observed, however, upon ISSR and RAPD analysis such variants proved to be genetically true to the parental type. During 9-12 months, ISSR and RAPD analysis revealed 10 to 24 percent dissimilarity to the parental type among ~0.5% observed phenotypic variations.

ESSENTIAL OIL AND CARVACROL CONTENT OF OREGANO (*ORIGANUM SPP.*) SPECIES GROWN IN WILD AND CULTIVATED CONDITIONS OF ANTALYA

Kenan Turgut, Saadet Tugrul Ay

Akdeniz University, Antalya, Turkey, kturgut@akdeniz.edu.tr
BATEM, Antalya, Turkey

The objective of this study was to investigate variability of essential oil and carvacrol content of five oregano species (*O. saccatum*, *O. solymicum*, *O. majorana*, *O. onites* and *O. vulgare subsp. hirtum*) collected from the natural flora of Antalya, Turkey. Samples were collected from early inflorescences, inflorescences and late inflorescences stages of 5 species grown in wild and cultivated conditions. Essential oils were obtained via steam distillation using Clevenger-type apparatus and they were analyzed with Gas Chromatography (GC). The highest average essential oil rate was obtained from inflorescences stage of wild *O. majorana* (8.2%) and followed by late inflorescences stage of wild *O. majorana* (6.1%) and inflorescences stage of cultivated *O. onites* (5.7%). Although cultivated *O. majorana* plants revealed lower essential oil rate than wild *O. majorana*, in the other species cultivated plants gave higher essential oil percentage. Eighteen compounds were identified in the essential oil of oregano species under studied conditions. The highest rate of carvacrol was found at the early inflorescences stage of cultivated *O. majorana* (53.90%) and followed by inflorescences stage of wild *O. majorana* (52.40%) and early inflorescences stage of wild *O. majorana* (50.10%). In general, rate of carvacrol in the wild and cultivated *O. majorana*, *O. solymicum*, *O. onites* and *O. vulgare subsp. hirtum* plants were appeared to be quite similar. However, percentage of carvacrol was found to be higher in the cultivated *O. saccatum* plants (average 40%) than wild *O. saccatum* plants (average 30%).

Acknowledgements: Funded by Akdeniz University Scientific Research Project Unit

AGRONOMIC CHARACTERISTICS OF OREGANO (*ORIGANUM SPP.*) SPECIES COMMONLY FOUND IN THE FLORA OF ANTALYA

Kenan Turgut, Saadet Tugrul Ay

Akdeniz University, Antalya, Turkey, kturgut@akdeniz.edu.tr
BATEM, Antalya, Turkey

In this study, some agronomic traits of *Origanum* species (*O. saccatum*, *O. solymicum*, *O. majorana*, *O. onites* and *O. vulgare subsp. hirtum*) collected from the natural flora of Antalya were investigated. The *Origanum* plants were propagated by stem cuttings from collection garden and planted at the experimental field with three replicates "Randomized Complete Plot

Design". In this research, green and dry herbage yield (kg/da), plant height (cm), branch number (number/plant) and essential oil yield (kg/da) of species were investigated. Air-dried aerial parts of the plants were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The percentage yields of the oils were calculated on a dry weight basis. The research results showed that *O. saccatum* gave the highest mean values on plant height (64.8 cm), branch number (56.8), green and dry herbage yield (834.3 and 382.0 kg/da, respectively) and essential oil yield (11.5 l/da). In terms of essential oil yield, *O. onites* (7.6 l/da) and *O. majorana* (7.1 l/da) statistically made up second group and followed by *O. vulgare* subsp. *hirtum* (2.4 l/da) and *O. solymicum* (0.7 l/da). After all, *O. saccatum* appeared to be good candidate for breeding and cultivation studies.

Acknowledgements: Funded by Akdeniz University Scientific Research Project Unit

MICROPROPAGATION OF *HYPERICUM TRIQUETRIFOLIUM*

Akçam Oluk E., Orhan S.

Ege University, Engineering Faculty, Faculty of Science, Biology Department, Bornova, 35100 Izmir, Turkey, Ege University, Bioengineering Department, Bornova, 35100 Izmir, Turkey

Hypericum perforatum is commonly used as a medicinal plant for its anti-depressant properties. Several constituents of the plant, such as hypericin and hyperforin, seem to be important for these properties. Another *Hypericum* cultivar, *Hypericum triquetrifolium* natives to eastern Europe and the Mediterranean area is also known to carry same anti-depressant compounds. Harvesting the medicinal plants from the wild is causing loss of genetic diversity. On the other hand one of the main characteristics desired for pharmaceutical application is stable content of active compound as not in the raw material obtained from wild plants. Also some medicinal plants have very small seeds like *Hypericum triquetrifolium* can not be cultured with traditional methods. For solving this problems *in vitro* culture methods are suggested by many researchers. We aim to establish a micropropagation protocol for *Hypericum triquetrifolium*. As starting material native seed were used. After an affective germination period on water-agar, seedlings were transferred 10 different shoot multiplication medium including Murashige&Skoog (MS) basal media supplemented with different concentrations of [*N*-phenyl-Nb-(1,2,3 thidiazoly) urea], benzyladenine and indoleacetic acid. The best shoot development in explants was obtained on MS medium supplemented with 1.25 mg/L thidiazuron and supplemented with 0.5 mg/L indoleacetic acid. Root development was obtained on MS medium supplemented with IAA or hormone free medium. Micropropagated plants were transferred to greenhouse successfully. All cultures were incubated at $27 \pm 4^{\circ}\text{C}$ under a light intensity of 4000 lux, with 16 hour photoperiod.

Key words: *Hypericum triquetrifolium*, micropropagation, medicinal plant.

IN VITRO MICRO PROPAGATION OF ORNAMENTAL AND MEDICINAL PLANTS *FRITILLARIA IMPERIALIS* AND *F. PERSICA*

S. Özcan, D. Gürlek, S. Çöçü, S. Uranbey, A. İpek, C. Sancak and N. Arslan

Department of Field Crops, Faculty of Agriculture, University of Ankara, 06110, Dışkapı, Ankara, Turkey.

Fritillaria imperialis and *F. persica* belonging to Liliaceae family are important ornamental plants because of their attractive and large flowers. Bulbs of *Fritillaria* species have also been used as herbal remedies in Turkish, Japanese, Pakistani and south-east Asian folk medicines for centuries. However, *F. imperialis* and *F. persica* species are endangered species of Turkey and threatened by extinction. The natural propagation rate of most geophytes including *F. imperialis* and *F. persica* is relatively low, which often inhibits the large-scale cultivation of these plants. For *in vitro* micro propagation of these species, many explants including bulb scales and immature embryos were cultured on media containing various combinations of auxins and cytokinins using different culture protocols. Prolific shoot regeneration and bulblet formation was observed from immature embryo explants after one year of culture initiation. Regenerated bulblets were rooted and then transplanted to a potting mixture.

ACTIVITIES ON DPPH FREE RADICAL SCAVENGING AND ANTIOXIDANT ENZYMES FROM THE FRUITS OF BITTER GOURD (*MOMORDICA CHARANTIA* L.) ACCORDING TO CULTIVARS

Ock Boo H., Hwa Lee H., Won Lee J., Jin Hwang S.

Department of Biology, Chosun University, Gwangju, South Korea, swboo@hanmail.net
Department of Biology, Chosun University, Gwangju, South Korea, Institute of Health Science,
Jeonju University, Jeonju, South Korea, Department of Biology, Chonnam National University,
Gwangju, South Korea

In the Asian sub-continent bitter gourd is extensively used as food and is popular in herbal medicine. This study was carried out in order to determine the biological activities such as antioxidant activities in eight bitter gourd cultivars (Korea, China, Japan and Philippine native cultivars). Free radical scavenging activities of various cultivars of bitter gourd were the strongest in Korea native bitter gourd as RC50 102.6 ug/ml. And its activity was similar to synthetic antioxidant BHT as RC50 95.6 ug/ml. The activity of SOD and CAT were higher in Korea and Japan bitter gourds than Philippine bitter gourd. Activation of APX activity according to cultivars were significantly increased in Korea native bitter gourd (7.82 U/mg protein), Japan cultivar "Nikko" (8.55 U/mg protein), and Philippine cultivar "Galaxy" (8.17 U/mg protein). Antioxidative enzyme activities were increased in the order of SOD > APX > CAT. These results suggest that the bitter gourd had the potent biological activities, and that their activities exhibited differently depending on cultivars.

SUSTAINABLE HARVESTING OF *TERMINALIA ARJUNA* (ARJUNA) AND *LITSEA GLUTINOSA* (MAIDA) BARK IN CENTRAL INDIA

Ashok Pandey, Asim Mandal

Tropical Forest Research Institute, INDIA, akpandey10@rediffmail.com

Terminalia arjuna (Arjuna) is a well-known medicinal plant whose bark is extensively used in ayurvedic medicine, particularly as cardiac tonic. Demand for Arjuna bark, both in India and abroad has been growing rapidly for over a decade. *Litsea chinensis* (Maida) is a medium sized tree. Its bark is used to treat joint pain, fracture, sprain, arthritis, back pain, indigestion,

cough and dryness of skin. Presently the bark of Arjuna and Maida is being extracted through unscientific and destructive harvesting practices. This is the first study on development of sustainable harvesting practices of Arjuna and Maida bark. The study revealed that the regeneration of bark in young trees was faster in comparison to old trees. In Arjuna, the bark was regenerated in two years whereas in Maida it took only one year. The study also showed that the stage of bark recovery (regrowth) varied from tree to tree, age of tree, harvesting technique (e.g. blaze making) and factors like temperature, relative humidity and time of stripping influenced wound healing in Arjuna and Maida. In Arjuna, the quality of trunk bark was superior in comparison to bark of other plant parts (stem, twigs) whereas no significant difference was found in Maida. Thickness of Arjuna bark at breast height varied from 8.12 to 20.96 cm and was found to be irrespective of the age/girth of tree. The medium aged trees gave better quality of bark. The best time to harvest bark was found between December and March. The study recommends that for sustainable harvest, only $\frac{1}{4}$ or $\frac{1}{3}$ of the mature bark of total girth of the tree should be stripped by removing only outer and middle bark leaving the inner bark for regeneration from opposite quarters of the trunk. Thus sustainable bark harvesting can be done after every two years (In Arjuna) and one year (In Maida) by removing opposing quarters of trunk bark rather than girdling the trees.

EFFECTS OF ARBUSCULAR MYCORRHIZA AND PHOSPHORUS APPLICATION ON GROWTH AND COLCHICINE CONCENTRATION IN *GLORIOSA SUPERBA*.

Devendra Pandey, Rathindra Mohan, Banik

School of biochemical engineering, IT, BHU, Varanasi, India, dkpandey1974@yahoo.com

Gloriosa superba produces an array of alkaloids including colchicine, a compound of current interest in the treatment of various diseases. The tuber of *Gloriosa superba* is a rich source of colchicine which has shown anti-gout, anti-inflammatory, and antitumor activity. However, this promising compound remains expensive and *Gloriosa superba* is good source in global scale. Increase in yield of naturally occurring colchicine is an important area of investigation. The effects of inoculation by four arbuscular mycorrhizal (AM) fungi, *Glomus mossae*, *Glomus fasciculatum*, *Gigaspora margarita* and *Gigaspora gilmorei* either alone or supplemented with P-fertilizer, on colchicine concentration in *Gloriosa superba* were studied. The concentration of colchicine was determined by high-performance thin layer chromatography. The four fungi significantly increased concentration of colchicine in the herb. Although there was significant increase in concentration of colchicine in nonmycorrhizal P-fertilized plants as compared to control, the extent of the increase was less compared to mycorrhizal plants grown with or without P-fertilization. This suggests that the increase in colchicine concentration may not be entirely attributed to enhanced P-nutrition and improved growth. Among the four AM fungi *Glomus mossae* was found to be best. The study suggests a potential role of AM fungi in improving the concentration of colchicine in *Gloriosa superba* tuber.

ASSESSMENT OF THE AGRONOMIC VALUES OF GENETICALLY MODIFIED CMV-RESISTANT HOT PEPPER (*CAPSICUM ANNUUM L.*) IN KOREA

Kuen Woo Park, Jun-Hong Kim, Sung Ok Park, Dong Sik Yang

Division of Biotechnology, College of Life Sciences and Biot., Korea University, South Korea, kuenwp@korea.ac.kr, Division of Biotechnology, College of Life Sciences and Biot., Korea

University, South Korea, joonong@korea.ac.kr, Division of Biotechnology, College of Life Sciences and Biot., Korea University, South Korea, parkisland@korea.ac.kr, Division of Life Science & Natural Resources, Korea University, South Korea, dsyang@uga.edu

Hot pepper is one of the most important cash crops in Korea with an economic impact of 1.3 billion US\$ annually since it is widely used for its phytochemicals with medicinal properties such as capsaicin as well as for its spicy taste. However, the cucumber mosaic virus (CMV) has declined the production of the hot pepper in Korea. To overcome the problem, the CMV-resistant hot pepper lines (H15 and B20) were introduced, transforming the coat protein (cp) gene from the isolate of CMV into the conventional hot pepper line (P2377 and P915) using *Agrobacterium*, respectively. The agronomic values of conventional and genetically modified (GM) lines (P2377 vs H15; P915 vs B20) were assessed on the basis of 17 items for vegetative growth (e.g., plant height, plant width, stem diameter, leaf length) and 21 items for fruit development (e.g., total harvest yield, dry weight, fruit length, stalk length, fruit colour). The hot pepper lines were seeded on the 19th of April 2007 and the transplants of each line were planted on the 23rd of May at the Deokso Research Farm in the Korea University (37° 34' N/127° 14' E) and they were grown under a general cultivation system for hot pepper. The experiment was performed in a completely randomized design in 3 replications. In the vegetative growth, the plant height in two conventional and two GM lines was not different, however, a GM line, B20 showed smaller leaf length and leaf width than other lines. In the fruit development, GM lines had slightly higher total number and weight of harvested fruits than conventional lines. In dry weight (%), P2377, a conventional line was not different from H15, a GM line, however, B20, a GM line showed higher dry weight than P915, a conventional line. Further studies on medicinal and nutritional quality will be performed to compare the contents of capsaicin and vitamin C in conventional and GM hot pepper lines.

Acknowledgements: BioGreen 21 provided funding.

CHARACTERIZATION AND GENETIC IMPROVEMENT OF *ASPARAGUS RACEMOSUS* (WILLD.): AN IMPORTANT MEDICINAL PLANT

Parveen and Ashok Kumar

Division of Genetics and Tree Propagation, Forest Research Institute (Indian Council of Forestry Research & Education), Dehradun 248195, Uttarakhand, India

Asparagus racemosus (Willd.) is an important medicinal plant known as Shatawari, means who possesses more than hundred husbands. It is the main Ayurvedic medicinal tonic especially for the female, as is *Withania somnifera* for the male. It is commonly mentioned as a rasayana in the Ayurveda, the drug which promotes general well being of an individual by increasing cellular vitality or resistance. *A. racemosus* reached endangered in nature due to its over-exploitation, as the fleshy roots of the species contain saponin having therapeutic value. It is one of the 32 plant species identified as priority species for cultivation and conservation by the National Medicinal Plant Board (NMPB), India. Owing to increased demand, the species has attracted the attention for its genetic improvement, conservation and cultivation. Accordingly, germplasm has been collected from different geographical regions of India, and a scientific field trial of 20 sources has been established at the Forest Research Institute, Dehradun to evaluate comparative performance of economic traits, stability and adaptability. The significant genetic variations for different morphological traits including plant type, maturity period, growth and yield characters and size / shape of tubers was determined and the potential genotypes for higher

productivity have been identified. The morphological traits identified at nursery stage could be utilized for screening of plants with maximum tubers production at early age. As *A. racemosus* is a cross pollinated species, attempts have also been made for production of F₁ hybrids. The programme is useful for genetic conservation for realization of future breeding strategies.

FACTORS AFFECTING IN VITRO SHOOT AND MICRORRHIZOME INDUCTION OF *CURCUMA LATIFOLIA* ROSC. AND *CURCUMA COMOSA* ROXB., THAI MEDICINAL PLANTS FOR WOMEN'S HEALTH

Sompop Prathanturarug, Sureerat Lo-apirukkul, Promchit Saralamp, Thaya Jenjittikul

Department of Pharmaceutical Botany, Faculty of Pharmacy, Ma, Thailand,
pyspr@mahidol.ac.th, Department of Pharmaceutical Botany, Faculty of Pharmacy, Ma,
 Thailand, Department of Pharmaceutical Botany, Faculty of Pharmacy, Ma, Thailand
 Department of Plant Science, Faculty of Science, Mahidol Uni, Thailand

In vitro propagation methods have for the first time been developed for wan chak motluk (*Curcuma comosa* Roxb. and *Curcuma latifolia* Rosc.), which is widely used for women's health in Thai traditional medicine. The effects of plant growth regulators (TDZ, BA, and NAA) have been investigated for multiple shoot induction using undivided and divided terminal bud explants. High frequency shoot multiplications were obtained when the undivided explants of *C. latifolia* Rosc. and *C. comosa* Roxb were cultured on semi-solid Murashige and Skoog (MS) medium supplemented with 36.32 and 18.16 μ M TDZ, respectively, for 8 weeks prior to transfer to MS medium without PGR for 4 weeks. Shoot regeneration rates were 18.38 \pm 2.28 and 11.82 \pm 1.03 shoots/response explant, respectively. Rooting was spontaneous achieved. Rooted plantlets were successfully transferred to soil. Microrrhizome induction was influenced by plant species, carbohydrate source, and plant growth regulators. Liquid MS medium with 8.87M BA and 70 g/l sucrose or 17.76M BA and 50 g/l sucrose were optimal for the microrrhizome induction of *C. latifolia* Rosc and *C. comosa* Roxb respectively. After 12 weeks of culture, Microrrhizome induction rates were 4.92 \pm 0.28 and 3.36 \pm 0.44 microrrhizomes/response explant, respectively. An anatomical study demonstrated that starch accumulation in microrrhizomes increased when the concentration of sugar was elevated and duration of culture extended. The microrrhizomes were germinable under greenhouse conditions and further developed into normal plants. The established protocols will be used for the production of uniform plantlets suitable for field plantation for the herbal industry.

AN INVESTIGATION INTO THE EFFECTS OF ORGANIC FERTILISER APPLICATION ON THE CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF MEDICINAL PLANTS

G. Prinsloo, M. Motsei, J. Viljoen, M. Mofokeng and L. Phala

Agricultural Research Council – Vegetable and ornamental Plant Institute (ARC-VOPI), Private Bag X 293, Pretoria, 0001, South Africa.

The use of medicinal plants has increased significantly due to their known biological activity against common human, animal and plant pathogens. The increased use of these plants has led to some of these plants facing extinction in the wild due to a lack of cultivation to maintain sustainable populations. When plants are removed from their natural environment, changes in their chemical composition can be observed. Changes may occur due to

environmental and geographical changes that the plants are exposed to. Cultivation of these plants under irrigation with the application of fertilisers also changes the chemical composition of their active compounds. A selection of important medicinal plants were subjected to organic fertiliser treatments, which included bone meal, gypsum, and an organic nitrogen source that was applied separately and in different combinations to obtain seven treatments. The fertiliser trials were harvested for two years. For all the treatments, ethanolic water and petroleum ether extracts were prepared. Successive fractions of ethyl acetate and methanol for the petroleum ether extracts were also prepared. All these extracts were evaluated for chemical differences by TLC and HPLC analysis. Many differences were observed, but it was not clear if the changes affected the biological activity of the plants. Bioassays using three different human bacteria were performed to test the activity of the extracts. Distinct differences in the biological activity were observed with extracts from the different organic fertiliser treatments.

DESTRUCTION OF HIMALAYAN MEDICINAL AND AROMATIC PLANTS (HMAPS); A THREAT TO LOCAL LIVELIHOOD IN NEPAL HIMALAYA

K.C. POUDEL Shanti

Graduate School of International Development, Nagoya University, Furo-cho, Chikusaku,
Nagoya, Japan, poudel_shanti@yahoo.com

Nepal is a mountainous country situated in the central Himalayas. Medicinal and Aromatic Plants (MAPs) found in Nepal Himalaya is an important sources of livelihood in the mountainous communities in Nepal. However, these resources are not utilized as sustainable source of livelihood in many mountainous communities in Nepal. Previous researchers have argued that collectors' strong market orientation¹ is the main cause of the destruction of biodiversity and loss of the MAPs resources. In addition, based on field research in mountainous communities, this author identifies that low income and low production from other sources, heavy pressure only on few commercially valuable MAPs, lack of local capability and social awareness about the sustainable wild harvesting, and weak local institutions exacerbate destruction of HMAPs, which in turn hurts the livelihood of mountainous communities. Though there are various kind of valuable MAPs available and utilized for their health care need, commercial collection pressure is only on few MAPs; Jatamasee (*nardostachys grandiflora*), Katuki (*neo-picrorhiza scrophulariifloa hong*), Guchichyau (*morchella conica*) and Atis (*aconitum heterophyllum*) in a Himalayan district, Humla, Nepal. The field research was conducted in mountainous communities in western Nepal. Methodology included household survey, focus group interview, key informant interview, village meeting and participant observation. The paper analyses the causes of the destruction of HMAPs, contribution of HMAPs in the livelihood of mountainous communities, and some possibilities of sustainable HMAPs utilization in the mountainous communities in Nepal.

¹Ghimire S. K et.al (2004). *Heterogeneity in Ethnoecological Knowledge and Management of Medicinal Plants in the Himalayas of Nepal: Implication for Conservation. Ecology and Society* 9 (3):6 PP. 1-17

Acknowledgements: Funded by Nagoya University, GSID, Grant for Overseas Field Research

PRELIMINARY CHARACTERISATION OF THREE *FILIPENDULA* SPECIES AS A POSSIBLE SOURCE FOR NUTRACEUTICALS

Milda Pukalskienė and Petras R. Venskutonis

Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas, LT-50254, Lithuania **Ona Ragažinskienė**, Kaunas Botanical Garden of Vytautas Magnus University, Žiliberio str. 6, Kaunas, LT-46324 Lithuania

Filipendula is a genus of 12 species of perennial herbaceous flowering plants in the family Rosaceae, native to the temperate regions of the Northern Hemisphere. The aim of this study was to determine essential oil composition and extract radical scavenging properties of the three species: European meadowsweet (*F. ulmaria*), dropwort (*F. vulgaris*), and Siberian meadowsweet (*F. palmata*). The plants have been used both for medicinal and food purposes; however publications on their biological properties and essential oil composition are rather scarce. The plants were subjected to the subsequent extractions with hexane, ethyl acetate, acetonitrile, methanol and water, i.e. in order of increasing solvent polarity. The radical scavenging capacity (RSC) was expressed as the EC₅₀ value, which is defined as the amount of antioxidant necessary to decrease the initial concentration of a stable free radical DPPH by 50%. The highest RSC possessed methanol extracts followed by acetonitrile, ethyl acetate and water extracts. The RSC of hexane extract was negligible. The EC₅₀ values of *F. ulmaria*, *F. palmata* and *F. vulgaris* methanol extracts were 0.21 mg/ml, 0.21 mg/ml, and 0.38 mg/ml, respectively. The extracts were analyzed by HPLC/MS and the differences in their composition were clearly observed. The essential oils were isolated by hydrodistillation, analyzed by GC/MS and the differences between the studied species were evaluated. Preliminary results show that *Filipendula* species are promising plants for expanding their uses, however further studies are needed to determine most valuable bioactive components and to assess the feasibility of their commercial cultivation and processing.

IN VITRO CULTIVATION OF RUBUS SANCTUS SCHREB.**Smaranda Vântu**

Al. I. Cuza University Faculty of Biology, B-dul Carol I, 20 A, 700505 Iasi, Romania

The plants are a major source of natural products used as pharmaceuticals, agrichemicals. Most likely plants will continue to provide novel natural products as well as chemical models for new drugs. In vitro biology and technology have been proposed as contributing toward production of plant natural products, via micropropagation, cell culture (1, 2, 3). The paper studies "in vitro" behaviour of *Rubus sanctus Schreb.*, a native of Central Asia, used in traditional medicine. It were tested, not only the dedifferentiation capacity, but also the regenerative potential for rapid micropropagation. The initiation of in vitro cultures at *Rubus sanctus Schreb* was achieved from axillary buds, cultivated on different variants of Murashige-Skoog medium. The MS medium supplemented with benzylaminopurine stimulated the direct caulogenesis at *Rubus sanctus Schreb.*, whereas the MS medium supplemented with kinetine generated an intensive proliferative reaction and callus development. The regeneration of whole plants was obtained in two steps: the shoots were excised and transferred to fresh medium and then rooting of these shoots was achieved on the same medium without growth regulators.

References: 1. Ayoub, N.A., Hussein, S.A.M., Reiß, C., Mentel, R., Lindequist, V. (2004)- Phenolics of interesting antiviral and antibacterial activities from *Rubus sanctus*, Bull. Fac. Pharm. Cairo, 42(1): 223-231

2. Connolly, J., Hill, R.A. (2005)- Triterpenoids, Nat. Prod. Rep. 22: 230-248

3. Erdemoglu, Nurgun, Kupeli, Esra, Yesilada, Erdem (2003)-Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine, Journal of ethnopharmacology, 89: 123-129

PRACTICAL APPLICATION OF CARBON DIOXIDE FOR THE EXTRACTION OF VARIOUS MEDICINAL AND AROMATIC SEEDS

Petras R. Venskutonis

Department of Food Technology, Kaunas University of Technology, Radvilėnų pl. 19, Kaunas, LT-50254, Lithuania

Medicinal and aromatic seeds were extracted with liquid carbon dioxide (L-CO₂) in order to measure extract yields, the content and composition of essential oils, which are the most important characteristics for the assessment of technological and economical feasibility of commercial applications of the extraction techniques. Preliminary assessment of some biological activities of the extracts, such as radical scavenging capacity and inhibition of microorganisms were also tested. Pilot plant scale extractions were performed using seeds from 9 species: coriander (*Coriandrum sativum*), caraway (*Carum carvi*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*), blue fenugreek (*Trigonella coerulea*), sicklefruit fenugreek (*Trigonella foenum-graecum*), amaranth (*Amaranthus lividus retroflexus*), black cumin (*Nigella sativa*) and white mustard (*Sinapis alba*). Different extraction cycles were applied to increase the yield of the extract. Depending on the process time, particle size and extraction cycle the yields varied from 2.3 (sicklefruit fenugreek) to 18 % (black cumin), while the content of the essential oil in extracts was from the traces to 27.5 %. The highest yields were obtained from the seeds containing remarkable amounts of essential oil and lipids. Essential oil composition was analyzed by using gas chromatography and mass spectrometry and the differences between hydrodistilled oils from the whole ground seeds and their extracts were evaluated. It was concluded extraction of some plant seeds with L-CO₂ is economically and technologically feasible process for commercial applications, e.g. producing ingredients for the formulations of different products, particularly flavorings, functional foods and food supplements.

LEONTOPODIUM ALPINUM CASS. 'HELVETIA', A NEW HYBRID EDELWEISS

José Vouillamoz, Claude-Alain Carron, Catherine Aryelle Baroffio, Christoph Carlen

Agroscope Changins-Wädenswil ACW, Centre de Recherche Conth, Switzerland

jose.vouillamoz@acw.admin.ch

Edelweiss (*Leontopodium alpinum* Cass. Asteraceae) is the most emblematic plant of the Alps. Wild collections is strictly forbidden or limited. Since natural populations show a high degree of morphological variability, a breeding program was carried out to obtain a high quality, productive and homogenous variety for cultivation. Breeding was facilitated by a particular reproduction system. Edelweiss "flowers" are in reality a capitulum of capitula: a star-shape involucre of bracts surrounds 5-10 capitula (inflorescences containing numerous tiny flowers) clustered in one capitulum. As a rule, an Edelweiss plant contains both hermaphrodite and female flowers (gynomonoecy). However, a few rare occurrences of hermaphrodite only and

female only individuals (gynodioecy) have been observed. We used this biological feature to create hybrid clones from five female only plants (mother-plants) pollinated by four hermaphrodite plants (father-plants). We obtained 19 hybrids and compared them to three selected hermaphrodite and two wild populations from the Swiss Alps. Hybrid plants turned out to be much more homogenous, with a higher average dry weight as well as a higher level of leontopodic acid, a recently discovered antioxidant with DNA protecting properties. The most interesting Edelweiss hybrid was named 'Helvetia' after the Latin name of Switzerland. 'Helvetia' is now successfully cultivated in mountainous regions of Switzerland and demand for Edelweiss 'Helvetia' extracts by food (beverage, liquor, chocolate, etc.) and cosmetic (sunscreen, anti-aging lotions, etc.) companies has greatly increased in recent years.

Acknowledgements: DSM/Pentapharm/Alpaflor, Valplantes, Weleda and Ricola for their logistic and financial support; Charly Rey for his helpful collaboration.

RED-LISTED ETHNOMEDICINAL PLANTS: IDENTIFYING GEOGRAPHIC "HOT SPOTS" OF CONSERVATION CONCERN

Vivienne Williams, N.R. Crouch, J.E. Victor, A.P. Dold

School of Animal Plant & Environmental Sciences, University of the Witwatersrand, Private Bag 3, 2050 Wits, South Africa, vivienne.williams@wits.ac.za, Ethnobotany Unit, SANBI, PO Box 52099, Berea Road 4007, South Africa/School of Chemistry, University of KwaZulu-Natal, 4041 Durban, South Africa, National Herbarium, SANBI, Private Bag X101, 001 Pretoria, South Africa
Selmar Schonland Herbarium, Rhodes University Botany Department, c/o Albany Museum, Somerset Street, 6139 Grahamstown, South Africa

Exploitation and excessive harvesting is a determining factor in the extinction risk of plants harvested for the traditional medicinal trade. This concerns especially the species that are in high demand. Harvesting has led to notable declines in the abundance and distribution of several species, and has thereby increased their level of vulnerability to unsustainable harvesting and extinction. Approximately 321 medicinal plant species have been assessed according to the 2001 IUCN Red List criteria, and more than 800 species remain to be assessed (but are mostly not threatened). Of the 321 assessed taxa, about 40% (± 123 species) are of conservation concern (i.e. have been listed as critically endangered, endangered, vulnerable, near-threatened and least concern-declining). At the distribution level of one quarter-degree grid square (QDS), there are very few areas of KwaZulu-Natal and Mpumalanga that don't have threatened or near-threatened medicinal plants. Most of the critically endangered species occur in the Mpumalanga and Limpopo Provinces, and there is a trend towards more vulnerable species being restricted to a band along the eastern coastline of South Africa, whereas less vulnerable species extend from the coast to the interior. "Hot spots" (i.e. QDSs that have the highest number of threatened medicinal taxa) include the Barberton and Durban-Pietermaritzburg areas, as well as parts of northern KwaZulu-Natal and the Eastern Cape. Reasons for the observed distribution patterns are being discussed.

AN ANALYSIS OF TWO BIOACTIVE SECONDARY METABOLITES IN *HARPAGOPHYTUM PROCUMBENS* DC; HARPAGOSIDE AND VERBASCOSIDE *IN VITRO* AND *IN VIVO*

Graham Wilson

University College Dublin, Ireland, graham.wilson@ucd.ie

Extracts of Devils Claw (*Harpagophytum procumbens*, Pedaliaceae) are used widely for the relief of muscular pain. The vulnerability of wild plant populations of *Harpagophytum* to commercial harvesting of the tubers focuses attention on alternative methods of sustainable production of these bioactive compounds, such as the use of plant cell cultures. The anti-inflammatory activity has been attributed to free and glucoside forms of iridoid monoterpenes harpagoside, harpagide, procumbine and harpagogenine present in the tubers of the plant. There are, however, additional bioactive compounds present in *Harpagophytum* such as the caffoyl derivative verbascoside, reported to have anti-bacterial, anti-viral, anti-hypertensive and immunosuppressive properties. The purpose of this study was to compare the accumulation of these two classes of metabolites in cell suspension cultures and in hairy root cultures with that present in leaves and tubers from the intact plants. The results, based on spectroscopic analysis, show that harpagoside type compounds were almost undetectable in cell suspension or hairy root cultures (0.12mg.g⁻¹ DW) compared to the plant (tuber 16.07mg.g⁻¹ DW). However levels of verbascoside type compounds were similar *in vitro* (cell suspension 0.078g.g⁻¹ DW) to the plant (tubers 0.056g.g⁻¹ DW). It is clear that in these *in vitro* conditions cultures of cells or roots of *Harpagophytum procumbens* lack a complete biosynthetic pathway for iridoid glycoside biosynthesis. However the caffoyl derivatives, verbascoside type compounds, are accumulated substantially. Taken together, *in vitro* produced material contains a markedly different profile of bioactive compounds compared to that present in the intact plant.

THEME 3 PERSPECTIVES IN NATURAL PRODUCTS CHEMISTRY

MAKING NATURAL PRODUCTS RESEARCH EXCITING AND PURPOSEFUL IN AFRICA

Berhanu Abegaz

Department of Chemistry, University of Botswana, Private Bag 00704, Gaborone, Botswana,
abegazb@mopipi.ub.bw

Natural products research has by-and-large been a South-North collaboration with the African contribution often reduced to either supplying the plant material or being confined to the rudimentary aspects of the research. The remaining, exciting part usually came from the Northern counterpart. Many of the international funding agencies, including the official bilateral and multilateral cooperation agreements started and maintained the forgoing status quo. Such framework for natural product research did not enhance the capacity building efforts in Africa. Thankfully this has been changing over the last decade as African scientists found new ways of cooperation among themselves and because a number of institutions began to realize the need to invest in major scientific equipment and other research facilities. This presentation will highlight the joint efforts of phytochemists, biochemists and synthetic organic chemists from various institutions in Africa to do exciting, and development-oriented research in natural products. Results of a broad screening of 200 plants using the screens-to-nature bioevaluation technologies will be presented. We will also present the most recent results revealing novel natural products from medicinal plants used in Botswana and Cameroon. These include a number of novel triterpenes from Cameroonian *Dubosia* species, novel phenolic compounds from *Morus* spp. and compounds that have the potential to potentiate chloroquine in malaria

therapy

EFFECT OF CO-CULTURE OF *ORIGANUM VULGARE* AND *THYMUS VULGARIS* ON ESSENTIAL OIL PRODUCTION

Gholamreza Asghari, Mohamadreza Shams Ardakani, Amir Hossein, Jamashidi Said Shakibai

Isfahan Pharmaceutical Research Centre, Iran, asghari@pharm.mui.ac.ir
Isfahan Pharmaceutical Research Centre, Iran
Isfahan Pharmaceutical Research Centre, Iran
Isfahan Pharmaceutical Research Centre, Iran

Origanum vulgare and *Thymus vulgaris* are plants containing essential oils. In this study the effect of co-culturing *O. vulgare* and *T. vulgaris* on essential oil production was investigated. *O. vulgare* and *T. vulgaris* seeds' surfaces were sterilized, and were transferred to the Petri dishes to produce seedlings. Callus culture was established from the seedlings and a cell suspension culture was obtained from the callus. The cultures were established in MS media supplemented with sucrose. A Co-culture of two plants was established. Dichloromethane extract of the cultured cells was prepared and subjected to GC analysis. Only Thymol was produced in cultured cells of *O. vulgare* and *T. vulgaris* as well as in their co-culture. It seems that enzymes involved in biosynthetic pathways of the volatile oils in these plants are not able to influence the terpen-biosynthesis of another plant.

PRELIMINARY STUDIES OF THE ANTIBACTERIAL ACTIVITIES OF *COMBRETUM VENDAE* LEAF EXTRACT

Aroke Ahmen, Chima Igwe, J. N. Eloff

Dept of Phytomedicine Programme, Faculty of Veterinary Science, South Africa,
shahid_arokey@yahoo.com

The Combretaceae family has many pharmacological activities and bioactive compounds have been isolated from some of the species.¹ No information is available on the anti-bacterial activities of *Combretum vendae*. This study investigates the effect of more polar leaf extracts of *Combretum vendae* on some bacteria associated with diarrhoea and inflammatory diseases (*Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). Milled samples of air dried leaves were extracted with 70% acetone acidified with 1% HCl, concentrated under vacuum and freeze-dried. The polyphenolic content of the extract was evaluated by Folin-Ciocalteu method. The antibacterial activities of the extract were determined *in vitro* and the minimum inhibitory concentrations (MIC) were assessed using the microplate dilution method². Serial dilutions with a maximum concentration of 2.5 mg/ml and a minimum concentration of 0.02 mg/ml of the extract were made. Distilled water was used as negative control and gentamicin as positive control. Yield of extract was 38.88%; total phenol, 38.55 mg catechin equivalent/g; total tannin, 22.39 mg catechin equivalent/g; non tannin, 16.39 mg catechin equivalent/g. The MIC of extract against *E. coli*, *E. faecalis*, *P. aeruginosa* and *S. aureus* were 0.312 mg/ml, 0.156 mg/ml, 0.156 mg/ml and 0.156 mg/ml respectively. The results shows that polar *Combretum vendae* leaf extracts have some anti-bacterial activities that can be explored as alternative to existing antibacterial drugs. Tannins are implicated in non-specific antibacterial activity and tannin content in this extract is relatively high, the antibacterial activity

may be attributed in part to the tannin content.

References:

- 1 Eloff JN, Katerere DR, McGaw LJ (2008) J. Ethnopharmacology 119, 686-699
- 2 Eloff JN (1998) Planta Medica 54,711-714

SPICATIC ACID: A 4-CAROXYGENTISIC ACID FROM A GENTIANA SPICATA EXTRACT WITH POTENTIAL HEPATOPROTECTIVE ACTIVITY

Nahla Ayoub

Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, ayoub.n@link.net

Investigation of the protective mechanisms of aqueous alcoholic extract of *Gentiana spicata* (Gentianaceae) in carbon tetrachloride intoxicated rats was undertaken. Rats were treated with carbon tetrachloride at the dose of 1 ml/kg body weight intraperitoneally once in every 24 hrs for 14 days. The hepatoprotective activity of the extract of *Gentiana spicata* was evaluated by measuring levels of serum marker enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Administration of the extract (250 mg/kg, b.wt.) significantly ($p < 0.05$) prevented CCl₄-induced elevation of levels of serum AST and ALT. Treatment of rats with CCl₄ led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with a significant reduction of the hepatic antioxidant system e.g. reduced glutathione (GSH). These biochemical alterations resulting from CCl₄ administration were significantly ($p < 0.05$) inhibited by pretreatment with the extract of *Gentiana spicata*. These results suggest that the aqueous alcoholic extract of *Gentiana spicata* may act as a hepatoprotective and antioxidant agent. Phytochemical study demonstrated a high phenolic content and led to the isolation and identification of the new carboxygentisic acid, 1,4-dicarboxy 2,5-dihydroxybenzene, for which we suggest the name spicatic acid, together with the two known flavonoids, quercetin 3-O-[(2,3,4-triacetyl- α -rhamnopyranosyl)-1^{''}? 6^{''}]-3-acetyl- β -galactopyranoside and quercetin 3-O-[(2,3,4-triacetyl- α -rhamnopyranosyl)-1^{''}? 6^{''}]-4-acetyl- β -galactopyranoside. All structures were elucidated on the basis of conventional analytical methods and confirmed by high resolution FTMS, 1D- and 2D-NMR data. The new phenolic carboxylic acid, spicatic acid is of special interest as it represents the first phenolic acid which bears two carboxyl functions in one aromatic ring elucidated on the basis of conventional analytical methods and confirmed by FTMS, 1D- and 2D-NMR data. The new compound was shown to have a slight positive effect on in vitro mineralization of SaOS-2 human osteosarcoma cells. However, it showed a significant increase of mineralized area at 20 μ g/mL, while at lower concentrations the effect was not of significant value. It should be noted however that an increase of the number of mineralized spots (nodules) at all concentrations tested was observed. This can be taken as an evidence for increased cell maturation and osteoblasticity. This data give rise to the notion that the compound has a contribution to the significant effect exhibited by the *Feijoa sellowiana* crude extract.

References: 1- Hussein, S. A. M., Nawwar, M. A. M. (2006) Pharmazie 61: 2993-2996 2- Hussein, S. A. M., Nawwar, M. A. M. (2007). Phytochemistry 68: 1464-1470 Extra authors: Michael Linscheid, Vice President for research, Humboldt Universität zu Berlin, 12489 Berlin, Germany Email: michael.linscheid@cms.hu-berlin.de Manuela Harms, Institut für Pharmazie, Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany Email: Harms@uni-greifswald.de Kristian Wende, Institut für Pharmazie, Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany Email: wende@uni-greifswald.de Ulrike Lindequist Institut für Pharmazie,

Comment [A1]: confirm what ?
is from the authors

Comment [A2]: confirm what ?
is from the authors

Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany Email: lindequi@uni-greifswald.de Presenter: Mahmoud A. M. Nawwar Department of Phytochemistry, Division of Pharmaceutical Industries, National Research Center, Dokki, Cairo, Egypt

CHEMICAL COMPOSITION AND ANTIBACTERIAL EVALUATION OF THE ESSENTIAL OILS OF *FERULAGO AUCHERI* BOISS. FROM DIFFERENT LOCALITIES

K. Husnu Can Baser¹, G. Özek¹, F. Demirci¹, G. Iscan¹, E. Hamzaoglu², A. Duran³

Anadolu University, Turkey, khcbaser@anadolu.edu.tr, Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University 26470 Eskisehir, Turkey, Department of Biology, Yozgat Faculty of Science and Letters, Bozok University, Yozgat, Turkey, Department of Biology, Faculty of Education, Selcuk University 42090 Meram-Yeniyol, Konya, Turkey

Air dried and crushed fruits of *Ferulago aucheri* Boiss. (*Apiaceae*) from Southern and Western regions of Turkey were subjected to hydrodistillation to obtain essential oils, which were subsequently analyzed by GC-FID and GC/MS. Seventy seven compounds representing 95.9% and 127 compounds representing 86.5% of the fruit oils have been characterized for samples of *F. aucheri* from Antalya (oil A) and Manisa (oil B) provinces, respectively. Monoterpenes comprised 83.6% of the oil A with limonene (43.1%), alpha-pinene (18.3%) and myrcene (7.0%) as major constituents. Sesquiterpenes (72.7%) prevailed in the oil B, with germacrene D (25.7%) and (2E,6E)-farnesol (8.0%) as major constituents. Both essential oils were also evaluated for their antibacterial activity against eight Gram (+) and Gram (-) strains by microdilution method. The oils displayed moderate to weak inhibitory activity (MIC 0.25-3 mg/mL). The oils were found to be the most active against *Pseudomonas aeruginosa* when compared with antimicrobial standards. Antibacterial activity of the oils appeared non-selective when comparing Gram (+) and Gram (-) strains.

THE CHEMICAL COMPOSITION OF *STENOTAENIA MACROCARPA* ESSENTIAL OIL AND BIOACTIVITY

K.H.C. Baser, T. Kiyan, B. Demirci, F. Demirci, H. Duman

Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskisehir, Turkey, Gazi University, Faculty of Science & Letters, Department of Biology, Ankara, Turkey

The genus *Stenotaenia* of *Apiaceae* is represented by only one species, namely *Stenotaenia macrocarpa* Freyn & Sint ex. Freyn in Turkey. The subject plant material was collected from Central Anatolia: Konya – Hadim. The essential oil of the crushed seeds of *S. macrocarpa* were initially obtained by hydrodistillation, which was further analyzed by both GC-FID and GC-MS systems. More than hundred constituents were identified where the main constituents were revealed as octyl acetate (32.6%), octanal (16.7%), and octanol (4.3%), to the best of our knowledge for the first time. Furthermore, the essential oil was screened for its antimicrobial activity and radical scavenging activity by TLC bioautographic techniques against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*, *Candida albicans* and the DPPH radical, respectively. As a result of the bioactivity screens the oil showed microbial inhibitory and radical scavenging activities at high concentrations (>100 mg/ml).

COMPOSITION OF THE ESSENTIAL OIL OF TWO ENDEMIC *CENTAUREA* SPECIES FROM TURKEY

Yavuz Bulent Kose¹, Ayhan Altintas², Osman Tugay³, Tuna Uysal³, B. Demirci², K. Ertugrul³, K.H.C. Baser²

Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, ybkose@anadolu.edu.tr, Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, aaltintas@anadolu.edu.tr, Department of Biology, Faculty of Science and Art, Selcuk University, 42075 Selcuklu-Konya, Turkey, otugay@selcuk.edu.tr, Department of Biology, Faculty of Science and Art, Selcuk University, 42075 Selcuklu-Konya, Turkey, tuna_uysal@hotmail.com

Almost 600 species belonging to genus *Centaurea L.* are naturally distributed throughout Asia, North Africa and America (1). In the flora of Turkey, *Centaurea* is one of the richest genera. There the genus is represented by 189 species and 120 of them are endemic (2-3). *Centaurea* species have been widely used for their anti-dandruff, anti-diarrheic, anti-rheumatic, anti-inflammatory, choleric, diuretic, digestive, stomachic, astringent, antipyretic, cytotoxic and antibacterial properties in folk medicine (4). Aerial parts of *Centaurea sericeae* Wagenitz and *Centaurea ensiformis* P.H. Davis were hydrodistilled for 3 hours using a Clevenger-type apparatus to produce a small amount of essential oil, which was trapped in n-hexane. Oils were analyzed by gas chromatography-mass spectrometry (GC-MS). Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to the series of n-alkanes. Computer matching against commercials (5-6), in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (7-9), were used for the identification. Seventy-three and seventy-four compounds representing 84.7% and 91% of the essential oils were characterized from *C. sericeae* and *C. ensiformis*, respectively. Caryophyllene oxide (10.6%), nonacosane (8.6%) and hexadecanoic acid (7%) were the main constituents in the oil of *C. sericeae* and β -eudesmol (29.8%), hexadecanoic acid (8%) and caryophyllene oxide (7.6%) were the main constituents in the oil of *C. ensiformis*.

References:

1. Brummitt, R. K., 2004, Report of the Committee for *Spermatophyta*: 54, *Taxon*, 53 (3), 813-825.
2. Wagenitz G. 1975. *Centaurea L.* in: *Flora of Turkey and the East Aegean Islands*. 5, pp. 465-585, Edit. P.H. Davis, Edinburgh Univ. Press, Edinburgh, UK.
3. Uysal, T., 2008, *Ann. Bot. Fen.*, 45, 137-140.
4. Arif R, Küpeli E., Ergun F. 2004., *Gazi Üniversitesi Fen Bilimleri Dergisi*, 17: 149-164.
5. McLafferty FW, Stauffer DB. 1989, *The Wiley/NBS registry of mass spectral data*, J Wiley and Sons: New York.
6. Joulain D. et al. 2001, *Terpenoids and related constituents of essential oils*. Library of MassFinder 2.1, Hamburg, Germany.
7. Joulain D., König W.A., 1998, *The Atlas of spectra data of sesquiterpene hydrocarbons*, EB-Verlag, Hamburg.
8. ESO 2000, 1999, *The complete database of essential oils*, Boelens Aroma Chemical Information Service, The Netherlands.
9. Jennings W.G., Shibamoto T., 1980, *Quantitative analysis of flavour and fragrance volatiles by glass capillary GC*, Academic Press, New York.

GC-MS ANALYSIS OF THE ESSENTIAL OIL FROM THE SEED OF SWEET FENNEL (*FOENICULUM VULGARE* MILL. VAR. *DULCE*) GROWN IN TURKEY

Belgin Cosge, Bilal Gurbuz, Arif Ipek

ABant Izzet Baysal University, Turkey, b_cosge@yahoo.com
Ankara University, Turkey, gurbuz@agri.ankara.edu.tr
Ordu University, Turkey, ipek@agri.ankara.edu.tr

Fennel (*Foeniculum vulgare* Miller, Apiaceae family) is an annual, biennial or perennial plant, depending on the variety, and it is native to the Mediterranean area. Fennel has been cultivated since ancient times and the leaves, stems, roots, seeds and oil are used for medical and culinary purposes. This research was carried out to evaluate the content and chemical composition of essential oil from the dried seed at the experimental fields of the Field Crops Department at the Agricultural Faculty of Ankara University, Ankara, Turkey. 10 sweet fennel lines, developed by single plant selection method in this department, and a sweet fennel population (control) grown at the experimental field of the same department were used as material. The seeds of lines and control population were sown on April 4th 2006. Seeds were sown spaced 40 cm apart and row length was 3 m. The experiment was not watered and no fertilizer was given. Harvest was made by hand on the 28th September 2006. The essential oil content was determined using an average 30 g of dried seeds and a Clevenger-type apparatus. The uncrushed seeds were distilled for 3 h in 500 ml water. The obtained essential oils were analyzed by GC-MS. The essential oil contents from the seeds of the ten sweet fennel lines ranged from 1.68 to 2.74%. The principal components in the oils of the lines were trans-anethole (92.04-95.81%) and estragole (2.59-3.80%). In seeds of the sweet fennel population 1.62% of essential oil containing 94.90% trans-anethole and 3.27% estragole were also recorded.

NEW JATROPHANE DITERPENES FROM *EUPHORBIA MONGOLICA* WITH MULTIDRUG RESISTANCE MODULATING ACTIVITY

Peter Forgo, Dora Redei, Joseph Molnar, Judit Hohmann

University of Szeged, Hungary, pforgo@pharm.u-szeged.hu, University of Szeged, Hungary,
redei@pharm.u-szeged.hu, University of Szeged, Hungary, molnarj@comser.szote.u-szeged.hu
University of Szeged, Hungary, hohmann@pharm.u-szeged.hu

Diterpenes of the genus *Euphorbia* based on different skeletal types attracted interest because of their promising multidrug resistance reversing activity. Many compounds of the macrocyclic jatrophane-type significantly inhibit the overproduction of permeability glycoprotein (P-gp) in the plasma membrane of resistant tumour cells, reducing the intracellular accumulation of drugs. In our previous work we have reported the isolation of three jatrophane polyesters from *Euphorbia mongolica* Prokh. with anti-MDR activity. With the aim to find further efficient compounds, the minor diterpene constituents of *E. mongolica* were examined. From the dried, powdered plant material, originating from Govi Gurvan-Sajhan, Omnogovi (Mongolia), a lipophylic chloroform extract was prepared. Five compounds were isolated from this extract with a combination of multiple chromatographic methods (CC, VLC, CPC and HPLC). The structures

were elucidated by HREIMS and NMR spectroscopy, including ¹H NMR, JMOD, 1H-1H COSY, NOESY, HSQC and HMBC experiments. The isolated compounds, including four new natural products, are jatrophone-12-ene-9,14-diketo-polyesters esterified with acetic, propanoic, n-butyrac, isobutyric and benzoic acids. The anti-MDR activities of the isolated compounds were assayed on human MDR gene transfected L5178 mouse lymphoma cells using the rhodamine exclusion test, and it was found that all compounds modulated the intracellular drug accumulation. Comparing the structures with the MDR modifying activity, expressed by fluorescence activity ratio, some structural requirements of the anti-MDR effect of the jatrophone polyesters were established.

Acknowledgements: This work was supported by grant OTKA K72771.

COMPARATIVE ANALYSIS OF ESSENTIAL OILS FROM THE LEAF, FRUIT AND STEM BARK OF *HARUNGANA MADAGASCARIENSIS* (HYPERICACEAE)

Adebayo Gbolade, Viana Tira-Picos, Joseph Nogueira, Adekunle Gbolade

Olabisi Onabanjo University, Sagamu campus, Nigeria, adegbolade@yahoo.com

University of Lisbon, Portugal University of Lisbon, Portugal, nogueira@fc.ul.pt

Obafemi Awolowo University, Ile-Ife, Nigeria, kunleladele@yahoo.com

Leaves and stem bark of *H. madagascariensis* are used in African traditional medicine as a remedy for anaemia, wounds and malaria. Leaf aqueous extracts of the plant were reported to show antimicrobial and antitrichomonal activities among others. No report has been documented on the essential oil constituents of the plant. As part of the efforts to generate a data bank for essential oils of Nigeria, we undertook for the first time a comparative analysis of hydro-distilled oils of the leaf, stem bark and fruit of the plant by combined gas chromatography-mass spectrometry (GC-MS). Both qualitative and quantitative differences existed in the composition of the three oils which comprised mainly sesquiterpene hydrocarbons (66.8 - 69.6 %). α -caryophyllene (32.4 % and 18.4 % respectively for leaf and fruit oils only), α -humulene (10.4 %, 9.8 % and 7.3 % respectively for leaf, stem bark and fruit oils), germacrene D (8.7 % for leaf oil only), and α -farnesene (37.4 % and 10.4 % respectively for stem bark and fruit oils only) are the predominant constituents, which may possibly account for the various reported biological activities of the plant.

ISOLATION OF ANTIPROLIFERATIVE COMPOUNDS FROM *CENTAUREA JACEA* L.

Judit Hohmann, Istvan Zupko, Peter Forgo

University of Szeged, Hungary, hohmann@pharm.u-szeged.hu, University of Szeged, Hungary, zupko@pharm.u-szeged.hu, University of Szeged, Hungary, pforgo@pharm.u-szeged.hu

Antiproliferative screening of 53 Asteraceae species against human tumour cell lines demonstrated a high cell proliferation inhibitory activity of the *Centaurea* species native to Middle and Eastern Europe. The highest activity was recorded for *C. jacea*, whose chloroform extract, prepared from different plant parts, significantly inhibited the growth of HeLa (57-86 %), MCF7 (44-64 %) and A431 (43-69 %) cells in 10 μ g/ml concentrations. This species is a perennial herb occurring widespread in Europe. Its anti-tumour constituents have not been investigated earlier. Previous studies revealed the presence of flavones, flavonols, sesquiterpenes and cinnamic

alcohol glucosides. The aim of the present work was the isolation and identification of the antiproliferative compounds from the aerial parts of *C. jacea* using bioactivity guided fractionations. The chloroform extract was chromatographed by CC on silica gel and polyamide, and further separated by CPC, PLC and RP-HPLC, to yield 10 pure compounds. Structure determinations were carried out by means of UV, MS and NMR spectroscopy and the comparison of the spectral data with literature values. The results allowed the identification of the flavonoid apigenin, axillarin, centaureidin, cirsiolol and isokaempferide, the sesquiterpene 4'-acetylcnicin, one new dibenzylbutyrolactone-type lignane, and three aliphatic glucose diesters, including the new natural product 1-beta-isobutanoyl-2-angeloylglucose. All compounds were isolated for the first time from this species. The isolated compounds were evaluated for their tumour cell inhibitory activity on HeLa, MCF7 and A431 cells and it was found that besides the extremely active centaureidin (IC₅₀ 0.0819–0.3540 µM), cirsiolol, isokaempferide, apigenin and 4'-acetylcnicin also exerted remarkable effects.

Acknowledgements: This work was supported by grant OTKA K72771.

STILBENOID CONSTITUENTS IN *WELWITSCHIA MIRABILIS*

I. **Iliya, T. Tanaka, H. Murata and M. Linuma**

II.

Gifu Pharmaceutical University, 5-6-1, Mitahora-Higshi, Gifu 502-8585, Japan
National Institute for Pharmaceutical Research and Development, Idu Industrial Area, Abuja, Nigeria, iliyas@niprd.org, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge, Hirakata, Osaka 573-0101, Japan

Welwitschia mirabilis is an endangered and unique gymnosperm of the Namibian Desert of South West Africa. It is a monotypic member of the Genus *Welwitschia*. Since its discovery about 140 years ago, very little is known about its chemical constituents. In the present study we report the isolation and structure elucidation of 10 new stilbenoids from the stem and root of the plant along with some known compounds. The structures of the compounds were assigned by spectroscopic analysis

EVALUATION OF ANTIBACTERIAL ACTIVITY OF SEVERAL SOUTH AFRICAN TREES AND ISOLATION OF TWO BIFLAVONOIDS WITH ANTIBACTERIAL ACTIVITY FROM *GARCINIA LIVIGSTONEI*

A. A. Kaikabo, B. B. Samuel, M. M. Suleiman and J. N. Eloff

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

The acetone leaf extracts of eleven South African plant species were screened for their antibacterial activity by determining the minimum inhibitory concentrations (MIC) against 4 nosocomial bacterial pathogens and bioautography. MIC screening was at concentrations of 0.02 to 1.25 mg/ml using two-fold serial microdilution against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*¹. The extracts of *Macaranga capensis*, *Garcinia livingstonei*, *Diospyros rotundifolia* and *Dichrostachys cinerea* had good antibacterial activity with minimum MIC values of 0.03, 0.04, 0.06 and 0.08 mg/ml, respectively. The average MIC values of the plant extracts against the tested pathogens ranged from 0.23-1.77 mg/ml. *S. aureus* was the most susceptible bacterial pathogen with average MIC

of 0.36, the extract of *Diospyros rotundifolia* was the most active with an average MIC against all the organisms of 0.23 mg/ml. The extracts of *Buxus natalensis*, *Dracaena mannii* (natal), and *Pittosporum viridiflorum*, *Acacia sibeiriana*, *Erythrina lattissima*, *Cassine papillosa* and *Pavetta schumanniana* (savoti) had a low antibacterial activity. *G. livingstonei* was selected for further work and two biflavonoids with antibacterial activity was isolated from a leaf extract.

Keywords: *Garcinia livingstonei*, biflavonoids, antibacteria, traditional medicine

References:

1. Eloff JN, 1988 Planta Medica 64, 711-714.

COMPOSITION AND BIOLOGICAL ACTIVITY OF THE ESSENTIAL OIL FROM *CYMBOPOGON NARDUS* (L.) RENDLE

Thangaraj Kandasamy, Bharti Odhav, Himansu Baijnath

Durban University of Technology, thangaraj@dut.ac.za

Durban University of Technology, odhavb@dut.ac.za

University of KwaZulu Natal, odhavb@dut.ac.za

The essential oil from the leaves, culms and rhizomes of *Cymbopogon nardus* were extracted and volatile oils were investigated for the first time using Gas Chromatography-Mass Spectroscopy (GC/MS). Alpha-Cubebene, Camphene, Geraniol, Limonene, Myrcene, Palmitic acid and Sabinen were the major compounds identified. The essential oil was investigated for its antioxidant (DPPH assay), anti-inflammatory (5-lipoxygenase assay), antimicrobial (disk diffusion) and anti-mosquito properties (insecticidal, larvicidal and repellency assays). The oil showed poor antimicrobial activity and inhibited the growth of *Bacillus*, *Micrococcus* and *Staphylococcus* with minimum inhibitory concentration of 0.0625 (vol/vol). The oil showed antioxidant activity, scavenging more than 80% of DPPH free radicals and did not show any anti-inflammatory activity. It also showed good adulticidal activity (53.7% mortality) and excellent larvicidal (100% mortality) and repellent activity (100% repellency) against *Anopheles arabiensis* mosquitoes which is a malarial vector. The results of this study show that the essential oil from *C. nardus* possesses, antioxidant, antibacterial and anti-mosquito activities which may be associated with its predominant compounds viz., α -cubebene, camphene, geraniol, limonene, myrcene, palmitic acid and sabinen. These results lead the way for exploiting *C. nardus* oil as a multi-functional agent.

ANTIMICROBIAL ACTIVITY OF STILBENOIDS AND FLAVONOIDS FROM THREE SPECIES OF *COMBRETACEAE*

David Katerere, Alexander Gray, Robert Nash, Roger Waigh

PROMEC Unit, MRC, South Africa, david.katerere@mrc.ac.za, University of Strathclyde, Scotland, UK, a.i.gray@strath.ac.za, VASTox Wales, Plas Goggerdan, Aberystwyth, Dyfed, UK, Robert.Nash@summitplc.com, University of Strathclyde, Scotland, UK, r.d.waigh@strath.ac.uk

Four phenanthrenes, one of them new, were isolated from the fruit of *Combretum hereroense* and one from the leaves of *C. collinum*. Three of them were moderately active against *Candida albicans*, *Mycobacterium fortuitum* and *Staphylococcus aureus* in the

microplate assay. Four bibenzyls, one of which is novel, two chalcones, three flavanones and a flavone, all previously unreported from *Combretaceae*, were isolated from the leaves of *C. apiculatum* Engl. & Diels and two were isolated from the leaves of *C. collinum*. Pinocembrin, one of the ten compounds from *C. apiculatum*, had good activity against *S. aureus* and excellent activity against *C. albicans* in agreement with literature reports.

FLAVONOID CONTENTS, ANTIOXIDANT AND NEUROPROTECTIVE ACTIVITY OF EXTRACTS OF RUSSIAN PLANTS

Sergey Klochkov, Sergey Bachurin, Elena Shevtsova, Oksana Novikova

Institute of Physiologically Active Compounds RAS, Russia, klochkov@ipac.ac.ru, Institute of Physiologically Active Compounds RAS, Russia, bachurin@ipac.ac.ru, Institute of Physiologically Active Compounds RAS, Russia Institute of Physiologically Active Compounds RAS, Russia

Plant flavonoids cause active interest in connection with their authentic influence on the decrease of the risk of cardiovascular diseases and with their potential neuroprotective properties. Antioxidant and free radical-scavenging abilities of flavonoids can be a basis of positive effects of many plant extracts (1). The aim of our work was to study interrelation of antioxidant and neuroprotective activity with the contents and structure of flavonoids in plants extracts. About 200 extracts were investigated. In extracts we defined general flavonoid content and antioxidant activity. HPLC–ESI-MS/MS was used to study flavonoids in the extracts. Analysis of the interaction of plant extracts and isolation by HPLC of pure flavonoids with the mitochondria was done the following way: we defined the influence on calcium- and t-butylhydroperoxide-induced occurrence of lipid peroxidation and mitochondrial membrane permeability transition (MMPT). Three basic structural requirements for the presence of antioxidant activity - hydroxylated C3, an unsaturated C ring, and hydrophobicity were defined. Influence on calcium- and t-butylhydroperoxide-induced MMPT of plant extracts has complex concentration dependence. It was authentically shown, that extracts of plants and individual flavonoids reduce lipid peroxidation in mitochondria irrespective of used substrates of breath as in conditions of "ageing mitochondria in vitro", and at the presence of t-butylhydroperoxide. We also investigated the influence of flavonoid-rich plant extracts on glutamate-induced neurotoxicity. Some extracts inhibit MMPT and Ca²⁺-induced swelling with an IC50 similar to MK-801, a known antagonist of glutamate receptors, and they protect rat brain neurons in culture from glutamate toxic action.

References: 1. Dean et al. Open Natural Products Journal, 2008, 1, 1-6

SESQUITERPENE LACTONES FROM PLANTS OF THE *ASTERACEAE* FAMILY AS THE POTENTIAL SOURCE OF ANTI-TUMOUR AGENTS

Sergey Klochkov, Sergey Bachurin, Yury Bulychev, Svetlana Afana´seva

Institute of Physiologically Active Compounds RAS, Russia, klochkov@ipac.ac.ru, Istitution of Physiologically Active Compounds RAS, Russia, bachurin@ipac.ac.ru, Institute of experimental diagnostics and therapy of tumors, Russia, Institution of Physiologically Active Compounds RAS,

Russia

The plants of the Asteraceae family are actively applied in national medicines for the treatment of a wide spectrum of diseases, including oncological ones. In this work chemical compositions of *Inula britannica*, *caspiaca*, *hirta*, and *helenium* have been studied. The high quantity of sesquiterpene lactones amounting up to 3-6 % of dry plant biomaterials was shown. In addition to the essential chemical diversity, this fact gives a real base for large scale isolations of various scaffolds followed by their chemical modification [1]. More than 150 novel modified sesquiterpene lactones, including water-soluble forms and containing different pharmacophoric fragments, were synthesized. Synthesized derivatives were tested as potential cytotoxic agents against a standard panel of human tumour cell lines using the MTT assay. Compounds with a high level of non-specific cytotoxic activity may be found very frequently within all lactones, natural and modified ones. More potent was SLD-41730 with cytotoxicity towards leukemia cell lines - GI50 values were 10^{-7} – 10^{-8} M. Probable mechanism of sesquiterpene lactones action is the apoptosis induction through the inhibition of the key enzyme farnesyltransferase. Natural and modified lactones were tested using the original spectrofluorometric method, the evaluation of the farnesyltransferase activity. Inhibition activity of hit-compounds was comparable to the level of known inhibitors: IC₅₀ range was about 0,3-5 mM. High levels and frequency of cytotoxic activity of sesquiterpene lactones demonstrated the outlook of further modification of these scaffolds and hit within the hit-to-lead program and on their screening based on further mechanisms.

References: 1. Klochkov et al., Chemistry of Natural Compounds, Vol. 42, No. 4, 2006, p.400

ANTIOXIDATIVE ACTIVITY OF UGANDAN MEDICINAL PLANTS

Kuglerova M., Marsik P., Vanek T., Kokoska L., Wanyana-Maganyi O., Van Damme P. and Grade J.

Department of Crop Science and Agroforestry, Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, Kamycka 129, 165 21 Prague 6-Suchdol, Czech Republic; Laboratory of Plant Biotechnologies, Joint Laboratory of Institute of Experimental Botany AS CR, v.v.i. and Research Institute of Crop Production, v.v.i., Rozvojova 263, 165 02 Prague 6 Lysolaje, Czech Republic; Department of Botany, Makerere University, PO Box 7062, Kampala, Uganda; Laboratory for Tropical and Subtropical Agriculture and Ethnobotany, Department of Plant Production, Faculty of Bio-Science Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

Plant drugs used in African traditional medicine are important source of compounds with various biological activities [1]. For the specific situation of Uganda, there is little information on medicinal plant use by traditional healers in Karamoja region (north-east Uganda). Moreover, even though there are some reports on pharmacology of Ugandan medicinal plants [2], their antioxidative activities are still poorly documented. Thus we decided to investigate antioxidative activity of seven Ugandan tree species selected according to their use in traditional medicine as evidenced by ethnobotanical research. The crude ethanolic extracts of seven barks traditionally used by Karamojong healers, namely *Capparis tomentosa* (Capparaceae), *Dregea rubicunda* (Asclepiadaceae), *Fagaropsis angolensis* (Rutaceae), *Trichilia prieuriana* (Meliaceae), *Turraea floribunda* (Meliaceae), *Warburgia ugandensis* (Canellaceae), and *Zanthoxylum chalybeum* (Rutaceae) was tested for antioxidative activity using 2,2-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging assay. The most promising results showed extract of *W. ugandensis*, which exhibited DPPH scavenging activity with IC₅₀ value (7 µg/ml) very closed to the effect achieved

by reference compounds Trolox (IC_{50} = 4 μ g/ml) and ascorbate (IC_{50} = 2 μ g/ml). Among the other plants tested, *Z. chalybeum*, *F. angolensis* and *T. prieuriana* exhibited certain antioxidative potential with IC_{50} values of 23 μ g/ml, 175 μ g/ml and 377 μ g/ml, respectively. *C. tomentosa*, *D. rubicunda* and *T. floribunda* showed no free radical scavenging activity in the assay.

Acknowledgements: Czech Ministry of Education, Youth and Sports (Project No. 6046070901 and ME08070).

References: 1. Katerere, D.R. et al. (2003) Phytochemistry 63:81-88. 2. Katuura, E. et al. (2007) Afr J Ecol 45:48-51.

SIMILARITIES AND DIFFERENCES IN THE ESSENTIAL OILS OF LAMIACEAE SPECIES

Máthé I., Veres K., Háznagy-Radnai E., Szabó M.K., Máthé Á.

Institute of Ecology and Botany of the Hungarian Academy of Sciences, H-2163 Vácrátót
Institute of Pharmacognosy, Szeged University, H-6720, Szeged, Eötvös Str. 6.
Department of Botany, West Hungarian University, Fac. Agric. and Food Science, H-9200,
Mosonmagyaróvár, Vár 2, Hungary

Lamiaceae is an important essential oil bearing family within the Class Dicotyledonopsida. Numerous species, predominantly medicinal plants, belonging to the Subfamily Nepetoideae have relatively high essential oil content, while those with a low or no essential oil content belong to the Subfamily Lamioideae, in Erdtman's two subfamilial system (1). Recent investigations into the also 'non volatile oil containing' species have revealed that all of them contain volatile components, even if only in trace amounts. The goal of the investigations was to contribute to the overview of the essential oil content and composition of several species of the genera *Salvia*, *Stachys*, *Ballota*, *Marrubium*, *Lavandula*, *Melissa*, *Ocimum*, *Hyssopus*, etc. of both subfamilies. Experimental plants grown in Central Europe (in Vácrátót, Hungary), were analyzed after steam distillation by GC, GC/MS. The investigations confirmed that all of the species contain some essential oils. Their composition does not significantly differ from the results published by the scientific references, even despite the fact that many of the species were grown beyond their natural growing area. Among the more than 100 ingredients found in the essential oils, many, like α -, β -pinen, β -caryophyllene, etc. could be detected in almost all species, while others like α -, β -thujone (*Salvia*), thymol, carvacrol (*Thymus*, *Satureja*, *Origanum*, etc.), phenylpropane derivatives (*Ocimum*), etc. were dominant only in certain groups of plants. The results, together with data from the special literature, are evaluated also in view of the recent systematic considerations of the Lamiaceae family.

References

1. Cantino, P.S., R.W. Sanders, 1982. Subfamilial classification of Labiatae. Systematic Botany, 11. 163-185.

Acknowledgement: This work was sponsored by the National Grant (OTKA T43148)

FLAVONOLS AND AN OXYCHROMONOL FROM *PILIOSTIGMA RETICULATUM*

Wilfred Mabusela

University of the Western Cape, South Africa, wmabusela@uwc.ac.za

The leaf extract from the plant *Piliostigma reticulatum* was found to exhibit antimicrobial activity against some bacteria and fungi such as *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 10418), *Bacillus subtilis* (NCTC 8236), *Proteus vulgaris* (NCTC 4175), *Aspergillus niger* (ATCC 10578) and *Candida albicans* (ATCC 10231). Upon investigation of the chemical constituents present in the leaf extract, a total of seven compounds were isolated and their structures were unambiguously established by spectroscopic methods including HR-MS and NMR spectrometry. Four of the isolated compounds were novel, namely 6-C-methyl-2-p-hydroxyphenyloxymethyl (piliostigmol) (1), 6,8-di-C-methylquercetin-3,3',7-trimethylether (2), 6,8-di-C-methylquercetin-3,3'-dimethylether (3) and 3',6,8-tri-C-methylquercetin-3,7-dimethylether (4). The other three were known C-methylated flavonols and they were isolated from *P. reticulatum* for the first time. These were 6-C-methylquercetin-3-methylether (5), 6,8-di-C-methylkaempferol-3-methylether (6) and 6-C-methylquercetin-3,3',7-trimethylether (7). All the isolated compounds were tested for cytotoxicity using the brine shrimp toxicity assay and all of them were active albeit at different levels. With respect to the antibacterial activity, piliostigmol (1) showed the highest activity against *E. coli* (MIC = 2.57 µg/ml, 0.006 mmol), which is three times more than that of amoxicillin, whereas 4 and 7 showed the least activity.

BONE MINERALIZATION ENHANCING ACTIVITY OF A METHOXYELLAGIC ACID GLUCOSIDE FROM A *FEIJOA SELLOWIANA* LEAF EXTRACT

Mahmoud A. M. Nawwar, Nahla A. Ayou, Sahar A. Hussein, Amani N. Amani¹, Nesrine M. Hegaz¹ and Ulrike Lindequist

Department of Phytochemistry¹, Division of Pharmaceutical Industries, National Research Center, Dokki, Cairo, Egypt; Faculty of Pharmacy² Department of Pharmacognosy, Ain-Shams University, Cairo, Egypt; Institut für Pharmazie³, Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany

As part of an ongoing study to discover potential bioactive phenolics from terrestrial plant sources, the capability of an aqueous methanol extract obtained from the leaves of *Feijoa sellowiana* Berg. on possible prevention and treatment of osteoporosis has been examined by evaluating its stimulating effect on the two human osteoblastic cell lines HOS58 and SaOS-2. The extract was found to increase significantly the mineralization of cultivated human bone cell, whereby a clear increment (15.3 ± 2.7 %) in von Kossa positive area was determined when administering 25 µg/ml leaf extract. Besides, a phytochemical investigation of the extract has led to the isolation and identification of twenty three phenolics, among which the new 3-methoxyellagic acid 4-O-β-glucopyranoside was fully identified. The new compound was shown to have a slight positive effect on in vitro mineralization of SaOS-2 human osteosarcoma cells. However, it showed a significant increase of mineralized area at 20 µg/mL, while at lower concentrations the effect was not of significant value. It should be noted however that an increase of the number of mineralized spots (nodules) at all concentrations tested was observed. This can be taken as an evidence for increased cell maturation and osteoblasticity. This data give rise to the notion that the compound has a contribution to the significant effect exhibited by the *Feijoa sellowiana* crude extract.

ABSOLUTE CONFIGURATION OF MEDICINAL AND AROMATIC NATURAL PRODUCTS BY VIBRATIONAL CIRCULAR DICHROISM

Pedro Joseph-NathanCINVESTAV-IPN-MEXICO, Mexico, pjoseph@nathan.cinvestav.mx

Vibrational circular dichroism (VCD) is an emerging spectroscopic method for the absolute configuration determination of organic molecules, either isolated from nature or derived by synthetic procedures. VCD is a general method in which an optically active measurement can be compared to calculations performed using quantum mechanical equations in the ground state of the molecule. Comparison of both results, the measured spectrum and the one calculated using density functional theory (DFT), directly provides for molecules of moderate complexity the absolute configuration of the studied compound and its conformational preference in solution. Thus, it is a method that allows determining the absolute configuration without reference to a rule or to the need of structural correlation. VCD can be seen as the extension of classical circular dichroism, now also called electronic circular dichroism, from the ultraviolet and visible regions of the electromagnetic spectrum into the infrared region, or alternatively it can be seen as the determination of an optically active infrared spectrum. The main difficulty in measuring VCD spectra resides in the fact that bands that are being optically active in the infrared region are of very low intensity, in fact ten thousand to hundred thousand times less intense than classical infrared bands, thereby placing great demands on optical and electronic requirements of an instrument. Associated with this is the mercury-cadmium-telluride detector, used in VCD spectrophotometers, which operates at liquid nitrogen temperature. Results that allow highlighting some of the benefits and limitations of this methodology applied to several natural products will be presented. These include aromatic molecules like camphor, limonene, menthol, myrtenal or cedrene, and medicinal molecules like the distinction of both diastereoisomers of the tropane alkaloid 6-hydroxyhyoscyamine, the evaluation of verticillanes, which are precursors of taxol, and physostigmine. Regarding calculations, it has to be mentioned that a reasonable balance between computer time and accuracy can be settled when the DFT calculations are performed using the hybrid functional B3LYP and the 6-31G(d) or 6-31G(d,p) basis sets, although in some cases more complex functional and higher basis sets could be required. For the herein studied molecules, this results in very variable computer times, ranging from 3 to 40 h per conformer, when using commercial Gaussian 03 software on a PC system operated at 3 GHz with 2 Mb RAM.

QSAR STUDY OF ANTIOXIDANT AND VASODILATORY ACTIVITY OF PHENOLIC ACIDS

Marica Medic-Saric, Vesna RastijaDepartment of Medicinal Chemistry, Faculty of Pharmacy and B, Croatia, bebamms@pharma.hr
Faculty of Agriculture, Josip Juraj Strossmayer University o, Croatia, Vesna.Rastija@pfos.hr

Phenolic acids are aromatic secondary plant metabolites that are widely spread throughout the plant kingdom. The recent interest in phenolic acids comes from their potential protective role against oxidative damage diseases (coronary heart disease, stroke, and cancers) [1]. In order to explain the contribution of various structure-related parameters on biological activities of nine phenolic acids, antioxidative [Ferric Reducing Antioxidant Power (FRAP/mol L⁻¹), Trolox Equivalent Antioxidant Capacity (TEAC/mol L⁻¹)] and vasodilatory activities were correlated with "two-dimensional" (2D) topological indices, "three-dimensional" (3D) molecular descriptors, calculated physicochemical parameters and variable indicators of the number and

position of hydroxyl groups on the phenyl ring. The best diparametric QSAR model for the prediction of FRAP was obtained with Topological Polar Surface Area (TPSA) and mean distance degree deviation (MDDD) ($r^2 = 0.950$; $F = 60.93$). A statistically significant model for the prediction of TEAC values was obtained with two GETAWAY descriptors, R7p i HATS3e ($r^2 = 0.955$; $F = 64.00$). The best model for vasodilatory activity was obtained by multiple regression with TPSA and GETAWAY descriptor R+3(u) ($r^2 = 0.916$; $F = 32.76$). The results of the QSAR study have shown that biological activity of phenolic acids largely depends on hydrophylicity, electronegativity, polarizability, size and the shape of molecules.

References: [1] R J Robbins, 2003, J. Agric. Food Chem. 51 2866-2887.

BONE MINERALIZATION ENHANCING ACTIVITY OF A METHOXYELLAGIC ACID GLUCOSIDE FROM A *FEIJOA SELLOWIANA* LEAF EXTRACT

Mahmoud Nawwar, Nahla Ayoub, Sahar Hussein, Amani Amani, Nesrine Hegazi

Faculty of Pharmacy¹ Department of Pharmacognosy, Ain-Shams, Egyp, ayoub.n@link.net
 Department of Phytochemistry¹, Division of Pharmaceutical In, Egypt, drsahar90@yahoo.com,
 Department of Phytochemistry¹, Division of Pharmaceutical In, Egypt, drsahar90@yahoo.com,
 Department of Phytochemistry, Division of Pharmaceutical In, Egypt,
nesrinehegazi@yahoo.com,

As part of an ongoing study to discover potential bioactive phenolics from terrestrial plant sources [1,2], the capability of an aqueous methanol extract obtained from the leaves of *Feijoa sellowiana* Berg. on possible prevention and treatment of osteoporosis has been examined by evaluating its stimulant effect on the two human osteoblastic cell lines HOS58 and SaOS-2. The extract was found to increase significantly the mineralization of cultivated human bone cell, whereby a clear increment ($15.3 \pm 2.7\%$) in VON KOSSA positive area was determined when administering 25 ug/ml leaf extract. On the other hand a phytochemical investigation of the extract has demonstrated the high phenolic content and led to the isolation and identification of twenty three of them, among which the new 3-methoxyellagic acid 4-O-glucopyranoside was fully identified. All structures were elucidated on the basis of conventional analytical methods and confirmed by FTMS, 1D- and 2D-NMR data. The new compound was shown to have a slight positive effect on in vitro mineralization of SaOS-2 human osteosarcoma cells. However, it showed a significant increase of mineralized area at 20µg/mL, while at lower concentrations the effect was not of significant value. It should be noted however that an increase of the number of mineralized spots (nodules) at all concentrations tested was observed. This can be taken as an evidence for increased cell maturation and osteoblasticity. This data give rise to the notion that the compound has a contribution to the significant effect exhibited by the *Feijoa sellowiana* crude extract.

References: 1. Hussein, S. A. M., Nawwar, M. A. M. (2006) Pharmazie 61: 2993-2996 2- Hussein, S. A. M., Nawwar, M. A. M.(2007). Phytochemistry 68: 1464-1470 Extra authors: Michael Linscheid, Vice President for research, Humboldt Universität zu Berlin, 12489 Berlin, Germany Email: michael.linscheid@cms.hu-berlin.de Manuela Harms, Institut für Pharmazie, Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany Email: Harms@uni-greifswald.de Kristian Wende, Institut für Pharmazie, Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany Email: wende@uni-greifswald.de Ulrike Lindequist Institut für Pharmazie, Pharmazeutische Biologie, Ernst-Moritz-Arndt-Universität Greifswald, 17487 Greifswald, Germany Email: lindequi@uni-greifswald.de Presenter: Mahmoud A. M. Nawwar Department of

Phytochemistry, Division of Pharmaceutical Industries, National Research Center, Dokki, Cairo, Egypt

CHEMISTRY AND BIOLOGICAL EVALUATION OF CAMEROONIAN *DORSTENIA* SPECIES

Bonaventure Ngadjui, Merhatibet Bezabih, Berhanu Abegaz

Department of Organic Chemistry, Faculty of Science, University of Yaounde 1 BP 812, Yaounde, Cameroon, ngadjuib@yahoo.fr, Department of Chemistry, Faculty of Science, University of Botswana, Private Bag 0022, Gaborone, Botswana, Department of Chemistry, Faculty of Science, University of Botswana, Private Bag 0022, Gaborone, Botswana, abegazb@mopipi.ub.bw

Research in the area of natural products is an important scientific activity in Cameroon where a large variety of plants, especially, *Dorstenia spp* are used as anti-snakebite, anti-infection and anti-rheumatism remedies in medicinal plant therapy. The genus is recognized as a rich source of prenyl and geranyl-substituted coumarins and flavonoids [1]. There is now increasing interest in this genus. Almost 40 papers have appeared during the last decade, reporting on research results on over 25 *Dorstenia* species. As part of our program to study the chemical constituents of African *Dorstenia* species [1] we have investigated 14 species from Cameroon. More than hundred secondary metabolites, of which thirty are new derivatives, were isolated using various chromatographic techniques (VLC, CC, PTLC), and characterized using extensive spectroscopic methods. These compounds are regrouped into: triterpenoids, styrenes, coumarins and mainly flavonoids [1]. 3, 3'- bis [3, 7-Dimethyl-2, 6-octadienyl]-4, 5, 2', 4'-tetrahydroxylchalcone, isolated from the twigs of *D. prorepens*, is the first example of a bis-geranylated chalcone. Eleven diprenylated flavonoids have so far been isolated from *Dorstenia* and all of them are reported to be isolated from the twigs of *D. mannii* [1]. *D. psilurus* provided triprenylated flavonoids with two units in ring A and one in ring B [1]. The pharmacological data of this genus is scanty. Extracts and/or compounds from the investigated species show anti-inflammatory, analgesic, anti-oxidant and antimalarial activities [2]. Inhibition of matrix-metalloproteinase-2 secretion from brain tumour cells suggests chemo-preventive properties of furanocoumarin glucoside and chalcones isolated from *D. turbinata* [3]. To the best of our knowledge, prenylated flavonoids have only been reported from African *Dostenia* species so far.

References:

- [1] Abegaz B.M., Ngadjui B.T et al. 2000, Current Organic Chemistry, 4, 1079-1090.
[2] Ngameni B., Watchueng J. et al., 2007, Arkivoc (XIII) 116-123 [3] Ngameni B. Touaibia M. et al, 2007, Phytochemistry, 67, 2573-2579

THERAPEUTIC EFFECTS OF EXTRACTS OF ANNONA SENEGALENSIS AND EUCALYPTUS CAMALDULENSIS IN EXPERIMENTAL AFRICAN TRYPANOSOMIASIS.

Emmanuel Olofu Ogbadoyi, Adamu Kabiru, Aderonke Salako, Joseph Okogun

Federal University of Technology, Minna, Nigeria, eogbadoyi@yahoo.com
Federal University of Technology, Minna, Nigeria, Federal University of Technology, Minna, Nigeria, National Institute for Pharmaceutical Research and Developme, Nigeria

Human African trypanosomiasis caused by trypanosome is 100% fatal if untreated. Currently chemotherapy, is the major control measure but it is grossly inadequate. Thus there is an urgent

need for new drugs. Leaves, stem and root barks of *Annona senegalensis* and *Eucalyptus camaldulensis* were sequentially extracted with hexane, ethylacetate, methanol and water. Administration of crude and partially purified aqueous extracts of *A. senegalensis* leaves at a dose of 200 mg/kg BW to mice experimentally infected with *Trypanosoma brucei* cured the experimental infection. Blood and cerebrospinal fluid (CSF) infectivity tests failed to produce any infection 2 months after subinoculation of blood and CSF from the cured mice into healthy mice [1]. Hexane and aqueous extracts of the stem bark of *A. senegalensis* also cured experimental *T. brucei* infection in mice. Hexane, ethylacetate, and methanol extracts of leaves of *E. camaldulensis* each administered at a dose of 200mg/kg BW all cured experimental *T. brucei* infection in mice. Similarly blood and CSF infectivity tests failed to produce infection in mice. Administration of a combination of methanolic extracts of the leaves of *A. senegalensis* and *E. camaldulensis* completely cured experimental *T. brucei* infection in mice. We conclude that the extracts of *A. senegalensis* and *E. camaldulensis* used in combination therapies have enormous potentials of overcoming drug resistance problem in sleeping sickness chemotherapy.

BIOLOGICAL ACTIVITIES OF EXTRACTS OF *PYCNANTHUS ANGOLENSIS* (WELW.) WARB

Patricia Onocha, Elizabeth Otunla

Department of Chemistry, Faculty of Science, University of Ibadan Ibadan, Nigeria,
triciaonocha@yahoo.com

Chloroform and methanolic extracts of the leaves, roots and stem of *Pycnanthus angolensis* (Myristicaceae) were investigated with the goal of establishing its acclaimed potency as an anthelmintic and antimicrobial agent. The result of the agar diffusion studies revealed that the leaf methanol extract caused inhibition against two of the 5 bacterial strains namely, *Salmonella typhii* and *Pseudomonas aeruginosa* used for the study. It also exhibited marked inhibition against the three fungal strains used for the study, the order of sensitivity being *Aspergillus niger* > *Candida albicans* > *Dermatophyte sp.* The chloroform extract of the leaves and the methanol extracts of the leaves and stem exhibited considerable anthelmintic activities in vitro using *Fasciola gigantica*, *Taenia solium* and *Pheritima pasthuma*. The sensitivity was concentration-related and comparable to that of the reference compound piperazine citrate. These results are consistent with the folklore use of the plant in treatment of helminthic and microbial infections.

Acknowledgement: Senate Research Grant (SRG/FSC/2000/22A).

INSECTICIDAL, ANTIMICROBIAL, PHYTO- AND CYTOTOXICITY OF *CHASSALIA KOLLY* WHOLE PLANT EXTRACT

P.A. Onocha, S.M. Ali

Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria,
triciaonocha@yahoo.com, H.E.J. Research Institute of Chemistry, International Centre for
Chemical Sciences, University of Karachi, Karachi 75270, Pakistan, shaiq303@hotmail.com

Chassalia kolly (Schumach.) Hepper (Rubiaceae) is used in ethno-medical practices,

particularly in Nigeria and the West African sub-region, for the treatment of typhoid and fevers that are endemic in Nigeria as well as in the tropics. The methanol extract of the dried whole plant *Chassalia kolly* was investigated for insecticidal, antimicrobial, phyto- and cytotoxicity activities. The extract inhibited the growth of all the bacteria tested in the agar cup plate diffusion technique, exhibiting a concentration dependent activity. Cytotoxicity was evaluated using the brine shrimp lethality assay. The extract was found to be relatively non-toxic as it had an LD50 value greater than 1000 µg/ml. Phytotoxicity tests using the Lemna bioassay revealed a moderate growth inhibitory effect against *Lemna minor*. The insecticidal assay by contact toxicity method also revealed a moderate insecticidal rate of 40% against *Rhizopertha dominica* at the concentration of 1572.7 µg/cm². The extract contained glycosides, alkaloids and flavonoids.

Acknowledgement: Senate Research Grant (SRG/FSC/2000/22A), H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan.

CHEMICAL COMPOSITION AND BIOLOGICAL EVALUATION OF *HERACLEUM PASTINACIFOLIUM* SUBSP. *TRANSCAUCASICUM* AND *H. PASTINACIFOLIUM* SUBSP. *INCANUM* ESSENTIAL OILS

Gulmira Ozek, Fatih Demirci, Gokalp Iscan, Nurhayat Tabanca, DE. Wedge, A. Duran, E. Hamzaoglu, K.H.C. Baser

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, gozek@anadolu.edu.tr, Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, fdemirci@anadolu.edu.tr, Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, giscan@anadolu.edu.tr, USDA-ARS-NPURU, University of Mississippi, University, MS 38677, USA, ntabanca@olemiss.edu, Department of Biology, Faculty of Education, Selcuk University 42090 Meram-Yeniyol, Konya, Turkey, Department of Biology, Yozgat Faculty of Science and Letters, Bozok University, Yozgat, Turkey

A study was conducted to evaluate the chemical composition and biological activities of *Heracleum pastinacifolium* C. Koch subsp. *transcaucasicum* (Manden.) Davis and *H. pastinacifolium* C. Koch subsp. *incanum* (Boiss. et Huet) Davis (Apiaceae) essential oils. Fruits and stems were separately subjected to hydrodistillation followed by simultaneous analysis by GC-FID and GC/MS. Fruit oil yields from each subspecies were 1.34% and 1.70%, respectively and stems from both subspecies produced very poor oil yields (0.03% and 0.04%, resp.). Fruit oils were found to be rich in esters (79% and 81%) with octyl acetate (35% and 5.6%), octyl butyrate (14% and 38%), and hexyl butyrate (8% and 30%) as major constituents of subsp. *transcaucasicum* and subsp. *incanum*, respectively. Over half of the stem oil of subsp. *transcaucasicum* was comprised fatty acids (53%) with hexadecanoic (27%), tetradecanoic (15%) and (Z)-9-hexadecenoic (11%) acids as main constituents. Phenylpropanoids (55%) with myristicin (50%) as the major compound comprised more than half of the stem oil of subsp. *incanum*. Fruit oils were subsequently investigated for antimicrobial, antifungal, antileishmanial, antimalarial, cytotoxic, and anticancer activities. Oils from both subspecies demonstrated weak antimicrobial activity in micro-dilution broth assays. Antileishmanial activity of the oil of subsp. *incanum* was determined to be IC₅₀= 50 µg/mL and IC₉₀= 90 µg/mL, while the oil of subsp. *transcaucasicum* was inactive towards leishmanial cells. There was no antimalarial activity or cytotoxicity against mammalian kidney fibroblasts (Vero). Oils were also ineffective against the plant pathogens *Colletotrichum acutatum*, *C. fragariae* and *C. gloeosporioides* tested by direct bioautographic assay.

CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *UMBELLIFERAE* SPECIES FROM THE CENTRAL AND NORTHEASTERN REGIONS OF KAZAKHSTAN

Gulmira Özek, Yerlan Suleimenov, Margarita Yu. Ishmuratova, Sluken B. Rakhmadiyeva, K.H.C. Baser

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskisehir, Turkey, gulmiraozek@gmail.com, Department of Chemistry, L.N. Gumilyov Eurasian National University, 010008 Astana, Kazakhstan, syerlan75@yandex.ru, Research and Production Centre "Phytochemistry", 100009 Karaganda, Kazakhstan, randr@hotbox.ru, Department of Chemistry, L.N. Gumilyov Eurasian National University, 010008 Astana, Kazakhstan, rakh.sluken@mail.ru

Essential oils of the Umbelliferae species from the Central and North-eastern regions of Kazakhstan were analyzed by GC-FID and GC/MS methods. *Eryngium planum* L., *Cnidium dubium* (Schkuhr) Schmeil et Fitschen, *Conioselinum tataricum* Hoffm, *Heracleum sibiricum* L. and *Ferula soongarica* Pall. ex Schult., collected at flowering stage, were subjected to hydrodistillation in a Clevenger type apparatus to obtain essential oils. Most of the studied oils were predominated by monoterpene type constituents with alpha-terpinyl acetate (24.8%), alpha-pinene (18.2%) and limonene (12.6%) in *Cnidium dubium*; gamma-terpinene (39.7%), p-cymene (19.4%) and beta-pinene (15.7%) in *Conioselinum tataricum*; (E)-beta-ocimene (41.5%), myrcene (31%) and (Z)-beta-ocimene in *Heracleum sibiricum*; alpha-pinene (17.9%) and myrcene (16.0%) in *Ferula soongarica*, as main constituents. The oil of *Eryngium planum* was dominated by germacrene type sesquiterpenes with germacrene D (25.7%), germacrene B (5%) and bicyclogermacrene (3.7%) as major representatives. Among the monoterpenes group, trans-chrysanthenyl acetate (18.9%) was the major constituent.

COMPOSITION OF THE VOLATILE METABOLITES FROM DIFFERENT PARTS OF *TORILIS LEPTOPHYLLA* (L.) REICHB. AND *T. UCRANICA* SPRENGEL

Temel Özek¹, Gulmira Özek¹, Michael G. Pimenov², Eugenij V. Kljuykov², K.H.C. Baser¹

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, tozek@anadolu.edu.tr, Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir, Turkey, gozek@anadolu.edu.tr, Moscow State University, Botanical Garden, 119992, Moscow, Russia, mgpimenov@mail.ru, Moscow State University, Botanical Garden, 119992, Moscow, Russia, mgpimenov@mail.ru

The volatile metabolites obtained from different aerial parts of *Torilis leptophylla* (L.) Reichb. and *T. ucranica* Sprengel (Umbelliferae) were analyzed by GC-FID and GC/MS methods. Plants were collected at the end of June 2007 in the Eskisehir province of the Central Anatolian region of Turkey. The leaves, fruits and flowers were separately subjected to micro-distillation, using an Eppendorf MicroDistiller to obtain volatile metabolites. The leaf and fruit volatiles of *T. leptophylla* were characterized by high percentages of myrcene (8.9% and 15.6%, respectively), germacrene D (13.9% and 13.7%, resp.) and spathulenol (6.5% and 3.6%, resp.). The leaf and flower volatiles of *T. ucranica* were characterized by high amounts of sesquiterpenes with germacrene D (31.7% and 27.5%, resp.), β -ylangene (6.8% and 5.9%, resp.) and bicyclogermacrene (5.3% and 6.9%, resp.) as major constituents. Among the fruit volatiles of *T. ucranica* himachalene-type sesquiterpenes, namely 1- α -H-himachal-4-en-1- β -ol

(50.6%), β -himachalene (10.2%) and 1-himachalen-7-ol (0.8%) were predominant. The major monoterpene of the flowers and fruits of *T. ucranica* was found to be β -pinene (15.1% and 11.3%, resp.). To the best of our knowledge, this is the first report on the volatiles of *T. ucranica*.

COMBINED MOLECULAR AND CHEMICAL ASSESSMENTS OF DIFFERENT CULTIVARS OF *TAMARINDUS INDICA* IN THAILAND

S. Pongsamart, S. Sukrong, P. Khanthapok and P. Bhusawang

Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, 10330, Thailand
Program in Biotechnology, ³ Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

Tamarind pulp has long been medically used as mild laxative. There are many cultivars of *Tamarindus indica* L., in Thailand. This study, tamarind cultivars with laxative activity was identified. Sequence of *rbcl* gene of each cultivar was used to correlate with chemical contents in tamarind pulps. Fruits and leaves of sweet-tamarind cultivars, "Srichomphu" and "Khanti", and sour-tamarind "Priao-yak" were collected from Phetchabun province. Gene *rbcl* of "Srichomphu" and "Khanti" composed of 1,428 bp, where only part in *rbcl* gene 1,398 bp of "Priao-yak" was sequenced. The sequence of 1,368 bp in *rbcl* gene of all three cultivars were analyzed and compared with *rbcl* gene of *T. indica* (accession no.Z70160), there were 24 base-substitutions, and dendrogram was constructed using UPGMA method. Sweet-tamarind, "Srichomphu" and "Khanti", belonging to Group I whereas sour-tamarind "Priao-yak" belonging to Group II. Organic acids, laxative active component, were determined by HPLC. The major organic acids in tamarind were tartaric, malic, citric, and oxalic acids. Tartaric acid content in each tamarind cultivar was significantly different ($p < 0.05$), and "Priao-yak" had the highest content. Malic, citric and oxalic acids contents in sweet-tamarind, "Srichomphu" and "Khanti", were not significantly different ($p > 0.05$), however, these values were significantly different from those of sour-tamarind "Priao-yak". Carbohydrate, reducing sugar, protein, and fiber contents in each tamarind cultivar were significantly different. Ash contents in sweet-tamarind, "Srichomphu" and "Khanti", were not significantly different, but those values were higher ($p < 0.05$) than that of sour-tamarind "Priao-yak". Combined molecular and chemical assessments can be used to characterize and identify tamarind cultivars

Acknowledgements: Faculty of Pharmaceutical Sciences and Post Graduated Research Fund, Chulalongkorn University.

A RAPID *IN VITRO* METHOD FOR THE EVALUATION OF CANDIDATE REPELLENTS AGAINST *LEPTOTROMBIDIUM CHIGGERS*

W. Rodkvamtook, A. Prasartvit, J. Gaywee, C. Jatisatienr

Department of Biology, Faculty of Science, Chiang Mai Univer, Thailand, Itwutti@hotmail.com
Department of Disease Control Ministry of Public Health, Nonthaburi 11000, Thailand
Armed Forces Research Institute of Medical Science (AFRIMS), Bangkok 10400, Thailand

Scrub typhus is an acute febrile zoonotic disease resulting from infection with the gram-negative intracellular bacteria *Orientia* (formerly *Rickettsia*) *tsutsugamushi* (Hyachi) (Seong et al. 2001). The disease is endemic in much of south and central Asia, with approximately one million cases each year and more than a billion people at risk worldwide (Rosenberg 1997). Scrub

typhus is transmitted by several species of larval trombiculid mites which are commonly known as chiggers (Tanskul et al. 1998). Repellents provide an effective method of protecting individuals from arthropods (Gupta and Rutledge 1994). In this study 6 essential oils were tested to evaluate their repellent activity against the chigger, *Leptotrombidium imphalum* Vercammen-Grandjean and Langston. A rapid and economic in vitro procedure which requires only 5 min and a small number of chiggers was used to determine the median effective doses. The results showed that clove oil was significantly more effective than others with ED50 of 53.2 µg followed by vetiver oil (<4.0 mg), orange oil (6.31 mg), oil of *Zingiber cassumunar* Roxb. (6.40 mg) and turmeric oil (32.6 mg) whereas oil of *Boesenbergia rotunda* (Linn.) Mansf. expressed slight efficiency only.

SUSTAINABLE DEVELOPMENT OF AFRICAN NATURAL PLANT PRODUCTS

J.E. Simon, R. Juliani, D. Acquaye, E. Jefthas, J. Asante-Dartey, Bismarck Diawuo, M. Diatta B. Diouf, P. Langenhoven, N. Hitimana, Pierre Tannous, Kerry Hughes, Ramu Govindasamy and Jerry Brown

New Use Agriculture and Natural Plant Products Program, Rutgers University, New Brunswick, NJ 08901, USA (www.pfidnp.org)

ASNAPP-Ghana, Accra (www.asnapp.org)

ASNAPP-South Africa, Dennesig, Stellenbosch

ASNAPP-Zambia, Lusaka

ASNAPP-Senegal, Dakar

ASNAPP-Rwanda and World Relief, Kigali

EthnoPharm, Vallejo, California

Agricultural Economics and Marketing and the Food Policy Institute, Rutgers University, New Brunswick, NJ

USDA/FAS-Washington, D.C.

The development of sustainable agricultural businesses in high-value niche sectors can stimulate agricultural growth that could improve the livelihoods of rural Africa and have a multiplier effect on the rest of the economy. The natural plant product (NP) industry is one that can offer higher income and niche markets for resource limited farmers in developing countries compared to traditional agronomic crops. However, the NP industry is beset with challenges hindering the realization of its full contribution to economic development and poverty reduction. Among these challenges are: (1) limited appropriate germplasm; (2) lack of quality control and quality assurance systems for production and processing; (3) Lack of knowledge and understanding of international markets and markets channels; (4) Limited processing infrastructure which constrains value-added opportunities; (5) variable market prices, and (6) weak enterprises with low technical and managerial skills to meet the requirements of buyers. In 1999, we began to develop models for the sustainable commercialization of NP in sub-Saharan Africa using a market-first and scientific-driven approach. This program implemented under ASNAPP (www.asnapp.org) is conducted in partnership with the public and private sector to facilitate diversification of agricultural commodities and marketing channels. Sustainable development incorporates good environmental stewardship from the bush to final product, GAP and robust quality assurance and quality control systems for collection or cultivation to ensure high quality and food safety. This evolving model consists of a multi step value-addition process through the commodity chain to provide safe and high quality NPs and economic opportunities to those rural communities.

MOSQUITO REPELLENTS FROM ESSENTIAL OILS OF PLANTS FROM FRENCH POLYNESIA

Isabelle Vahirua-Lechat, Fanny Adam, Eric Deslandes, Ulrich Bernier

Louis Malarde Institute, French Polynesia, ilechat@ilm.pf
Lebham-UBO-ILM, France, FAdam@ilm.pf
University of Brittany, France, edeslandes@univ-brest.fr
USDA-ARS-CMAVE, USA

Filariasis and Dengue Fever are two mosquito-borne diseases threatening French Polynesia. The potential vectors for the transmission of these illnesses are *Aedes aegypti* (L.), the Yellow Fever mosquito, and *A. polynesiensis* Marks. A research program financed by the Ministry of Health of French Polynesia, the Establishment for Prevention and the Laboratory of Ecophysiology and Biotechnology of Halophytes and Marine Algae was undertaken with the ultimate goal of developing improved insect repellents and lures for insect traps. This goal became even more urgent due to the threat of mosquito-borne diseases. Several natural products from terrestrial plants of French Polynesia were evaluated as spatial repellents against *A. aegypti* mosquitoes, using a triple cage-dual port olfactometer, and as topical repellents, using a cloth patch assay test with DEET as the positive control. The attraction of *A. aegypti* to L-lactic acid combined with the natural sample extracts was evaluated using the dual-port olfactometer. A total of 10 aromatic plant species have been collected by the Laboratory of Natural Products of the Institute Louis Malard[©], and these have yielded 10 Essential Oils. 4 more samples of essential oils originating from New Caledonia and Madagascar were also tested. The fine chemical composition of some repellent essential oils were determined by gas chromatography and gas chromatography coupled with Mass Spectrometry at Malard[©] and at Montpellier. Their major volatile components are still to be tested and the preliminary results are presented. Bioassays were performed with *Ae. aegypti* mosquitoes at the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS). This mosquito is the commonly accepted standard species for laboratory bioassays. Bioassays were conducted in randomized sample order and were distributed among the three cages of the olfactometer over three separate assay periods per day (approximately 08.00, 11.00, and 13.00 local time). Each sample was analyzed at least 3 times to improve the statistical precision of the results. The cloth patch repellency assays were conducted using 5-6 individuals to provide adequate precision of the duration and minimum effective dosage measures of the repellent efficacy.

THEME 4**TARGETED SCREENING APPROACHES FOR DRUGS AND COSMETICS**

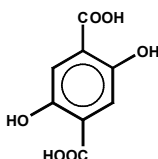
SPICATIC ACID: A 4-CARBOXYGENTISIC ACID FROM A *GENTIANA SPICATA* EXTRACT WITH POTENTIAL HEPATOPROTECTIVE ACTIVITY

Nahla Ayoub, Heba Handoussa, Laila Mahran

Faculty of Pharmacy, Department of Pharmacognosy, Ain-Shams University, Cairo, Egypt

Department of Pharmaceutical Biology, Faculty of Pharmacy and biotechnology, German University in Cairo, Egypt

Due to our interest in bioactive plant derived materials, investigation of the protective mechanisms of aqueous alcoholic extract of *Gentiana spicata* (Gentianaceae) in carbon tetrachloride intoxicated rats was undertaken in the present work. Animals were treated with aqueous alcoholic extract of *Gentiana spicata* (Gentianaceae) for 14 days IP at a dose of 1mg/kg followed by a single dose of carbon tetrachloride at the dose of 1 ml/kg body weight intraperitoneally. The hepatoprotective activity of the extract of *Gentiana spicata* was evaluated by measuring levels of liver serum marker enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Administration of the extract (250 mg/kg, b.wt.) significantly ($p < 0.05$) reduced CCl_4 -induced elevation of levels of serum AST and ALT. Treatment of rats with CCl_4 led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA). This was associated with a significant reduction of the hepatic antioxidant system e.g. reduced glutathione (GSH). These biochemical alterations resulting from CCl_4 administration were significantly ($p < 0.05$) inhibited by pretreatment with the extract of *Gentiana spicata*. These data suggest that the aqueous alcoholic extract of *Gentiana spicata* may act as a hepatoprotective and antioxidant agent. Also, in the course of the present work, a phytochemical study of this extract has demonstrated the high phenolic content and led to the isolation and identification of the new carboxygentisic acid, 1,4-dicarboxy 2,5-dihydroxybenzene, for which we suggest the name spicatic acid, together with the two known flavonoids, quercetin 3-O-[(2,3,4-triacetyl- α -rhamnopyranosyl)-1 \rightarrow 6]-3-acetyl- β -galactopyranoside and quercetin 3-O-[(2,3,4-triacetyl- α -rhamnopyranosyl)-1 \rightarrow 6]-4-acetyl- β -galactopyranoside. All structures were elucidated on the basis of conventional analytical methods and confirmed by high resolution FTMS, 1D- and 2D-NMR data. The new phenolic carboxylic acid, spicatic acid is of special interest as it represent the first phenolic acid which bears two carboxyl function in one aromatic ring.



The new natural phenolic: Spicatic acid

IDENTIFICATION OF NEW ADVANCED GLYCATION END PRODUCTS INHIBITORS:
DEVELOPMENT OF A 96-WELL PLATE SCREENING

Amelie Chaigneau, Valinov, France, amelie.chaigneau and Pascal Richomme

University of Angers, France (Presenter), pascal.richomme@univ-angers.fr

The non-enzymatic Maillard reaction is divided into two stages. The early one consists in the formation of a Schiff base between the amino group of a protein and the aldehyde group of a reducing sugar which then evolves into an Amadori rearrangement product. The later one involves several processes: complicated oxidation reaction, dehydrogenation and condensation followed by formation of fluorescent proteins through the so-called Advanced Glycation End Products (AGEs). This glycation of proteins causes structural as well as functional changes. Indeed, the accumulation of AGEs may contribute to the pathogenesis of diabetes, neurological diseases or vascular disorders. AGEs also play a predominant role in cellular aging. Therefore AGEs inhibitors and breakers (AGEIB) not only represent potential therapeutics for the aforementioned diseases^{1,2} but can also be used in the formulation of new food supplements or innovative cosmetics. In this aim a new 96-well plate screening of AGEIB was developed in our laboratory. The efficiency of this test will be discussed and illustrated by many examples dealing with natural products (crude plant extracts, secondary metabolites, structural analogues). Besides, in order to study specific inhibitors of the last and irreversible steps of the formation of AGEs, several improvements of this evaluation will be proposed.

SENSITIZING EFFECT OF CURCUMIN ON CISPLATIN-INDUCED APOPTOSIS INVOLVES SUPEROXIDEANION INDUCTION AND BCL-2 DEGRADATION

Pithi Chanvorachote, Varisa Pongrakhananon, Sudjit Luanpitpong, Ubonthip Nimmannit

Department of Physiology, Faculty of Pharmaceutical Sciences, Thailand,
pithi_chan@yahoo.com

The possibility of using curcumin as a chemotherapeutic sensitizing agent has been intensively demonstrated in some cancers. However, the effect of curcumin on non-small cell lung cancer (NSCLC), one of the most resistant cancers, is largely unknown. The aim of this study was to investigate the sensitizing effect of curcumin on cisplatin-induced apoptosis in NSCLC cells. Curcumin was shown to induce intracellular superoxide anion generation, down-regulate anti-apoptotic Bcl-2 protein, and subsequently sensitize NSCLC H460 cells to cisplatin-induced apoptosis. Amplification and overexpression of bcl-2 protein has been implicated in chemotherapeutic resistance in many cancers and overexpression of this protein strongly rendered H-460 cells resistant to cisplatin-induced apoptosis. The present study showed that co-treatment of the cells with curcumin and cisplatin resulted in increased apoptosis and reversal of Bcl-2-mediated cisplatin resistance. The mechanism by which curcumin down-regulates Bcl-2 and sensitizes cells to cisplatin-induced apoptosis involves proteasomal degradation of Bcl-2, since a specific proteasome inhibitor lactacystin reversed effect of curcumin on bcl-2 level. These findings indicate a novel pathway for curcumin regulation of Bcl-2, which benefits the development of a cisplatin sensitizing agent.

EXPRESSION OF MRNAS FOR TYROSINASE GENES ON EXPOSURE TO PLANT EXTRACT/COMPOUNDS

Wai Kuen Cheung, Namrita Lall, Brenda Mapunya, Sanushka Naidoo, Peter Houghton

University of Pretoria, South Africa, kenovec@yahoo.com.hk

Over production of melanin pigment results in hyperpigmentation disorders. Melanin is produced by melanocyte cells in the basal layer of the dermis. Ineffectiveness of the present depigmenting agent with side effects led to the exploitation of alternative solutions to the problem. Several South African plants were selected to test their activity on tyrosinase. Ethanol extracts were evaluated for their inhibitory effect on tyrosinase and antioxidant activities *in vitro*, using a colorimetric procedure. Active extracts were investigated for their inhibitory effect on melanogenesis in cultured B16 melanoma cells. IC₅₀ of *Sideroxylon inerme*, *Greyia flavaganii* and *Galenia africana* were found to be 63.12±1.2, 148±0.7 and 240±1.3 µg/ml respectively when L-tyrosine was used as a substrate. Two active compounds, epigallocatechin gallate and procyanidin B1, isolated from *S. inerme*, exhibited IC₅₀ values against monophenolase of 30 and >200 µg/ml respectively. *S. inerme* exhibited 37% reduction of melanin content at 6.2 µg/ml in melanocytes without being significantly toxic to the cells. *S. inerme*, epigallocatechin gallate and procyanidin B1 exhibited antioxidant DPPH radical scavenging activities with EC₅₀ values of 1.54, 1.33 and 1.68 µg/ml respectively. The inhibition of tyrosinase activity relative to the inhibition of its activity at transcriptional level was evaluated by determining the degree of expression of mRNAs for the tyrosinase gene by the extracts and the purified compounds with semi-quantitative RT-PCR. *S. inerme* extracts at 25 µg/ml was evidenced as a potent tyrosinase inhibitor in the mechanism studies. *S. inerme* showed promising results for skin-depigmentation and clinical trials are underway.

Acknowledgements: NRF, Royal Society.

THE EFFECT OF SEASONAL CHANGE ON THE ANTIBACTERIAL ACTIVITY OF *C. EDULIS*

P.K. Chokoe, P. Masoko, M.P. Mokgotho, R.L. Howard and L.J. Mampuru

Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa

Carpobrotus edulis (family Aizoaceae) has been used over the years to treat microbial infections. Previously, the antibacterial activity of the plant had only been qualitatively assessed. The antibacterial activity of the plant harvested during different times of the year was quantitatively evaluated by preparing extracts of varying polarities from the leaf debris and filtrate of a Spring and an Autumn harvest of *C. edulis*. Thin layer chromatography was used to analyze the phytochemicals of the extracts as well as to assay the plant for antioxidant compounds. The Spring harvest showed equal distribution of the phytochemicals within the leaf debris and the filtrate, but there was a high prevalence of phytochemicals within the leaf debris extracts of the Autumn sample. An antioxidant compound was intensely pronounced in the Autumn extracts of intermediate polarity and in the polar extract. The plant was evaluated for antibacterial activity against *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* by using a two-fold serial microdilution method as well as bioautography. *Staphylococcus aureus* was found to be the most sensitive bacterium to the plant extracts and *P. aeruginosa* was resistant to the extracts. The spring extracts were more potent against all test organisms, showing MIC values that were lower than those of the Autumn extracts. When taking the total activity of each extract into account, the autumn extracts showed higher efficacy than the extracts from the Spring sample. The antibacterial activity observed in extracts of both seasons somewhat validated the ethnomedicinal usage of *C. edulis* by traditional healers.

Acknowledgements: University of Limpopo Senate Research Fund and National Research Foundation provided funding.

α – AMYLASE INHIBITORY ACTIVITY OF *PARKIA BIGLOBOSA* (MIMOSOIDEAE) SEED**Adediwura Fred-Jaiyesimi, Kio Abo, Richard Wilkins**

Faculty of pharmacy, olabisi onabanjo university, Nigeria, adediwurajaiyesimi@gmail.com
 Faculty of pharmacy, university of ibadan, Nigeria, , school of biology, University of newcastle,
 England, U.K.

Parkia biglobosa (Jacq) Benth is a perennial legume tree grown in the Sudan and Guinea ecological Zones of West Africa. The fermented seed is used in West Africa to spice, flavour and thicken soups. Ethnomedically, it is reported as treatment for diabetes mellitus, snakebites, diarrhoeal infections, leprosy, hypertension and dental problems. Previous studies have reported the antidiarrhoeal, antihypertensive and antisnake venom activities. This bioactivity- guided study of *Parkia biglobosa* fermented seed using the alpha amylase inhibitory assay method adopted from Bernfeld (Bernfeld 1951; 1955) reports its activity. The methanol extract, its chloroform, chloroform/methanol and diethyl ether fractions were assayed for alpha amylase inhibitory potentials. The chloroform/methanol fraction exhibited a pronounced α – amylase inhibitory activity of 62% ($p < 0.05$) comparable to that of Acarbose. The active fraction was subjected to column chromatography using solvents of varying polarities, an active sub-fraction obtained significantly inhibited the alpha amylase enzyme activity by 64% ($P < 0.05$) compared to that of Acarbose (a known α -glucosidase inhibitor) which exhibited 59% inhibition. This study provides evidence that *P. biglobosa* and its fractions have ability to lower blood glucose by inhibiting the activity of the digestive enzyme, alpha amylase. Furthermore, it shows the nutraceutical potentials of *Parkia biglobosa* fermented seed (a West African spice).

References: 1. Bernfeld, P (1951). Enzymes of starch degradation and synthesis in, *Advances in Enzymology* XII, F. Noed, Interscience publ. NY 379. 2. Bernfeld, P (1955). *Methods in Enzymology* 1, 149 – 158

Comment [A3]: confirm from authors

ANTI-CANCER AND OXIDANT PROPERTIES OF DIFFERENT SESQUITERPENE LACTONES ISOLATED FROM INDIGENOUS LEBANESE PLANTS

Akram Ghantous, Hala Gali-Muhtasib, Najat Saliba, Nadine Darwiche

American University of Beirut, Lebanon, ayg01@aub.edu.lb

Most cancers are of epithelial origin of which skin cancer is the most frequent. Numerous naturally occurring compounds have been identified as skin chemopreventive agents, and many of the best-selling anticancer drugs are plant-derived. While screening for anti-cancer activities of forty-four Middle Eastern plant extracts used in folk medicine, we identified the Lebanese indigenous plants, *Centaurea ainetensis* and *Achillea falcata*, to possess potent and selective activities against cancer cells. Bioassay-guided fractionation of these plants' crude extracts led to the isolation of the sesquiterpene lactone family of plant secondary metabolites having the most potent anti-tumor activities. These included two guaianolides from *C. ainetensis*: Salograviolide A (Sal-A) and its hydroxy-isomer and four seco-guaianolide derivatives from *A. falcata*: Tanaphillin, 3-beta-methoxy-3-deshydroxy-iso-seco-tanaphthalide (beta-Tan), iso-seco-

tanaparholide, and 8-hydroxy-3-methoxy-iso-seco-tanaparatholide. We determined the growth inhibitory effects of the isolated sesquiterpene lactones on the human epidermal HaCaT cells, a well characterized model for early stage skin carcinogenesis, and correlated these activities with oxidant potential. We found that Sal-A and beta-Tan exhibited the most potent growth inhibitory effects at concentrations that were not cytotoxic to primary human keratinocytes. Sal-A caused pre-G0/G1 cell cycle arrest and reduced cyclin D1 proteins while beta-Tan induced G2/M arrest and decreased cyclin B1 proteins. Sal-A- and beta-Tan-induced growth inhibition was preceded by an early and progressive accumulation of reactive oxygen species (ROS). Pre-treatment with different anti-oxidants reduced drug-induced ROS generation and abrogated growth inhibition. These studies provide a rationale for designing plant-derived anti-cancer drugs from sesquiterpene lactones.

TRICHILIA EMETICA: ETHNOBOTANICAL SURVEY AND THE ANTITYROSINASE ACTIVITY RESULTS

Komane B, Olivier I.E., A.M.

Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa, Komanemb@tut.ac.za

African traditional medicine greatly contributes to the cosmetics industry. However scientific data confirming its use in improving post-inflammatory hyperpigmentation is sparse. *Trichilia emetica* is one of many medicinal plants used for dermatological purposes. Traditionally, the bark is used as part of a skin lightening treatment by Venda people (Limpopo Province). Tyrosinase is a multifunctional copper-containing enzyme that catalyzes tyrosine to form melanin which darkens the skin. In excess the process results in melanogenesis which promotes the formation of hyperpigmentation. An ethnobotanical survey was conducted by interviewing traditional healers and users. *Trichilia emetica* (methanol extracts, aqueous extracts of the bark and leaves) (20 – 200 µg/ml, pH 6.5) were tested in vitro using the colourimetric tyrosinase inhibition assay. Kojic acid and dimethyl sulfoxide were used as positive and negative controls, respectively. The results of the ethnobotanical survey showed that the powdered bark was mixed with lemon juice to reduce hyperpigmentation on the skin. Traditional healers reported that this paste has a skin-lightening effect and this was confirmed by the users of the treatment. The highest percentage inhibition on colourimetric tyrosinase assay was obtained with aqueous extract (12%) which was significantly lower than the positive control (98%). These studies confirmed that *T. emetica* has potential in reducing post-inflammatory hyperpigmentation, hence validating the use of the plant as a skin depigmenting remedy.

EMBLICA EXTRACT INDUCES TYPE I PRO-COLLAGEN SYNTHESIS AND INHIBITS COLLAGENASE ACTIVITY IN MOUSE FIBROBLASTS

Sudjit Luanpitpong, Pithi Chanvorachote, Varisa Pongrakhananon, Ubonthip Nimmannit

Pharmaceutical Technology, Faculty of Pharmaceutical Science, Thailand, suidjit@gmail.com

Decrease of type I collagen generation as well as the increase of its degradation via matrix metalloproteinases (MMPs; e.g. gelatinases and collagenases) are considered as a major cause of skin aging. A potent natural antioxidant emblica extract from emblica fruits (*Phyllanthus emblica*) was herein evaluated for collagen promoting activity in mouse fibroblasts. We

demonstrated that the level of intracellular type I pro-collagen increased following emblica extract treatment in concentration-dependent manner. *Emblica* extract at the concentration of 100 µg/mL exhibited the maximum effect on type I pro-collagen; approximately 7 fold-induction versus non-treated control determined by Western blot analysis. Moreover, our result indicated that emblica extract affected the MMPs function. Percentage of collagenase inhibition exceeded forty at the concentration of 100 µg/mL of emblica extract. Taken together, emblica extract has a promising anti-aging effect attributed to its positive effect on type I collagen formation as well as the protection against collagen degradation.

PROTECTIVE EFFECT OF A *PHYLLANTHUS EMBLICA* EXTRACT AGAINST CISPLATIN-INDUCED APOPTOSIS IN DERMAL PAPILLA FIBROBLASTS

Sudjit Luanpitpong, Ubonthip Nimmannit, Varisa Pongrakhananon, Pithi Chanvorachote

Pharmaceutical Technology, Faculty of Pharmaceutical Science, Thailand, suidjit@gmail.com

Cisplatin is a widely prescribed anticancer agent that causes hair loss in patients. Since the dermal papilla (DP) fibroblasts are known as a key mediator in controlling hair growth and loss, protection against cisplatin-induced cell damage on these cells may lead to a new strategy for hair loss protection in chemotherapy patients. We herein reported that cisplatin induced DP cell death in a concentration-dependent manner through apoptosis mechanism, which was further found to be related to the intracellular reactive oxygen species (ROS) generation. Therefore, extract from emblica fruits (*Phyllanthus emblica*), a known natural antioxidant, was examined for its protective effect on cisplatin-induced DP fibroblast cell death. Our results indicated that emblica extract attenuated the intracellular ROS induction following cisplatin treatment and subsequently protected against cisplatin-induced DP fibroblast apoptotic cell death. Co-treatment of emblica extract (250-500 µg/mL) and cisplatin 100 µmol/L (LD50) markedly increased the DP fibroblast relative cell viability to the same level as the non-treated control.

BIOLOGICAL ACTIVITIES OF *TYPHA CAPENSIS* (TYPHACEAE) FROM LIMPOPO PROVINCE (SOUTH AFRICA)

Masoko P., Mokgotho M.P., Howard R.L. and Mampuru L.J.

University of Limpopo, Department of Biochemistry, Microbiology and Biotechnology, Private Bag X1106, Sovenga, 0727, South Africa

Dried ground leaves and rhizomes of *Typha capensis* were extracted with different solvents of varying polarity (hexane, dichloromethane, acetone, methanol) to determine the best extractant for subsequent isolation and characterization of antibacterial compounds. Some extracts were active against *Escherichia coli* and *Enterococcus faecalis*, with at least one of them exhibiting minimum inhibitory concentration value of 0.04 mg/ml. Methanol was the best extractant with an average minimum inhibitory concentration (MIC) value of 0.75 mg/ml (rhizome) and 0.21 mg/ml (leaves) for the four pathogens. *Escherichia coli* and *E. faecalis* were the most sensitive with the average MIC values of 0.53 and 0.42 mg/ml, respectively. *Staphylococcus aureus* was the most resistant pathogen. The MIC values for the positive control

(ampicillin) were ranging from 0.08 mg/ml to 0.16 mg/ml. Average total activity, a measure of potency, was highest for methanol (4498 ml/g) leaves and (1838 ml/g) rhizomes extract followed by acetone (1795 ml/g) leaves and (1075 ml/g) rhizome extracts. The most active compounds were at the R_f value of 0.47 against all pathogens. In some extracts the antibacterial activity was high enough to consider extracts for isolation and characterization of antibacterial compounds.

Acknowledgements: The National Research Foundation (NRF) and Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo provided financial assistance.

THE ANTIMICROBIAL ACTIVITIES OF *SCLEROCARYA BIRREA* EXTRACTS AGAINST THREE STRAINS OF PATHOGENIC YEASTS

T.J. Mmushi, P. Masoko, M. Mogashoa, M.P. Mokgotho, L.J. Mampuru and R.L. Howard

Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private bag x 1106, Sovenga, 0727, South Africa

Sclerocarya birrea is used by some of South African traditional healers for the treatment of skin disorders and epilepsy. The leaves, barks and roots of this plant were scientifically investigated for antifungal activity against *Candida parapsilosis*, *Cryptococcus albidus* and *Rhodotorula mucilaginosa* which are opportunistic and systemic skin pathogens. The phytochemical constituents of finely ground plant parts were extracted using four different solvents (hexane, dichloromethane, acetone and methanol). The phytochemical analysis, antioxidant activity and bioautography were evaluated on chromatograms separated by eluting systems, BEA (benzene: ethanol: ammonia), CEF (chloroform: ethyl acetate: formic acid) and EMW (ethyl acetate: methanol: water). The antifungal activities were tested against all organisms and extracts. Amphotericin B was used as the positive control. Methanol yielded the highest quantity of extracts while acetone had the highest antifungal activity. The phytochemical analysis revealed the presence of different compounds on the chromatograms. The eluting system BEA had the best separation for all extracts. Acetone, ethanol and methanolic bark extracts had the lowest MIC values of 0.16 mg/ml against all tested pathogens. All bark extracts extracted with non polar or intermediate polar solvents were not active. Polar extracts had high activity, high enough to consider for clinical application and for isolation and characterization of antifungal compounds.

Acknowledgements: The National Research Foundation (NRF) and Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo provided financial assistance.

ISOLATION OF AN ANTIFUNGAL TRITERPENES FROM *COMBRETUM NELSONII* (COMBRETACEAE) LEAVES

Masoko P., Mdee L.K, Mampuru L.J, Eloff J.N.

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Sciences, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa.
University of Limpopo, Department of Biochemistry, Microbiology and Biotechnology. University of Limpopo, Private Bag X1106, Sovenga, 0727, South Africa

Antifungal bioassay-guided fractionation of *Combretum nelsonii* leaf extracts afforded two closely related triterpenes, asiatic acid and arjunolic acid. Antifungal activities of the mixture of asiatic acid and arjunolic acid were determined against five fungal animal pathogens. The MIC's of the mixture to the different pathogens varied from 0.2 – 1.6 µg/ml; *Candida albicans* (0.9 µg/ml), *Cryptococcus neoformans* (0.4 µg/ml), *Aspergillus fumigatus* (1.6 µg/ml), *Microsporium canis* (0.2 µg/ml) and *Sporothrix schenckii* (0.2 µg/ml). *Microsporium canis* and *S. schenckii* were the most susceptible followed by *C. neoformans*. *Aspergillus fumigatus* was the most resistant. The R_f value of the mixture of asiatic acid and arjunolic acid was 0.27 in CEF, 0.09 (BEA) and 0.55 (EMW) which was active against all pathogens. *In vitro* cytotoxicity of the mixture gave an LC_{50} of 10.58 µg/ml towards Vero monkey kidney cells. These results partly validate the ethnobotanical use of many *Combretum* species for conditions that may be of bacterial aetiology.

Acknowledgements: The National Research Foundation (NRF) and National Department of Agriculture (South Africa) provided funding. Dr L. McGaw (UP) for helping with toxicity studies.

EFFECT OF THE PHYTOESTROGEN DAIDZEIN ON TESTICULAR CELL FUNCTIONS

Thomas K. Monsees, Kathrin Bittorf, Sonja Hinkel, Jaleh Hayatpour

University of the Western Cape, South Africa, tmonsees@uwc.ac.za
JLU Giessen, Germany, , JLU Giessen, Germany, , JLU Giessen, Germany

The soy bean isoflavone daidzein has weak estrogenic properties. Estrogens can disturb male reproduction by inhibiting steroidogenesis in Leydig cells. They may also cause decreasing sperm counts by affecting Sertoli cells which are crucial in spermatogenesis. We investigated the effect of estrogenic compounds on testicular cells in vitro. Cultures of rat Sertoli (SC) or Leydig (LC) cells were incubated with estrogens (nM to µM) for 3 d. Cytotoxicity, production of lactate and secretion of inhibin B (SC) or steroids (LC) were determined. 0.1-1 µM of estrogen did not alter any Sertoli cell parameter investigated. At higher concentrations (3-36 µM), only daidzein significantly reduced cell viability whereas ethinylestradiol significantly enhanced secretion of lactate and inhibin B. In contrast, bisphenol-A reduced inhibin levels. In Leydig cells, a dose-dependent decrease in progesterone and testosterone production was induced by nM of estradiol and ethinylestradiol, or µM of daidzein. Cell viability was not affected. Estrogen receptors are present in Sertoli and Leydig cells. Inhibition of LC testosterone synthesis by estradiol and ethinylestradiol was observed at nM concentrations and thus possibly be related to genomic effects. Daidzein acted only at µM levels. Here, a non-genomic mechanism, e.g. by competitive inhibition of enzymes involved in steroidogenesis is likely. A receptor-independent action may also be responsible for the observed effects of daidzein, ethinylestradiol, and bisphenol A on Sertoli cells. A daily intake of 1 mg/kg BW (= 3.9 µM) of daidzein is regarded as safe. This concentration, however, caused a significant reduction of androgens in our test system.

WIL-2 NS LYMPHOMA CELL LINE SHOW APOPTOTIC FEATURES WHEN TREATED WITH TRADITIONAL MEDICINE *COMMELINA BENGHALESIS*

Mokgotho M.P., Masoko P., Mbazima V.G., Lebogo K.W., Mampuru L.J.

Biochemistry, Microbiology and Biotechnology Department, University of Limpopo, Sovenga, South Africa, Limpopo Province, 0727

Many natural occurring agents have shown chemo preventive and chemotherapy potentials, yet there are no effective drugs to treat most cancers. *Commelina benghalensis* has been traditionally used as a herbal plant whose leaves are believed to have anti-tumour properties. The aim of this study was to investigate the effect of semi-purified extracts from *C. benghalensis* on the proliferation of Wil-2 NS cells. An acetone extract was prepared from the leaves and defatted with n-hexane and fractionated with dichloromethane (F2). F2 was tested for anti-proliferative activities on Wil-2 NS cells. Results from the trypan blue exclusion and sulphorhodamine B assays showed F2 inhibited the proliferation of the Wil-2 NS cells in a dose and time dependent manner. The acridine orange/ethidium bromide dual stain showed that the treated cells die through a process of apoptosis. These results show that *C. benghalensis* has anti-proliferation properties against Wil-2NS lymphoma cells. Further studies are required to determine the components responsible from the extracts and elucidate the mechanism of action on growth inhibition.

Acknowledgements: NRF and University of Limpopo Research office.

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF THE ESSENTIAL OIL FROM *CYMBOPOGON NARDUS* (L.) RENDLE

N. Naidoo, K.Thangaraj, B. Odhav and H. Baijnath

Department of Biotechnology and Food Technology, Durban University of Technology, Durban, South Africa odhavb@dut.ac.za

The essential oil from the leaves, culms and rhizomes of *Cymbopogon nardus* were extracted and volatile oils were investigated for the first time using Gas Chromatography-Mass Spectroscopy (GC/MS). Alpha-cubebene, camphene, geraniol, limonene, myrcene, palmitic acid and sabinen were the major compounds identified. The essential oil was investigated for its antioxidant (DPPH assay), anti-inflammatory (5-lipoxygenase assay), antimicrobial (disk diffusion) and anti-mosquito properties (insecticidal, larvicidal and repellency assays). The oil showed poor antimicrobial activity and inhibited the growth of *Bacillus*, *Micrococcus* and *Staphylococcus* with a minimum inhibitory concentration of 0.0625 (vol/vol). The oil showed antioxidant activity, scavenging more than 80% of DPPH free radicals and did not show any anti-inflammatory activity. It also showed good adulticidal activity (53.7% mortality) and excellent larvicidal (100% mortality) and repellent activity (100% repellency) against *Anopheles arabiensis* mosquitoes, which are malarial vectors. The results of this study show that the essential oil from *C. nardus* possesses, antioxidant, antibacterial and anti-mosquito activities which may be associated with its predominant compounds viz., α -cubebene, camphene, geraniol, limonene, myrcene, palmitic acid and sabinen. These results lead the way for exploiting *C. nardus* oil as a multi-functional agent.

ANTIMICROBIAL, ANTIOXIDANT AND ANTICANCER ACTIVITIES OF THAI LOCAL VEGETABLE EXTRACTS: APPLICATION IN A CHINESE-STYLE SAUSAGE

Suree Nanasombat

Department of Applied Biology, Faculty of Science, King Mong, Thailand, nsuree@yahoo.com

Twenty species of Thai local vegetables were screened for their antioxidant and antimicrobial activities against 19 microbial strains. Methanolic extracts of four plant species, *Garcinia cowa*, *Cassia siamea*, *Limnophila aromatica* and *Polygonum ordoratum* showing high activities were selected to determine the minimum inhibitory concentration, and evaluate for their anticancer activity against human oral epidermal carcinoma (KB), breast adenocarcinoma (MCF-7) and small cell lung carcinoma (NCI-H187). The sensitive bacteria were *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*. Of all, the extract of *P. ordoratum* had the highest phenolic content and antioxidant activity with EC₅₀ value of 315.35 microgram extract/miligram DPPH (2,2-diphenyl-1-picrylhydrazyl), and was moderately active against breast adenocarcinoma (IC₅₀ value of 6.01 microgram/mililiter). Major types of active compounds were identified by HPLC method. Main types of flavonoids found in *P. ordoratum* were rutin, catechin, quercetin, kaempferol and isorhamnetin. Among all compounds, rutin was found in the highest amount (3.77% w/w dry extract). Then, three formulations of dried vegetable extracts were produced and applied as natural additives in a Chinese-style sausage called Gunchieng. These natural additives (0.1%) were added alone or in combination with sodium lactate (2.5%) in Gunchieng. All natural additives tested were able to retard lipid oxidation during storage at 4 C for 21 days. Treatments with natural additives in combination with sodium lactate resulted in decreasing of microbial counts in Gunchieng by 1.36-2.42 log units after 21-day storage, but the natural additive alone did not cause significant reduction of total microbial counts.

EFFECT OF CONCENTRATIONS AND CARRIERS ON CHARACTERISTICS OF SPRAY-DRIED TAMARIND PULP EXTRACT

Ubonthip Nimmannit, Rattana Songmek Pornthip, Nimmannit Sunanta Pongsamart

Chulalongkorn University, Thailand, nubonthi@chula.ac.th

Tamarind pulp comprises reducing sugar, organic acid, pectin, protein, fiber and cellulosic material. Organic acids in tamarind pulp are tartaric acid, malic acid and citric acid which are alpha hydroxy acids. The aims of this study were to prepare spray-dried tamarind pulp extract using different carriers, maltodextrin and acacia with different concentrations, 5 and 10% w/w with or without silicon dioxide 1% w/w. In the preparation of spray-dried tamarind pulp extract, 10% w/w acacia, inlet temperature 110 degree Celsius, fan setting 50 were successful for spray drying. Percent yield of spray-dried tamarind pulp extract was $55.73 \pm 4.75\%$ w/w. Percent moisture content was $6.41 \pm 0.64\%$ w/w, spherical and smooth surface with slightly aggregated particles were obtained with mean particle size of 16.20 ± 0.09 micrometers. The spray-dried tamarind pulp extract contained $7.83 \pm 0.13\%$ w/w of tartaric acid. Stability of spray-dried products were determined by keeping in tight, clean or light protected vials at accelerated temperature 40 ± 2 degree Celsius relative humidity $75 \pm 5\%$ for 6 months. The percent remaining of tartaric acid in spray-dried product in both vials was 93.16% w/w. The final product is still free flowing.

SCREENING FOR ANTIPROLIFERATIVE ACTIVITY OF A CRUDE ACETONE EXTRACT OF *SENNA* SPP ON HL-60 AND WIL-2 NS CELL LINES

A. Ngobeni, M. P. Mokgotho, P. Masoko, R. L. Howard and L. J. Mampuru

Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private Bag X 1106, Sovenga, 0727, South Africa

For many decades plants have provided mankind with herbal remedies against many infectious diseases. Cancer is one of the major public health problem in many developed countries. *Senna* species falls under the Fabaceae family and are well known for their therapeutic properties. They are being used as plant herbs in many countries including South Africa. The acetone crude extract prepared from the roots of *Senna* sp was investigated for its cytotoxic activity against two cell lines, HL-60 promyelocytes and WIL-2 NS lymphocytes cells. The changes in nuclear morphology brought by the extract on these cells were also investigated. To investigate the cytotoxicity of the extract, cells were treated with concentrations varying from 0-200 µg/ml for 72 hours and samples collected at 24 hours interval to evaluate cell proliferation and viability using the trypan blue dye exclusion method. The extract was shown to inhibit the proliferation of both HL-60 and WIL-2 NS; with WIL-2 NS being more sensitive to the treatment as compared to HL-60 cells. The nuclear morphological changes of the treated HL-60 and WIL-2 NS cells showed characteristics that are associated with apoptotic features. This could mean that the extract inhibited the proliferation of the cells through the programmed cell death pathway.

Acknowledgements: University of Limpopo senate research fund, NRF

BIOLOGICAL ACTIVITIES OF EXTRACTIVES OF SOME *ALPINIA* AND *HEDYCHIUM* SPECIES**Nuchnipa Nuntawong**

Chiang Mai University, Thailand, nuchnipa@chiangmai.ac.th

Air-dried rhizomes of *A. malaccensis*, *A. galanga* and *H. gardnerianum* were successively extracted with hexane, dichloromethane and methanol at room temperature and the solvent evaporated under reduced pressure to give hexane extract (2.02, 1.86 and 2.17 %), dichloromethane extract (1.92, 2.20 and 2.45 %) and methanol extract (1.48, 1.72 and 1.80 %). The crude extracts were evaluated for antiplasmodial, antimycobacterial and cytotoxic activities. The results showed that the hexane and dichloromethane extracts of all species exhibited antimycobacterial activity against *Mycobacterium tuberculosis*, with the hexane and dichloromethane extracts of *A. galanga* showing the strongest activity (MIC 0.39 µg/ml). Only the hexane and dichloromethane extracts of *A. galanga* showed antiplasmodial activity against *Plasmodium falciparum* (IC₅₀ 3.74 and 6.04 µg/ml, respectively). For cytotoxicity, the hexane and dichloromethane extracts of *A. galanga* and *H. gardnerianum* exhibited significant activity against the KB, BC and NCI-H187 cancer cell lines. However, the methanol extract of all species were inactive in all tests.

IN VITRO BIOACTIVITY OF *VENDA* MEDICINAL PLANTS USED IN THE TREATMENT OF RESPIRATORY CONDITIONS**Christopher Pallant, Vanessa Steenkamp**

Department of Pharmacology, University of Pretoria, South Africa, capallang@gmail.com

Infectious diseases, especially those affecting the respiratory tract, represent a critical problem to health. Crude methanol and water extracts of ten Venda plants reported to be used ethnomedically in the treatment of respiratory conditions were assessed for their antimicrobial activity against standard strains and clinical isolates of *Candida albicans*, *Haemophilis influenza*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Mycobacterium smegmatis* using the disc diffusion assay. Four of the ten plants tested demonstrated antimicrobial activity. No extracts showed activity against *K. pneumoniae*. Minimum inhibitory concentrations (MIC), as determined by the broth microdilution assay, showed three plants, *Securidaca longepedunculata*, *Syzygium cordatum* and *Tabernaemontana elegans*, to possess MICs \leq 1mg/mL. These extracts, however showed toxicity *in vitro* to lymphocytes. The crude extracts were tested for antioxidant activity using the TEAC assay, but only *S. cordatum* showed significant antioxidant activity. Phytochemical screening, performed by thin layer chromatography (TLC) using a variety of mobile phases and selective visualization reagents demonstrated various classes of compounds in the active extracts. While three plant species were identified with significant antimicrobial activity, there remains a need for identification of the bioactive constituents, as well as a further investigation of their toxicity.

Acknowledgements: NRF provided funding

ANTIDIABETIC ACTIVITY OF THE ETHANOLIC EXTRACT OF HEARTWOOD OF BIJASAR (*PTEROCARPUS MARSUPIUM ROXB.*) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

Umesh K. Patil

Vns Institute of Pharmacy, Bhopal (M.P.) 462003 M.P., India,
umeshpatil29@yahoo.com

The antidiabetic potential of the ethanolic extract of bijasar (*Pterocarpus marsupium Roxb.*) heartwood was evaluated in the streptozotocin-nicotinamide induced type 2 diabetic models. Graded doses of the ethanolic heartwood extract were administered to normal and experimental diabetic rats for 12 days. Significant ($p < 0.05$) reduction in fasting blood glucose levels were observed in the normal as well as in the treated diabetic animals. Serum insulin levels were not stimulated in the animals treated with the extract. STZ-nicotinamide induced diabetic rats administered with the extract (100 mg/kg) showed 30 and 42.2% decline in the blood glucose levels whilst a decline of 31.5 and 51.3% were observed in animals treated with 200 mg/kg of the alcoholic extract on days 5 and 12 respectively. In this test the 300 mg/kg dose of the extract showed a decline of 34.4 on day 5 and 56.4% on day 12. In addition, changes in body weight, serum lipid profiles, thiobarbituric acid reactive substance levels, glycosylated haemoglobin and liver glycogen levels assessed in the extract treated diabetic rats were compared with the diabetic control and normal animals. Significant results were observed in the estimated parameters, thereby justifying the use of the bijasar heartwood by Indian herbal practitioners for the treatment of diabetics in different parts of the country.

Acknowledgements: CDACR, Bhopal for financial assistance.

CURCUMIN INDUCES ANOIKIS IN H460 NON-SMALL LUNG CANCER CELLS THROUGH SUPEROXIDE ANION INDUCTION

Varisa Pongrakhananon, Suidjit Luanpitpong, Pithi Chanvorachote, Ubonthip Nimmannit

Pharmaceutical Technology Program, Faculty of Pharmaceutical, Thailand, iamrisa@gmail.com
Pharmaceutical Technology Program, Faculty of Pharmaceutical, Thailand
Department of Physiology, Faculty of Pharmaceutical Sciences, Thailand
Pharmaceutical Technology Program, Faculty of Pharmaceutical, Thailand

Anoikis or apoptosis triggered by loss of cell anchorage plays an important role in prevention of tumor cell metastasis. During metastasis process, anticancer drugs are not able to completely eliminate cancer cells that suspended in the blood stream; therefore, the use of natural safety compounds to enhance apoptosis of these suspended cells may improve the therapy. Curcumin (diferuloylmethane), a major active component in turmeric, *Curcuma longa*, has been shown to inhibit neoplastic initiation, promotion and progression of a wide variety of tumor cells. However, the effect of curcumin on anoikis process has not been well characterized. We herein demonstrated that 10 μM of curcumin triggered 1.2-folds anoikis of detached lung carcinoma H460 cells compared with controlled cells. Cell viability was detected by XTT assay and anoikis cells were indicated by annexin V staining assay. Mechanism of curcumin induced anoikis was associated with its ability to generate intracellular reactive oxygen species (ROS). Curcumin caused 1.6-folds induction of intracellular ROS level as detected by DCF-DA and flow cytometry. Our inhibitory study revealed that superoxide anion generated by curcumin was a principle ROS responsible for anoikis induction in these cells. Thus, our results showed that curcumin induced anoikis in detached-H460 cells resulted from increasing of intracellular ROS.

PROFILING OF CANNABIS EXTRACTS AND CONSTITUENTS FOR CYTOTOXIC AND NF-KAPPA B-MODIFYING EFFECTS**Wieland Peschel, M. Politi, G. Appendino, A. Constanti, M. Heinrich, J. M. Prieto**

SOP, University of London, United Kingdom, wieland.peschel@pharmacy.ac.uk

Cannabis is used as co-medication by patients with cancer or inflammatory diseases. Cannabinoids are linked to the Nuclear Factor kappa B (NF- κ B) which is pivotal in the cellular immune response [1]. Anti-inflammatory effects of 9-tetrahydrocannabinol (THC); cannabidiol (CBD), or cannflavin A and advantages of whole plant preparations have been reported [2,3]. Hydroethanolic traditional cannabis preparations (TCP) may thus combine effects of various co-constituents. We compared the profile of 7 cultivars by HPLC and $^1\text{H-NMR}$ fingerprints and screened for cytotoxicity in three cancer cell lines (MTT assay) and modulation of the PMA/ TNF- α induced NF- κ B activation (IL-6 reporter gene luciferase assay in stably transfected HeLaLuc cells). We also tested pure cannabinoids and combined effects with phenolics, anti-inflammatory/cytotoxic drugs, and CB1-, CB2- and TRPV1 receptor antagonists. TCP showed cannabinoid dominance and a high portion of cannabinoid acids from unheated materials. Four markers were defined: (M1) the THC content, (M2) the total cannabinoid content, (M3) the ratio between the major cannabinoids, and (M4) the ratio between cannabinoids, cannabinoid acids and phenolics. TCP toxicity was determined by M2 and M4 but not M1 or M3 (LC50 100 $\mu\text{l/ml}$ to 0.02 $\mu\text{l/ml}$). All cannabinoids proved to be equally toxic (LC50 < 5 μM). Interestingly, this cytotoxic effect could be reverted by some phenolics. The window for non-toxic inhibition of NF- κ B expression was wider for extracts than for cannabinoids. Unexpectedly, some compounds induced the activation of this nuclear factor. The interplay between polar/non-polar cannabis

constituents in the activation/inhibition of NF- κ B is discussed.

Acknowledgements: William Ransom & Son, PLC, Hitchin (UK), European Commission (grant code COOP-CT-2004-512696) for funding, G Grassi, Roviglio (IT), TNO, Zeist (NL) for material provision References: [1] Juttler E et al. (2004) *Neuropharmacol* 47: 580-592, [2] Barrett ML et al. (1985) *Biochem Pharmacol.* 34, 2019-2024, [3] Russo E, Guy GW. (2006) *Medical Hypotheses* 66:234-246

VARIABLE EFFECTS OF *LEUZZEA CARTHAMOIDES* (ASTERACEAE) EXTRACTS AND 20-HYDROXYECDYSONE ON NF-KAPPA B

Wieland Peschel, Marco Leonti, Jose Maria Prieto

SOP, University of London, United Kingdom, wieland.peschel@pharmacy.ac.uk

Leuzea carthamoides (Willd.) DC. (syn. *Rhaponticum carthamoides* (Willd.) Iljin, Asteraceae) has adaptogenic [1], anticancer [2] and immunomodulating [3] effects. The plant – traditionally hydroethanolic extracts- and its main ecdysteroid 20-hydroxyecdysone (20-E) are increasingly offered as natural anabolics for animal and human use. Considering the central involvement of the nuclear factor NF-kappa B within the cellular immune response [4] we investigated the effect of pure 20-E and *Leuzea* extracts using a reporter gene luciferase based model with a transfected HeLa cell line. Cytotoxicity and proliferative effects were controlled by the MTT assay and HPLC fingerprints including 20-E determination recorded. 20-E alone showed a minor inhibitory effect between 5 and 50 μ M upon PMA or TNF α stimulation. In contrast, the stronger effects by standard inhibitors such as parthenolide or dexamethasone were attenuated. Crude hydroethanolic/methanolic herb extracts lowered (IC50 around 20 μ g/ml), whereas root extracts increased NF-kappa B activation (plus 50% at 10 μ g/ml). Hydrophilic fractions both from root/herb containing 20-E and higher amounts of phenolcarbonic acids activated, whereas some of the medium polar fractions with higher portion of flavonoids and sesquiterpenolactones inhibited NF-kappa B more effectively (IC50 < 5 μ M). All effects were lowered by the transcription inhibitor dexamethasone. Spiking of the extracts with 20-E attenuated the effect of inhibitors and further increased those of activating extracts in most cases. *Leuzea* NF-kappa B effects are for the first time reported here. The results show that the standard marker 20-E alone is not primarily responsible, but may modify the effects exhibited by other compounds in either direction.

Acknowledgements: A. Kump, Linz (AT) for material provision References: 1Slama et al. (1996) *Experientia* 52(7):702-6, [2] Hamburger et al. (2006) *Planta Med* 2006; 72: 992 [3] Kormosh et al.(2006) *Phytother Res.* 20(5):424-5, [4] Li Q, Verma IM. (2002) *Nat Rev Immunol.* 2(10):725-34

EFFECT OF *NYPA FRUTICANS* STEM AND LEAF EXTRACT ON GLUCOSE TOLERANCE IN GLUCOSE-INDUCED HYPERGLYCEMIC MICE

Reza H., Jahan S., Wahid M., Rahmatullah M.M.

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
hmmmb1st@yahoo.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
sjmoushumi@gmail.com, Faculty of Life Sciences, University of Development Alternat,
Bangladesh, wahid.haa@gmail.com, Faculty of Life Sciences, University of Development
Alternat, Bangladesh, rahmatm@uoda.org

Nypa fruticans (Thunb.) Wurmb. (Palmae), also known as Golpata in Bangladesh, is the only palm considered a mangrove. In this study, we examined the effect of methanol extract of stems and leaves on serum glucose lowering ability in glucose-induced hyperglycemic mice (glucose tolerance test). Fasted mice were divided into five groups. Group 1 (control) received distilled water. Groups 2-4 received methanol extract at oral doses of 100, 250 and 500 mg/kg body weight (bw). Group 5 received glibenclamide at an oral dose of 10 mg/kg bw. After 60 min of extract administration, the mice of all groups were orally treated with 2 g/kg bw of glucose. Blood samples were collected at 60 and 120 min after glucose loading and serum glucose levels determined. The significance of the results was calculated using Student's t-test and were considered statistically significant when $P < 0.05$. The methanol extract of *Nypa fruticans* did not demonstrate any significant lowering of serum glucose at 60 min. However, at 120 min after glucose loading, the extract demonstrated highly significant glucose lowering activity. At 120 min, the serum glucose levels of Groups 1-5 were respectively, 80.4 ± 2.4 , 71.0 ± 0.6 , 54.7 ± 2.6 , 30.4 ± 2.4 and 59.5 ± 2.5 mg/dL (Mean \pm SEM). The extract, when administered at 250 mg/kg bw demonstrated equivalent glucose lowering activity compared to the standard drug glibenclamide and at 500 mg/kg bw demonstrated

ANTIMICROBIAL ACTIVITY AND CYTOTOXICITY OF *PTEROCARPUS ANGOLENSIS* EXTRACTS AND COMPOUNDS AGAINST SELECTED BACTERIAL ORGANISMS AND *ENTAMOEBIA HISTOLYTICA*

Amidou Samie

University of Venda, South Africa, samieamidou@yahoo.com

Pterocarpus angolensis also known as the bloodwood tree or Mutondo in Shi-Venda is well known to traditional doctors in the Venda region and commonly used. In the present study, extracts and compounds isolated from *P. angolensis* were prepared and tested against different bacterial organisms and *Entamoeba histolytica*. The cytotoxicity of the compounds was also determined using the MTT assay. The acetone extract was the most active against all the bacterial organisms tested with MIC varying between 0.0156 mg/ml against *Staphylococcus aureus* and 2 mg/ml against *Enterobacter cloacae*. Seven pure compounds were isolated and the structures of 5 were determined. The 5 isolated compounds included Phthalate and 4 derivatives of epicatechin. All the purified compounds were active against *S. aureus* as determined by bio-autography. Piperitenone (a compound isolated from *Lippia javanica* essential oil) and one compound out the eight obtained from *P. angolensis* appeared to be active against *E. histolytica* with IC₅₀ of 25 μ g/ml and 100 μ g/ml respectively. The other *P. angolensis* compounds were not active up to the concentration of 400 μ g/ml tested. The compounds isolated from *P. angolensis* were more toxic than Piperitenone and their IC₅₀ was found to be between 175 μ g/ml and 375 μ g/ml. In conclusion, the present study has demonstrated the presence of epicatechin and its derivatives from the stem bark of *Pterocarpus angolensis* and demonstrated their weak activities against *E. histolytica*. Further studies are needed to characterize the activity of the isolated compounds in the treatment of other diseases.

THE IN VITRO ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF HERBAL EXTRACTS

Maxleene Sandasi, Alvaro Viljoen, Carmen Leonard

Department of Pharmaceutical Sciences, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa, maxies24@yahoo.co.uk

The *in vitro* antimicrobial and antibiofilm activity of thirty-two extracts (water and dichloromethane/methanol) from sixteen dietary spices and medicinal herbs were investigated against three pathogenic microorganisms (*Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Candida albicans*). The antimicrobial activity was determined using the minimum inhibitory concentration (MIC) microtitre plate assay. The extracts that showed the highest antimicrobial activity against all the test organisms were turmeric, rosemary, thyme, echinacea, peppermint and tea tree with MIC values of ≤ 2 mg/ml. The growth and development of biofilms was assessed using the crystal violet (CV) assay and confocal scanning laser microscopy (CSLM) was used to visually assess the morphological changes and composition of the biofilm. The majority of the extracts tested prevented cell adhesion to the polyvinyl chloride (PVC) surface. The extracts that showed the greatest inhibition against all the test organisms were peppermint, rosemary and echinacea with inhibition values of $\geq 50\%$. In contrast, growth inhibition of an already established biofilm was more difficult to achieve. In most cases the extracts enhanced the growth of the biofilms. Peppermint was the only extract that showed antibiofilm activity against all the pathogens. The reduction of biofilm mass by use of plant extracts shows potential in anti-adhesion therapy thus reducing the incidence of diseases associated with biofilm formation.

INHIBITORY ACTIVITY OF FIVE TROPICAL PLANTS IN MALAYSIA AGAINST MEDICALLY-IMPORTANT MICROORGANISMS

N.W. Sit*, Y.S. Lau, P.Y. Soo, S.R. Teo, and K.S. Teoh

Universiti Tunku Abdul Rahman (UTAR), Faculty of Science, Engineering and Technology, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia.*Corresponding author:

sitnw@utar.edu.my

Development and spread of microbial resistance has necessitated a search for novel antimicrobial agents for treatment of infectious diseases. Thus, the present study was carried out to evaluate the potential antibacterial activity of five tropical plants against medically-important bacteria (Gram positive: *Bacillus cereus* and *Staphylococcus aureus*; Gram negative: *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) using colorimetric broth microdilution method with resazurin as a growth indicator. The crude ethanolic extract and its various fractions (hexane, chloroform, ethyl acetate, butanol and water) of each plant were evaluated at concentrations ranging from 5 to 0.04 mg/mL using two-fold serial dilution. All the extracts and fractions tested were found to have percent activity of $\geq 40\%$ except the ethyl acetate fraction of *Artocarpus integer* fruits (20%), chloroform fraction and aqueous fraction of *Euphorbia heterophylla* stem (both 0%). The lowest minimal inhibitory concentration (0.04 mg/mL, average of two replicates) was given by the ethyl acetate fraction of *Manihot esculenta* leaves against *B. cereus* and *P. aeruginosa*. In contrast, the highest total activity was recorded

on the crude ethanolic extract of *Citrus grandis* leaves against *P. aeruginosa* (171.9 mL/g). Among the bacteria studied, *E. coli* and *S. aureus* were the least susceptible bacteria, with the bacterial susceptibility index (BSI) of 36.7%. In addition, this study also demonstrated the limitation of using resazurin in natural product research, as auto-reduction of this indicator occurred in some of the fractions of *Spondias cytherea* fruits.

Acknowledgements: Universiti Tunku Abdul Rahman for financial support

SOUTH AFRICAN MEDICINAL PLANTS USED TO TREAT MENTAL ILLNESS

Gary Stafford, Mikael Pedersen, Anna Jäger, Johannes Van Staden

Research Centre for Plant Growth and Development, University, South Africa,
972150566@ukzn.ac.za
University of Copenhagen, Denmark, mep@farma.ku.dk, University of Copenhagen, Denmark,
ankj@farma.ku.dk, Research Centre for Plant Growth and Development, University, South
Africa, rcpgd@ukzn.ac.za

The majority of the population in South Africa use traditional health care to treat various mental conditions. Only a small number of the more than 300 southern African plant species reported to treat or affect the CNS have been scientifically evaluated and very few of the active compounds have been isolated and identified. Ethnobotanical information on plants used by the traditional healers in southern Africa to treat mental illnesses, specifically epilepsy, depression, age-related dementia and debilitating mental disorders will be highlighted. Details of the recent pharmacological studies conducted on some of these plants, including screening results from several *in vitro* and *in vivo* bioassays, as well as the isolation and identification of active compounds will be presented. Several plants have exhibited potential antidepressant activity by binding to the serotonin re-uptake transporter in the [3H]-citalopram binding assay. This includes the observed serotonin re-uptake inhibitory activity and acetylcholinesterase (AChE) inhibitory activity of several South African Amaryllidaceae alkaloids. Inhibitors of AChE are important in the symptomatic treatment of Alzheimer's disease. The ability of southern African medicinal plants to inhibit monoamine oxidase, an important enzyme in the metabolism of several neurotransmitters, has the potential to lead to new treatments for Parkinson's disease. Several plants have shown probable sedative or anticonvulsant activity by binding to the GABAA/benzodiazepine receptor. Active compounds in this and other assay, such as biflavones from *Searsia* (= *Rhus*) species, will be discussed.

Acknowledgements: National Research Foundation (NRF, Pretoria), University of KwaZulu-Natal.

ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT FROM SEED COAT OF MAJOR TAMARIND CULTIVATED IN THAILAND

Maneewan Suksomtip, Saowaluck Ukrisdawithid, Sunanta Pongsamart, Parichart Bhusawang

Chulalongkorn University, Thailand, maneewan.s@chula.ac.th

Methanolic extracts from the seed coat of five major tamarinds (TI-SP/P: Srichomphu, TI-

STN/P: Sithong-nak, TI-STB/P: Sithong-bao, TI-PY/P: Priao-yak, TI-K/P: Khanti) cultivated in Thailand were investigated for antioxidative properties and content of total phenols. The content of total phenols in the extracts were determined by Folin-Ciocalteu's method. Antioxidative activities of extracts were evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, anti-lipid peroxidation assay and reducing power. The strongest anti-lipid peroxidation activity (IC₅₀ = 68.24 µg/mL) was found in the extract of TI-STB/P. Although all extracts showed higher anti-lipid peroxidation (IC₅₀ between 68.24 and 311.53 µg/mL) compared to vitamin C (IC₅₀ = 322.66 µg/mL), a different efficacy as reducing power and scavenging ability was observed. Extracts of TI-PY/P and TI-STB/P were found to have stronger reducing power compared to butylated hydroxyanisole (BHA) at concentration between 1-100 µg/mL, whereas highest radical scavenging activity was observed with the extract of TI-K/P (IC₅₀ = 72.68 µg/mL). A statistically significant correlation ($p < 0.05$) between antioxidant activity and content of total phenols was not found. The results suggested that not only the amount of phenolic compounds but their chemical structures may also be important for the antioxidative activities. Furthermore, the seed coat extract of the TI-STB/P cultivar which showed marked antioxidant activities in most of the assays may be a potential source of natural antioxidant.

ANTIPLASMODIAL TRITERPENOIDS ISOLATED FROM *CANTHIUM MULTIFLORUM*

M.Traoré, H.L.Ziegler, C.E. Olsen, H. Millogo, J.B. Ouédraogo, I.P. Guissou, O.G. Nacoulma, T. R. Guiguemdé, and S.B. Christensen

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Copenhagen, Denmark

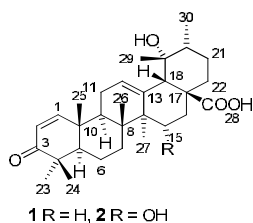
Direction Régionale de Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso

Laboratoire de Parasitologie-Entomologie, Centre Muraz, Bobo-Dioulasso, Burkina Faso
Department of Natural Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark

Département de Biochimie et de Microbiologie Appliquées, Université de Ouagadougou, Burkina Faso

Institut de Recherche en Science de la Santé, Ouagadougou, Burkina Faso

In Burkina Faso, roots of *Canthium multiflorum* (Thonn.) Hiern (Rubiaceae) are used for the treatment of malaria and hypertension [1]. This investigation was initiated because of the absence of published phytochemical reports on this specie and the possibility of the presence of antiplasmodial compounds. A bioactivity guided fractionation of roots of *Canthium multiflorum* led to the isolation of the 2 new ursenoic acid derivatives, 19 α -hydroxy-3-oxo-ursa-1,12-dien-28-oic acid **1** and 3-oxo-15 α ,19 α -dihydroxyursa-1,12-dien-28-oic acid **2** which were tested for antiplasmodial effect and for inducing change of the shape of membranes of erythrocytes. Assay for antiplasmodial activity was performed using chloroquine-sensitive *Plasmodium falciparum* strain 3D7 [2]. Chloroquine was used as a positive control. Investigation of membrane shape effects of pentacyclic triterpenes was performed according to a previous described method (Ziegler *et al.*, 2002) [3]. The structure of the two new compounds was elucidated using MS and NMR spectroscopy. The HR-ESI-MS gave a m/z 467.3161, calcd. for C₃₀H₄₃O₄ (M-H)⁻ 467.3167 for **1** and m/z = 507.3089 [M+ Na]⁺ (calcd. for C₃₀H₄₄NaO₅ :507.3081) for compound **2**. Application of the modified octant rule and comparison of the recorded CD spectrum with those of related pentacyclic triterpenoids with a 1-en-3-one system in the A-ring confirmed the absolute configuration of the two compounds. A moderate antiplasmodial activity (IC₅₀=26 µg/ml) was observed which was not related to the deformation of the shape of erythrocyte membranes.



1. Nacoulma, O.G. (1996). Plantes médicinales et pratiques médicinales traditionnelles au Burkina Faso: Cas du plateau central. Faculté des Sciences et Techniques, Université de Ouagadougou.
2. Ziegler, H.L., Staerk, D., Christensen, J. et al. (2002b). Antimicrobial Agents and Chemotherapy, 46, 1441–1446.
3. Ziegler, H.L., Jensen, T.H., Christensen, J., Staerk, D. et al. (2002a). Planta Medica, 68, 547–549.

Acknowledgments: This work was supported by Danida. We thank Dorte Brix for technical support.

ANTIMICROBIAL ACTIVITY OF *PIPER CAPENSE* L.F. EXTRACTS

Anzelle Thorburn, Duncan Cromarty, Vanessa Steenkamp

University of Pretoria, South Africa, thorburn@vodamail.co.za

Piper capense L.f. (Piperaceae) is a plant used traditionally for the treatment of infectious diseases. It therefore has potential as a source of novel antimicrobial compound/s. In this study antimicrobial activities of *P. capense* extracts were determined against various pathologically relevant microorganisms using disc diffusion and micro-broth dilution assays. Methanol and acetone extracts of root-bark exhibited MIC values < 1 mg/ml against *Staphylococcus aureus* (ATCC 12600) and *Candida albicans* (ATCC 10231). Phytochemical analyses of these extracts using various mobile phases on TLC and selective visualizing reagents revealed the presence of alkaloids, phenols, terpenes and flavonoids. Bioassay-guided fractionation of differentially solubilized or SPE sub-fractions indicated an enhanced antimicrobial activity in a hexane soluble sub-fraction (MIC of 0.02 mg/ml). HPLC analyses using a binary gradient of water (0.1% formic acid) and methanol (0.1% formic acid) revealed four major peaks for the hexane soluble sub-fraction. HPLC eluents were collected in a drop-wise fashion onto silica TLC plates and bioautography indicated that the compound that eluted at 13.6 minutes was responsible for the antimicrobial activity. This compound was further analysed using LC-MS/MS and GC-MS and identified as 5-hydroxy-2-methyl-1,4-naphthoquinone. This is the first time that this compound has been identified in this plant family. 5-hydroxy-2-methyl-1,4-naphthoquinone is reported to have highly toxic properties related to superoxide formation which confirms its antimicrobial activity but also implies non-selective *in vivo* toxicity. Acknowledgements: NRF and MRC provided funding.

TWO FACES OF THE QUATERNARY BENZO[C]PHENANTHRIDINE ALKALOID SANGUINARINE

Jitka Ulrichova, Vilim Simanek

Palacky University, Czech Republic, jitkaulrichova@seznam.cz

Mechanistic studies on normal or cancer cells testify to the effects of sanguinarine (SG) on apoptosis, angiogenesis, proliferation, differentiation and transformation. *In vivo* SG displayed mainly antimicrobial and anti-inflammatory effects. On the other hand, a well-known toxic effect attributed to SG and its dihydroderivative (DHSG) is "The Epidemic Dropsy Syndrome". SG has been reported to form DNA adducts *in vitro* and increase the levels of DNA single strand breaks in blood and bone marrow of mice treated i.p. with SG. The weakness of safety/toxicity SG and/or DHSG assessment has been the absence of data on the pharmacokinetics, biodistribution, metabolism and the possible genotoxicity *in vivo*. SG pharmacokinetics was studied in the rat after a single oral dose (10 mg.kg⁻¹ body weight). Alkaloid determination in plasma and liver was carried out by the validated HPLC/ESI-MS method. The pharmacokinetic parameters (t_{max}, c_{max}, AUC_{0-t} and AUC_{0-∞}) were determined for SG. The major metabolite detected in plasma was DHSG. Neither SG nor DHSG were detected in the urine. The formation of the less toxic DHSG might be the first step of SG transformation in the organism and its subsequent elimination in phase II reactions. Both compounds were completely eliminated from plasma and liver after 24 h. Benz[c]acridine, the only SG metabolite referred to in the literature, was not detected in the plasma, liver or urine. The rats consumed ad libitum either the standard diet or the diet containing 367 ppm of SG and chelerythrine (sanguiridin) for 90 days. The DNA adduct formation in liver was analyzed by ³²P-postlabeling technique and DNA single strand breaks in lymphocytes were evaluated by Comet assay. The results showed that sanguiridin induced no DNA damage to rat lymphocytes or hepatocytes after 90 days oral administration. Data from the studies described confirms the reduction of SG to DHSG in mammals and that orally administered SG displays no DNA damage.

Acknowledgements: This work was supported by the Grant Agency of the MSMT (grant No. MSM 6198959216) and by the Grant Agency of the Czech Republic (GA CR 525/07/0871).

PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITY OF *PRIMULA SPECTABILIS* TRATT., AN ENDEMISM OF VALVESTINO (ITALIAN ALPS)**Sara Vitalini, Gelsomina Fico, Aurora Valaguzza, Francesco Visioli and Franca Tomè**Dipartimento di Biologia, Università degli Studi di Milano, via Celoria, 26 – 20133 Milano (Italy)
UMR7079, Université Pierre et Marie Curie-Paris 6, Paris, France

Valvestino (Brescia) is a valley of Italian Central Southern Alps, recognized by European Union as "Site of Community Interest"; this site is characterized by the presence of numerous endemisms, some of them included in the IUCN Red List.

An ethnobotanical investigation in this valley has pointed out that some species belonging to *Primula* genus are commonly used for their medicinal properties. In this work we report the flavonoid composition of the endemism *Primula spectabilis* Tratt. and its antioxidant potential.

Samples of this species were collected during the summer of 2007 and determined according to "Flora d'Italia" by Pignatti. The dried powdered leaves were exhaustively extracted with increasing polarity solvents: *n*-hexane, dichloromethane, dichloromethane:methanol (9:1) and methanol. The methanol extract was chromatographed on Sephadex LH-20, using MeOH as eluent, to obtain fractions that showed NTS-PEG positive spots on TLC. Finally some of these

fractions have been submitted to RP-HPLC to obtain phenols, and the structural identification was determined on the basis of extensive NMR spectral studies, as well as by HPLC-MS analysis. Three compounds (C-glucosides of kaempferol, quercetin and apigenin), never isolated before in the *Primula* genus, were identified. The methanol extract, the richest in polyphenols, was also submitted, after the determination of polyphenolic content by the Folin-Ciocalteu method, to DPPH, total antioxidant capacity based upon the reduction of Cu^{++} to Cu^+ and FRAP assays. The obtained results showed a considerable antioxidant activity from *P. spectabilis*.

ANTIMICROBIAL ACTIVITY OF SOUTH AFRICAN MEDICINAL PLANTS ACCENTUATING ASPECTS RELATING TO METHODOLOGY

Sandy van Vuuren, Alvaro Viljoen

University of Witwatersrand, South Africa, Sandy.vanvuuren@wits.ac.za
Tshwane University of Technology, South Africa

A review of the antimicrobial research undertaken on South African medicinal plants validates the traditional medicinal practices used to treat infectious diseases. The various microbiological techniques such as disc diffusion, minimum inhibitory concentration, time-kill and synergy assays commonly used when assessing the antimicrobial activity will be presented with reference to their application in studies on South African medicinal plants. Antimicrobial investigations on extracts are presented where the most active plants are identified from a number of screening studies. Some extracts from indigenous medicinal plants (*Croton gratissimus*, *Tarchoanthus camphoratus*) have been selected to demonstrate antimicrobial activity. Antimicrobial activities on the essential oils of some of the most widely used indigenous medicinal aromatic plants are reviewed with emphasis on *Osmitopsis asteriscoides* and *Artemisia afra*. A summary of some bioactive compounds, isolated from South African medicinal plants, with antimicrobial activities $<200 \mu\text{g/mL}$ will be presented. A study on *Helichrysum cymosum* encompassing oil, extract and isolation of a bioactive compound (helihumulone) indicate activities 1–8 mg/mL, 78 – 313 $\mu\text{g/mL}$ and 16-125 $\mu\text{g/mL}$ respectively. Methods will be critically assessed and recommendations made, based on both literature reviews and practical experience. An overview is given on what antimicrobial activities should be considered noteworthy when reporting on efficacy of extracts, oils and compounds.

DIFFERENCES IN THE AMOUNT OF POLYPHENOLIC COMPOUNDS IN DIFFERENT AERIAL PARTS OF *PLANTAGO MAJOR*

Muhammad Zubair, Nybom Rumpunen Lindholm

Swedish University of Agricultural Sciences, Sweden, muhammad.zubair@ltj.slu.se
Department of Plant Breeding and Biotechnology Balsgard, Fjalkestads 459, Kristianstad 29194,
Sweden
Department of Clinical Nursing, Kristianstad University, 29188 Kristianstad, Sweden

The common weed *Plantago major* (common plantain) has been used as a wound

healing remedy in almost all parts of the world. It has also been used in the treatment of a number of other diseases in folk medicine. The biological activities of *P. major* leaves are wound healing, anti-inflammatory, analgesic, antioxidant, weakly antibiotic, immuno-modulating and anti-ulcerogenic properties. Polyphenols and polysaccharides in the leaves could possibly act synergistically to stimulate wound healing. The aim of the present study is to determine which of the aerial plant parts are most useful for production of specific phenolic compounds. Seed-propagated *P. major* plants were grown in a greenhouse. Leaves (both fresh and old), flowering stalks and seeds were harvested from five different populations obtained by one generation of controlled selfing (15 leaves per population, 15 flowering stalks and seeds from 15 spikes) and were freeze-dried. Leaves, flowering stalks and seeds were then ground and extracted with a 50% ethanol solution in an ultrasonic bath for 15 minutes. These extracts were analyzed using HPLC to quantify different phenolic compounds. The amount of some phenolic compounds (e.g. plantamajoside) in *P. major* decreases from young leaves to seed (young leaves - old leaves - flowering stalks - seeds), whereas other compounds (e.g. verbascoside) increase from young leaves to seeds (young leaves - flowering stalks and seeds). The maximum amount of plantamajoside was thus obtained in samples of young leaves while maximum content of verbascoside was obtained in flowering stalks and seeds (no significant difference between these).

THEME 5

QUALITY, EFFICACY AND SAFETY OF PHYTOMEDICINES AND PHYTOCOSMETICS

IN VITRO EVALUATION OF THE INTERACTIONS BETWEEN CRUDE LEAF EXTRACTS OF *HELICHRYSUM PEDUNCULATUM* AND SOME ANTIBIOTICS

Olayinka Ayobami Aiyeoro, Anthony Jide Afolayan, Anthony Ifeanyi Okoh

University of Fort Hare, Alice, South Africa, oaiyeoro@ufh.ac.za

University of Fort Hare, Alice, South Africa, aafolayan@ufh.ac.za, University of Fort Hare, Alice, South Africa, aokoh@ufh.ac.za

The effect of combinations of the crude acetone, methanol and water extracts of *Helichrysum pedunculatum* leaves and eight first-line antibiotics was investigated by means of fractional inhibitory concentration (FIC) indices as well as by the use of time kill assays. Using the FIC indices synergistic interactions were observed on most of the bacteria tested and this is independent of Gram reaction characteristics (FIC indices of ≤ 0.875) with combinations in the aqueous extract yielding largely antagonistic interactions (FIC indices of ≥ 2.0). The time kill assay detected synergy that is also independent of Gram reaction characteristics with a ≥ 1000 times ($\geq 3\text{Log}_{10}$) potentiation of the bactericidal activity of tetracycline and ciprofloxacin against *Klebsiella pneumoniae* as well as that of penicillin G and oxytetracycline against *Proteus vulgaris*. We conclude that acetone and methanol extracts of *H. pedunculatum* could be potential sources of broad spectrum antibiotics resistance modifying compounds.

ANTIOXIDANT AND PROOXIDANT EFFECTS OF EPIGALLOCATECHIN GALLATE AND TANNIC ACID ON DNA DAMAGE IN HUMAN LYMPHOCYTES

A. Ahmet Basaran¹, Dilek Tokaç², Sevtap Aydin², Nursen Basaran²

Hacettepe University, Faculty of Pharmacy, ¹Pharmacognosy Department
(abasaran@hacettepe.edu.tr)
Hacettepe University, Faculty of Pharmacy, ²Pharmaceutical Toxicology Department, TR06100
Ankara, Turkey, nbasaran@hacettepe.edu.tr

Dietary polyphenolic compounds are associated with a wide variety of beneficial health effects. The widely renowned biological actions of polyphenolic compounds are attributed to their antioxidant and free radical scavenging properties. They are however also reported to induce oxidative damage through the generation of reactive oxygen species. Epigallocatechin gallate (EGCG) and tannic acid (TA) are abundant and active polyphenolic compounds mostly found in herbal teas, fruits and beverages. EGCG is the main constituent of catechin polyphenols found in green tea. It is suggested that EGCG can reduce the risk of certain types of diseases and cancers because of its antioxidant properties. TA is also used as a food additive. In the present study the modulating effects of EGCG and TA against the oxidative DNA damage induced by H₂O₂ in human lymphocytes were investigated by COMET assay. Briefly after incubating human lymphocytes with low amounts of EGCG and TA at concentrations of 0.01-250 μ M, and 0.1-100 μ g/ml respectively with or without 0.05 μ M H₂O₂, DNA damage was determined. At the concentrations below 10 μ M of EGCG, and 100 μ g/ml of TA alone no significant DNA damage was observed. The concentrations above 10 μ M of EGCG alone seemed to induce DNA damage. However, at the concentrations of 0.01-30 μ M and 0.1-100 μ g/ml of EGCG and TA, respectively significantly reduced DNA strand breakage induced by 0.05 μ M H₂O₂ in human lymphocytes. In conclusion, it seems that our results highlight the potential benefit of EGCG and TA as natural antioxidants.

THE HAEMODYNAMIC RESPONSE TO THE CRUDE AQUEOUS EXTRACT OF *CRINUM MACOWANII* BULBS (CAEB) IN ANAESTHETIZED WISTAR RATS

Andries Burger, Kirstin Persson, Pierre Mugabo, Ivan Green

University of the Western Cape, South Africa, aburger@uwc.ac.za
University of the Western Cape, South Africa, kikipersson@hotmail.com
University of the Western Cape, South Africa, pmugabo@uwc.ac.za
University of the Western Cape, South Africa, igreen@uwc.ac.za

Crinum macowanii is used in traditional medicine. To increase efficacy and safety we evaluated the haemodynamic effect of the CAEB. CAEB was administered via the jugular vein after sodium pentobarbitone anaesthesia (40 mg/kg, i.p.) and tracheostomy. The rats were placed on a heating pad at 37°C. A Powerlab© using Deltran II© pressure transducers monitored femoral arterial pressure (BP). The heart rate (HR) was determined from BP. The data is reported as mean \pm SD (n=6) with significance set at P<0,05. Psys increased significantly from 125 \pm 3 mmHg at baseline to 180 \pm 19 mmHg at 0,2 mg/kg. After this dose there was a significant decrease in Psys with increased [CAEB] to 60 \pm 4 mmHg at 0,3 mg/kg. Pdiast peaked at 0,2 mg/kg (123 \pm 14 mmHg). This was not significantly relative to baseline (95 \pm 9 mmHg, n = 6). Pdiast did however decrease significantly (compared to the peak value at 0,2 mg/kg) at 0,25 mg/kg (90 \pm 13 mmHg) and 0,3 mg/kg (34 \pm 3 mmHg). The pulse pressure showed a non-significant tendency to increase up to 0,2 mg/kg followed by decreases at 0,25 and 0,3 mg/kg but due to large SDs this was not significant at any of the applied dosages. HR had a relatively flat response over the dose range but was significantly increased relative to the baseline at all

doses with the exception of 0,05 mg/kg. CAEB thus had a tachycardic effect at all doses with increased Psys up to and including 0,2 mg/kg, but it decreased Psys and Pdiast at doses higher than 2 mg/kg (0,25 mg/kg and 0.3 mg/kg).

INNOVATIVE PLANT IDENTIFICATION: PLANT REFERENCES IN A NEW ACCESSIBLE FORMAT: VIRTUAL HERBARIUM FOR ECONOMIC PLANTS AND THEIR RELATIVES

Trish Flaster

Botanical Liaisons, LLC, USA, tflastersprint@earthlink.net

Now that the FDA has passed the new GMPs it required manufacturers to qualify their starting materials and for the first time identify their plants "Plant identification has always been an area of weakness in the botanical industry". Now new innovative tools that assure product quality and consumer satisfaction are needed. Now there is an online tool that meets our criteria, Herbarium Online (SM). Since 1995 Botanical Liaisons, LLC, has focused on the plant identification as a core service. With over 600 plants common in commerce, it is difficult for manufacturers, researchers and regulators to know what plants they have on their premises. Botanical Liaisons, has created HerbariumOnlineSM, a virtual herbarium for economic plants. With this innovative tool you can identify your starting materials by viewing "zoomable" images of authenticated voucher specimens and their related TLC, microscopy and macroscopic samples. Multiple species of a genus are all online so a positive identification is possible. This presentation offers background information about the value of plant identification and a virtual walk through the virtual herbarium.

BIOCHEMICAL AND TOXICOLOGICAL EFFECTS OF *GLOBIMETULA BRAUNII* (LORANTHACEAE) LEAF

Adediwura Fred-Jaiyesimi, Temitope Onabanjo, Alfred Jaiyesimi

Faculty of Pharmacy, Olabisi Onabanjo University, Nigeria, adediwurajaiyesimi@gmail.com

Faculty of Pharmacy, Olabisi Onabanjo University, Nigeria

Faculty of Clinical Sciences, Olabisi Onabanjo University, Nigeria,

alfredjaiyesimi@yahoo.co.uk

Globimetula braunii (Engle) Van Tiegh, growing on the *Cola acuminata* tree is used in Nigerian traditional medicine as a laxative, against diabetes, as an antihypertensive and as an antimicrobial agent. Information on biological activity of this plant is scanty, however previous studies have reported the antidiabetic (Olagunju et al., 1999) and laxative effects (Fred-Jaiyesimi et al., 2008) of the leaf. This study aims at investigating the biochemistry and safety of the ethanol extract of *Globimetula braunii* leaf by determining its pharmaco-toxicological effects after administration in rats. After administering the extract to rats for 14 days, no deaths occurred. A single daily oral administration of *G. braunii* ethanol extract (2 g/kg) significantly ($p < 0.05$) lowered the triglyceride and cholesterol levels of the rats. A significant 22.2% and 58.7% reduction in the triglyceride level of the rats was observed on day 7 and 10 respectively while the blood cholesterol was lowered by 8.9% and 39.1%. The ethanol leaf extract had no

effect however on the Packed Cell Volume of the animals. The histopathology results showed no morphological alterations in the brains, kidneys, hearts, small intestines and spleens of the animals that got the leaf extract administered. The livers were normal without lesions but showed mild mononuclear cell aggregation. This study indicates that *G. braunii* is non-toxic when administered acutely. It may also be helpful in managing and controlling complications such as atherosclerosis, cholesterolemia, cardiovascular diseases often associated with diabetes as well as hypertension. Further studies to evaluate these potentials are required.

References:

1. Olagunju JA; Ismail FI; Gbile ZO (1999). The hypoglycaemic property of normal saline leaf extract of *Globimetula braunii* in alloxanized diabetic albino rats. Biomed. Lett. 60 (235): 83-89.
2. Fred-Jaiyesimi Adediwura, Onabanjo Temitope and Ademuyiwa Oluwakemi (2008). Phytochemical and laxative studies of *Globimetula braunii* (Engle) van Tiegh growing on *Cola acuminata* (Schott & Endl). AJTCAM (In press).

TRACE ELEMENT AND HEAVY METAL ANALYSIS OF COMMERCIAL GINSENG AND HYPOXIS SUPPLEMENT PRODUCTS

Gary Gabriels, Peter Smith

University of Cape Town, South Africa, gary.gabriels@uct.ac.za

Natural products extensive use as a source of primary health care requires routine quality control scientific testing methodologies to verify the safety, quality and efficacy of commercially available plant products. This will enhance public confidence in the use of such products in an unregulated environment, as adverse side effects often associated with the use of traditional/ herbal products, are generally due to misidentification of plants, lack of standardization and good manufacturing practices, botanical substitution, adulteration, and contamination. The therapeutic effect of plant medicine is alleged to be enhanced by essential trace elements. Some products may however also contain excessive amounts of trace elements and heavy metals. For this study the focus was two specific groups of product. Ginseng as a popular traditional Chinese medicine, used for its adaptogenic and restorative properties, and African Potato products used as an immune booster for patients infected with HIV/AIDS. Different commercial brands of Ginseng and African Potato products were purchased from pharmacies and health stores in Cape Town for this study. The products were evaluated for content validity and contamination for the following trace element and heavy metals, sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), Iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb) and mercury (Hg), Lithium (Li). The instrument utilized for the trace element and heavy metal analysis was the combination tandem system of Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) that is a powerful, accurate, fast and sensitive, analytical technique

A COMPARATIVE RANDOMIZED CLINICAL TRIAL OF NIPRD AM1 AGAINST A CHLOROQUINE AND SULPHADOXINE/PYRIMETHAMINE COMBINATION IN SYMPTOMATIC BUT UNCOMPLICATED MALARIA

Karniyus Gamaniel

National Institute for Pharmaceutical Research and Development, Nigeria, ksgama@yahoo.com

NIPRD 92/001/1-1 is a single plant preparation used in Africa to treat malaria and other ailments. The plant occurs widely in continental Africa where the various parts are used. The plant is also used extensively as a food source. The root extracts are usually preferred for the treatment of malaria. We studied the non-clinical efficacy and safety profile of NIPRD AM 1 against *P. berghei* in mice and found the product to be safe and effective. The results of our phytochemical studies on the plant extract revealed the presence of alkaloids, tannins and saponins and an aqueous extract yield of 3.6% w/w. The product had an LD₅₀ > 2000mg/kg p.o. in rats and mice and did not show any significant toxic activity within the organs or systems in the 28 day sub-acute toxicity study. However, there was a decrease in food consumption and weight loss associated with the product. NIPRD AM 1 also significantly reduced the spontaneous motor activity (SMA) in mice. A comparative randomized clinical trial of NIPRD AM 1 was carried out against symptomatic but uncomplicated Malaria in human volunteers at the two district hospitals located in Gwagwalada and Wuse, both in the Federal Capital Territory, Abuja, Nigeria. NIPRD AM1 was studied against a chloroquine and sulphadoxine/pyrimethamine (Fansidar) combination. The results indicated that NIPRD AM 1 was effective against uncomplicated malaria with its activity superior to that of CQ and SP. The parasite clearance was better than that of chloroquine and there were no threats of serious side effects affecting the organs or tissues. Acknowledgements: WHO TDR provided funding.

PLANTS AS SOURCES OF HUMAN HEALTH PRODUCTS

Peter Houghton

Kings College London, United Kingdom, peter.houghton@kcl.ac.uk

Plants continue to provide novel molecules for therapeutic use but there is also an increasing interest in the acceptance of extracts or mixtures of plants in Western medicine. Examples of molecules achieving clinical importance in recent years are galantamine, huperzine A and artemisinin whilst other natural products are valuable as lead compounds. Many clinical trials are now being carried out on herbal products e.g. ginkgo, black cohosh, Job's tears and some encouraging results are being obtained which substantiate their acceptance in mainstream Western medicine as well as in their traditional cultural environments. Regulatory systems are becoming more sympathetic but also recognising the need for good quality and adequate safety testing. Novel analytical and drug discovery approaches are consolidating the scientific appraisal of plants as sources of new medicines.

MOLECULAR IDENTIFICATION OF *HYPERICUM PERFORATUM* L. BY PCR AMPLIFICATION OF THE RDNA ITS REGION

Caroline Howard, Paul Bremner, Mark Fowler, Adrian Slater

De Montfort University, United Kingdom, caroline.howard@learner.dmu.ac.uk

De Montfort University, United Kingdom, PBremner@dmu.ac.uk

De Montfort University, United Kingdom, mrfowler@dmu.ac.uk

De Montfort University, United Kingdom, ads@dmu.ac.uk

The increase in usage of medicinal plant products has highlighted the necessity for authentication and quality assurance in commercial products. The development of a DNA-based method for the identification of St John's Wort (*Hypericum perforatum* L.) was used as a pilot study to determine the feasibility of using DNA barcode data in the future to design simple authentication tests. The nuclear-encoded rRNA coding regions are highly conserved throughout plant species but the ITS sequences have an evolutionary rate which results in inter-species variation and intra-species conservation, and have been identified as potential DNA barcode regions. PCR primers were designed to recognise the most divergent regions of the ITS1 sequence in *Hypericum* species. One primer pair designed to selectively identify St John's Wort within this region was able to discriminate *H. perforatum* from vouchered DNA samples of 7 different *Hypericum* species (DNA Bank, Royal Botanic Gardens, Kew). St John's Wort could also be distinguished from related ornamental *Hypericum* species obtained from seed companies and nursery suppliers. Three commercial medicinal products claiming to contain St John's Wort were also tested. The PCR assay identified *Hypericum perforatum* in only two of three commercial products at a level of detection corresponding to 0.75ng DNA (0.1% of the total genomic DNA present). The method utilised in this study has enabled the design of a rapid and reliable authentication method for St John's Wort. This has the potential to become a model for molecular identification design, and may be reproducible in other economically valuable plants.

QUALITY AND CHEMISTRY OF AFRICAN BIRDS EYE CHILI (*CAPSICUM FRUTESCENS*) FROM ZAMBIA

H. Rodolfo Juliani, Julia Coppin, Kwansu Kim, Halimu Malumo, Bismarck Diawuo, P. Langenhoven and Jim Simon

New Use Agriculture and Natural Plant Products Program. Rutgers University. Foran Hall. 59
Dudley Road. 08901 New Brunswick, NJ, USA.
ASNAPP-Zambia, Lusaka, Zambia
ASNAPP-South Africa, Stellenbosch, South Africa (www.asnapp.org)

African Birds Eye Chili (*Capsicum frutescens*) is a specialty hot pepper of increasing interest to consumers and to those who enjoy and rely on hot peppers. Assessing of the chemistry and quality of this spice is becoming increasingly important to increase its market access and better define the product variation from different producing regions. The objective of this study is to assess the chemistry and quality of African Birds Eye Chili grown in Zambia. Fruit samples from this plant were grown and harvested (2006-2007 seasons) from the Chinyanja Triangle area and from the 2008 season from Chongwe, Kalomo and Mpongwe and subjected to proximate and chemical analysis. The deep orange color of the Zambian African Birds Eye Chili was found to be very positive. All the samples showed low levels of moisture (<10%) indicating proper drying and showed no contamination with sand. The samples from Chongwe and Mpongwe showed the highest levels of capsaicinoids (by HPLC), with capsaicin levels of >1%, and 1%, respectively; and dihydrocapsaicin reaching 0.5-0.7% and 0.5%, respectively from these sites. Samples from the Chinyanja Triangle, 2006-07 were found to be lower (0.6-0.8% capsaicin, 0.3-0.5% dihydrocapsaicin) but direct comparisons are not possible as those studies were conducted in an earlier growing season. In 2008 season, the application of Good Agricultural Practices, higher quality seeds and improved production practices (improved weeding and drying) which appeared to result in the production of high quality Chili. A study to compare Zambian African Birds Eye Chili to other hot pepper commercial samples found on the USA market found that the Zambian product contained far higher levels of capsaicin and were of superior quality to the commercial hot pepper products.

ASSESSMENT OF THE NUTRITIONAL VALUE OF *FADOGIA TRIPHYLLA* HERBAL TEA OF ZAMBIA**H. R. Juliani, A. Koroch, Y. Fonseca, H. Malumo, B. Diawuo, J. E. Simon**

New Use Agriculture and Natural Plant Products Program, Rutgers, The University of New Jersey, 59 Dudley Road, New Brunswick, NJ 08901-8520 (pfidnp.org). ²ASNAPP Zambia. Lusaka, (www.asnapp.org)

Fadogia triphylla, a perennial shrub that can reach up to 120 cm is used both as a local herbal tea and in traditional medicine. Traditional healers have reported the use of *Fadogia* spp. in the treatment of coughs, ascariasis, abdominal pains and stomachs. There is limited information about the nutritional and medicinal value of the leaves of this local tea. Thus, the objective in this study was to assess the nutritional value and herbal tea characteristics of *F. triphylla* leaves from Zambia. *Fadogia triphylla* leaves were collected from Shatumbu district, Lusaka at two different times (June 2006 and August 2006). The dry *Fadogia* leaves were then subjected to foreign matter analysis and moisture content analysis. Total ashes and total insoluble ashes, total phenols, antioxidant activity, and elemental and caffeine. The leaves are high in antioxidant components (8%) and rich in minerals (7%), potassium (1%), calcium (1.3-1.8%) and manganese (90-71 mg/100 g). No caffeine was detected in the leaves based upon HPLC analysis and pure caffeine as the standard, permitting the labelling of this bush tea to be caffeine-free infusion. Initial quality standards were developed for the first time to define quality in *Fadogia* tea. While a preliminary study, we found the leaves to be high in antioxidant components, rich in minerals (e.g. potassium, calcium and manganese), caffeine-free and the herbal tea to have a pleasant taste. *Fadogia* leaves contain interesting functional properties that show the leaves to be healthy and nutritional and which may assist in the commercialization of this indigenous tea into non-local markets.

PLANT CULTIVATION, SCALED UP PREPARATION AND BIOLOGICAL TESTING OF NON ETHANOLIC EXTRACTS OF *PELARGONIUM SIDOIDES DC***Oliver Kayser, Wilfried Kohnen, Ludwig Nyhuis, Riaan van Breda**

University of Groningen, the Netherlands, o.kayser@rug.nl
 EmsPharm GmbH, Germany, w.kohnen@emspharm.de EmsPharm GmbH, Germany,
 l.nyhuis@emspharm.de
 Afriplex (Pty) Ltd, South Africa, riaan@afriplex.co.za
 Fifth Author Danien J.C. Nel Afriplex (Pty) Ltd South Africa, danien@afriplex.co.za

Ethanollic extracts of *Pelargonium sidoides* DC and *Pelargonium reniforme* CURT are used for the production of the market authorized herbal remedy Umckaloabo®. In various studies related ethanollic extracts have been proven to be successful for high activity against upper airway infections. As most relevant constituents a variety of highly oxygenated coumarins, diverse phenolic compounds and hydrolysable and condensed tannins have been identified and tested. Despite its documented safe use in clinical studies, there has been a debate about the application of ethanollic extracts on children as one target group for ethanollic extract prescription. In this study we have invented an extraction protocol using PEG400, sorbitol, glycerol and water

to produce phytoequivalent extracts with remaining high biological activities in the claimed therapeutic fields (1). Extracts were prepared from sustainable cultivated plants by industrially scaled up percolation of root material from three different batches and the qualitative and quantitative profile was determined by HPLC-UV/DAD and HPLC-MS. In non ethanolic PEG extracts the same metabolite profile as it is known in ethanolic extracts was confirmed based on scopoletin and umckalin as reference compounds. To validate biological activity and equivalency non ethanolic extracts were tested against *S. aureus*, *H. influenzae*, *P. mirabilis*, and *E. coli*. All extracts showed moderate antimicrobial activity. In extended immunomodulating assays interleukin-like activity has been demonstrated in macrophages infected with *L. donovani* as an intracellular parasite.

Reference:

[1] Kohnen, W. (2007) Verfahren zur Extraktion von Wurzeln der Pflanzengattung *Pelargonium*, danach hergestelltes Extrakt und Verwendung desselben, DE 2005 034227 A1

STABILITY OF *PHYLLANTHUS EMBLICA* EXTRACT IN LIPOSOMES**Pornpen Kongiampitee, Ubonthip Nimmannit**

Chulalongkorn University, Thailand, pornpen_oung@hotmail.com
Chulalongkorn University, Thailand, Ubonthip.N@Chula.ac.th

Phyllanthus emblica L. (*P. emblica*) has many benefits in its biological effects, including anti-oxidant activity, anti-tyrosinase activity, anti-microbial activity and protection against radiation-induced chromosome damage. However, the stability is a major concern of the *P. emblica* extract. Therefore, the objective of this research is to compare the stability of the *P. emblica* extract in liposomes and buffer solutions at pH 5.5 and 7.4. The lipid compositions of liposomal membranes were egg phosphatidylcholine (Lipoid E80) and cholesterol at 2:1 molar ratio with cholesterol ester as a stabilizer. The concentrations of *P. emblica* extract were varied at 1, 2, 3, 4 and 5 mg/mL. The encapsulation efficiency and chemical stability were determined by measuring the amount of total phenolic compounds using UV-VIS spectrophotometer. The highest percent encapsulation efficiency was found at 52.83% obtained from liposome formulation containing 1 mg/mL of *P. emblica* extract prepared at pH 5.5. In addition, the above preparation was stable at least 12 weeks when stored in a refrigerator at 4°C. The particle size of the liposomes stored for 0 and 12 weeks was not significantly different with the mean particle sizes of 5.790 + 0.756 and 5.553 + 0.647 µm respectively. Summarily, *P. emblica* extract in liposomes is more stable than that in buffer solutions.

EFFICACY OF ASTAXANTHIN, A NATURAL ANTIOXIDANT FROM ALGAE, IN THE TREATMENT OF FUNCTIONAL DYSPEPSIA: A PROSPECTIVE, RANDOMIZED, DOUBLE BLIND, AND PLACEBO-CONTROLLED STUDY

Limas Kupcinskas, Ake Lignell, Leif Andersen, Torkel Wadström

Kaunas University of Medicine, Lithuania, likup@takas.lt
AstaReal, Sweden, ake.lignell@astareal.com

University of Copenhagen, Denmark, leif.p.andersen@rh.hosp.dk
Lund University, Sweden, torkel.wadstrom@mmb.lu.se

The aim of this study was to evaluate the efficacy of the natural antioxidant astaxanthin from algae in functional dyspepsia in different doses and compared with a placebo.

Materials and methods: The study was a controlled, prospective, randomized, and double blind trial. Patients with functional dyspepsia were divided into three groups with 44 individuals in each group (placebo, 16 mg, or 40 mg astaxanthin respectively). Participants were asked to accept gastroscopy before treatment and they had to fill out questionnaires: GSRS and SF-36. A urea breath test (UBT) was done before the treatment.

Main outcome: The primary objective was to test the hypothesis that the antioxidant astaxanthin at two doses regimens compared to the placebo should ameliorate gastrointestinal discomfort, measured as GSRS, in patients with functional dyspepsia, who were either positive or negative for *Helicobacter pylori*, after 4 weeks of treatment.

Results: At the end of the therapy (week 4) no difference between the three treatment groups was observed regarding the mean Gastrointestinal Symptom Rating Scale (GSRS) scores of abdominal pain, indigestion and reflux syndromes. The same results were observed at the end of the follow-up. However the reduction of reflux syndrome before treatment to week 4 was significantly pronounced in the higher (40 mg) dose compared to the other treatment groups (16 mg and placebo, $p=0.04$). **Conclusion:** In general, no curative effect of astaxanthin was found in functional dyspepsia patients. Significantly greater reduction of reflux symptoms were detected in patients treated with the highest dose of the natural antioxidant astaxanthin. The response was more pronounced in *H. pylori*-infected patients.

HYPOTENSIVE EFFECTS AND ACUTE TOXICITY PROPERTY OF METHANOL EXTRACT OF *BAISSEA AXILLARIS* HAU. LEAF ON ANIMAL MODELS

MacDonald Idu, Odaro Timothy

University of Benin, Nigeria, macdonaldidu@hotmail.com
University of Benin, Nigeria, odtimo@yahoo.com

The hypotensive and acute toxicity activities of methanol extract of *B. axillaris* leaves, obtained by Soxhlet extraction, were analyzed using animal models. The extract exhibited a dose-dependent reduction in rabbit blood pressure. Basal mean arterial pressure of 115.67 ± 3.60 mmHg was progressively reduced to 106.22 ± 3.13 and 69.3 ± 2.59 mmHg by 2.5 and 20 mg kg⁻¹ doses of extract respectively. Haematological parameters in Wistar rats treated with 1, 2 and 4 g kg⁻¹ of extract showed no significant difference ($P > 0.05$). However, lethal dosage was obtained at 8 g kg⁻¹. The results obtained showed that the extract has a blood pressure lowering effect.

DEVELOPMENT OF A QUALITY CONTROL PROTOCOL FOR *PELARGONIUM SIDOIDES* USING NEAR INFRARED SPECTROSCOPY (NIRS)

Joanet Maree, Alvaro Viljoen, Marena Manley

Tshwane University of Technology, South Africa, mareeje1@tut.ac.za
Tshwane University of Technology, South Africa, viljoenam@tut.ac.za
Stellenbosch University, South Africa, mman@sun.ac.za

Quality control analysis is vital in the pharmaceutical industry to guarantee the authenticity and the quality of products. A major challenge in quality assurance of herbal material is the vast variation of active constituents in plants from the same species. As a result of this variation, the selection of only a few compounds as criteria for quality control is inadequate. Identification is one of the first steps in quality and safety assurance of herbal medicinal products. Identification tests should be able to discriminate between related species and/or potential adulterants. *Pelargonium sidoides* is indigenous to South Africa with a centre of diversity in the Eastern Cape Province. It is highly valued in South Africa by traditional healers. For hundreds of years various ethnic groups have used root extracts of *P. sidoides* as a remedy for coughs, upper respiratory tract irritations and gastrointestinal conditions. An ethanol extract of *P. sidoides* is used in the proprietary herbal tincture known as Umckaloabo® that is currently being successfully marketed in Germany with sales that have escalated over 700%. For many years the true taxonomic identity of this herbal medicine was debated. In 1974 the origin of the decoction was claimed to be *P. reniforme*. It is now speculated that identification was wrong and that *P. sidoides* was used. Despite the commercial interest in *P. sidoides* very few studies have been conducted to document the full phytochemical range of the variation for natural populations and to construct a fast accurate quality assurance method for the validation of raw material. High performance liquid chromatography (HPLC) and thin layer chromatography (TLC) are being used for the validation of *P. sidoides* raw material. In this project near infrared profiles of *P. sidoides* and *P. reniforme* were constructed and compared. Plant samples were collected from different locations in the Eastern Cape Province of South Africa. The NIRS data were used to do qualitative analysis in order to detect the relationships among all samples. Quantitative analysis was done to construct a calibration curve that can accurately predict the percentage of contamination of *P. sidoides* with *P. reniforme*.

CHEMICAL PROFILING OF *SUTHERLANDIA FRUTESCENS* AND *S. MICROPHYLLA*

Nontobeko Mncwangi, Alvaro Viljoen, Marena Manley

Tshwane University of Technology, South Africa, mncwangin@tut.ac.za
Tshwane University of Technology, South Africa, viljoenam@tut.ac.za
Stellenbosch University, South Africa, mman@sun.ac.za

Sutherlandia frutescens and its taxonomic ally, *S. microphylla* are indigenous to southern Africa and both boast an impressive history as an ethnomedicinal plant. The uses are diverse, spanning from the use against infectious diseases to the use against wasting to the treatment of diabetes and inflammation. More recently *S. frutescens* has been used in the treatment of conditions associated with HIV. The various pharmacological properties are ascribed to the presence of amino acids, triterpenes and glycosides. Despite the extensive use, published scientific studies focusing on the phytochemical profile and variations are scarce. This is crucial in unravelling the scientific rationale for the past and present day uses and possibly provides valuable information to optimise harvesting protocols and could contribute to good manufacturing practices. The aerial parts of *S. frutescens* (n=125) and *S. microphylla* (n=20) were collected from natural and cultivated sites. Near infrared spectroscopy (NIRS) was used to determine the potential of this technique to distinguish between the two species. The amino acids and pinitol were quantified with high performance liquid chromatography (HPLC). High-pressure thin layer chromatography (HP-TLC) was used to assess the consistency in the chemical fingerprint for the various samples. NIRS shows no difference between the two species. This technique was

however not sensitive enough to quantify the amino acid content through NIRS calibration. The quantitative variation of amino acids and pinitol within and between natural populations and individual plants in a cultivation site was noted and these results will be discussed.

QUALITY, EFFICACY AND SAFETY OF PHYTOMEDICINES AND PHYTOCOSMETICS
FUMIGANT AND REPELLENT ACTIVITY OF *OCIMUM AMERICANUM* L. ESSENTIAL OILS
AGAINST FOUR INSECT PESTS OF STORED FOOD COMMODITIES

J.O. Ogendo, A.L. Deng, M. Kostyukovsky, U. Ravid, J.C. Matasyoh, E.O. Omolo, S.T. Kariuki, A.W. Kamau, E. Shaaya

Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536 Egerton 20115
Kenya

Department of Food Science, ARO, The Volcani Center, Bet Dagan 50250, Israel.

Department of Aromatic, Medicinal and Spice Crops, ARO, Newe Ya'ar Research Center,
Ramat Yishay 30095, Israel

Department of Chemistry, Egerton University, P.O. Box 536 Egerton 20115 Kenya

Department of Biological Sciences, Egerton University, P.O. Box 536 Egerton 20115 Kenya

Laboratory studies were conducted to evaluate the fumigant and repellent properties of essential oils extracted from the leaves of *Ocimum americanum* L. against five storage insects, *Sitophilus oryzae* L., *Rhyzopertha dominica* F., *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* F. Each essential oil was tested at four (0, 1, 5 and 10 µl/L air) and five (0, 0.5, 1.0, 1.5 and 2.0 µl oil/ g grain) rates in space fumigation and choice bioassay studies, respectively, with four replicates per treatment. In space fumigation studies, leaf essential oil at 10 µl/L air 24 h after treatment, caused 88.8, 87.5, 11.3 and 100% kill of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis* insects, respectively, whose corresponding LC₅₀ values were 0.38, 0.49, NS and 0.18 µl/L air, respectively. The LC₅₀ values varied with plant part from which the essential oil was extracted. Leaf essential oil was most efficacious (0.18-0.49 µl/L air) followed by fruit (1.15-15.07 µl/L air) and stem (3.70-34.68 µl/L air) essential oils, respectively. In the choice bioassay studies, fruit essential oil (at 2.0 µl oil/ g grain and 24 h) produced highest repellence against *S. oryzae* and *R. dominica* with PR values of 81 and 94%, respectively. Under the same conditions, the leaf essential oil had PR values of 51 and 83% against adult *T. castaneum* and *C. chinensis* insects, respectively. Results of this study demonstrate the potential use of plant volatiles as alternatives to the synthetic fumigants and their applicability for preservation of stored food commodities in subsistence agriculture.

Key words: *Callosobruchus chinensis*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum*, *Ocimum americanum*, Essential oil, Fumigant toxicity, Repellence,

*Corresponding author: Email: ogendojoshua@yahoo.co.uk (J.O. Ogendo)

Fax: +254-51-62145/62442

Cell phone: +254-722-892504; +254-738-412218

EFFECT OF CD ACCUMULATION ON GROWTH AND BIOLOGICAL ACTIVITY OF
MERWILLA PLUMBEA (HYACINTHACEAE) – A WIDELY USED MEDICINAL PLANT

Rene Street

University of Kwazulu-Natal, South Africa, renee.street@mrc.ac.za

Merwillia plumbea (Lindl.) Speta is one of the most popular plant species sold at medicinal markets in South Africa. Due to its high demand, *M. plumbea* is highly recommended for cultivation. Cadmium (Cd) content in the soil has drastically increased due to pollution and is an important metal to consider in terms of food-chain contamination. The aim of this study was to determine Cd uptake and accumulation and its effect on growth and antibacterial activity in *M. plumbea*. Cadmium (2, 5 or 10 mg l⁻¹) was supplied to the growth media of potted *M. plumbea* plants for 6 weeks after which various growth parameters were recorded. Elemental concentration (mg kg⁻¹) was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES). Plant extracts were bioassayed against two Gram-positive and two Gram-negative bacteria using the microdilution method for minimum inhibitory concentration (MIC) determination. Compared with control, Cd had a negative effect on the growth parameters of *M. plumbea*. The medicinally used bulbs accumulated Cd 7.1, 5.9 and 11.6 mg kg⁻¹ when supplied with Cd at 2, 5 and 10 mg l⁻¹ respectively. When supplied with Cd at 10 mg l⁻¹, *M. plumbea* bulbs showed higher antibacterial activity against three bacterial species. However, compared to control, no change in activity was seen against *E. coli*. Due to Cd accumulation in *M. plumbea*, consumer safety may be compromised. In addition, both horticultural yield and phytochemical composition may be negatively affected when grown in Cd-contaminated soils.

PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *BERKHEYA BERGIANA* LEAVES

Olukayode Odeleye, Adebola Oyedeji, Andy Opoku

Department of Chemistry, University of Zululand, South Africa, odeleyeom@yahoo.com

Department of Chemistry, University of Zululand, South Africa

Department of Biochemistry and Microbiology, University of Zululand, South Africa

The use of medicinal plants in the world and especially in South Africa, contributes significantly to primary health care. The genus *Berkheya* belongs to the family of the Asteraceae. There are about 75 species of *Berkheya*. 71 species are widespread in Southern Africa and 30 species are spread in Natal. The plant material was air-dried, powdered and extracted with methanol. The fractions were obtained by successive extraction to yield n-Hexane (Hex), Chloroform (CHCl₃), Ethyl acetate (EtOAc), and n-Butanol (BuOH). Phytochemical screening was carried out using standard methods. The antioxidant activity was investigated by DPPH radical scavenging effect, by reducing power, by the metal Chelating effect on ferrous ion and by the ABTS scavenging effect. Phytochemical screening carried out on *B. bergiana* revealed the presence of carbohydrates, flavonoids, terpenoids, saponins and tannins, but the absence of anthraquinones and alkaloids. The result demonstrated that the methanol extract of *B. bergiana* had excellent antioxidant activities and also showed that the crude extract and fractions were found to have different levels of antioxidant activity in all the systems tested. Results revealed that the BuOH fraction exhibited the best performance in DPPH and the Metal chelating assay, 93.7% and 63.0% respectively. IC₅₀'s were determined for the fractions and EtOAc showed the lowest IC₅₀ (110.73 µg QE/mg) Total phenolic, proanthocyanidins and flavonoid contents in the crude extract and the fractions were also determined. The EtOAc fraction (12.29±0.11GAE mg g⁻¹) had the highest total phenolic content. A strong correlation was recorded between DPPH/GAE (R²= 0.85). This study of phytochemical characteristics and antioxidant activity of the methanol extract and the fractions of *B. bergiana* was being conducted for the first time.

Acknowledgements: National Research fund (NRF), University of Zululand research committee.

QUALITY AND COMPOSITION OF *SCELETIUM* PLANT MATERIAL FOR USE AS PHYTO-MEDICINES

S. Patnala, I. Kanfer

Division of Pharmaceutics, Faculty of Pharmacy, Rhodes University, Grahamstown, 6140 South Africa

The *Sceletium* plant has been reported to contain psychoactive alkaloids, specifically mesembrine, mesembrenone, mesembrenol and other related alkaloids. *Sceletium* is marketed on the internet as dried plant powder that is claimed to have mood elevation and anti-anxiety properties. Furthermore, the plant, locally known as “kougoed” or “œchanna”, is advertised as having increased potency after it has been fermented. The absence of quality control (QC) requirements for potency and purity of herbal preparations is a major concern due to variation in chemical content and composition between samples and some may not even contain the assumed active contents. The present study investigated the quality and composition of five samples of *Sceletium tortuosum* plant material and two samples of *S. emarcidum*, obtained from cultivators and which were intended for use as phytomedicines. The results obtained from HPLC analyses of the five *Sceletium tortuosum* samples showed large variation in the alkaloidal content and composition with only one sample having quantifiable alkaloids, mesembrine (0.6%) and mesembranol (0.5%) and lower quantities of the minor alkaloids such as mesembrenone and epimesembranol. The *S. emarcidum* samples showed complete absence of the relevant alkaloids. These results thus reflect a major issue generally associated with herbal products in that there could be phytopharmaceutical products containing species which either are devoid of presumed chemical components such as alkaloids in the case of *Sceletium* or whose chemical constituents are too low thereby resulting in inappropriate raw material for use as phytomedicines. Acknowledgements: The authors acknowledge the Medical Research Council, South Africa for a post-doctoral fellowship (S Patnala).

EFFECT OF POLYSACCHARIDE GEL FROM THE FRUIT-RIND OF “DURIAN” AS AN IMMUNOMODULATOR IN *PENAEUS MONODON* (BLACK TIGER SHRIMP)

Komsil Pholdaeng, Sunanta Pongsamart

Chulalongkorn University, Thailand, kpholdaeng@yahoo.com
Chulalongkorn University, Thailand

Polysaccharide gel (PG) isolated from the fruit-rind of “durian” (*Durio zibethinus* Murr.) has been characterized to be a pectic polysaccharide with immunomodulating and bactericidal activities. This study aimed to evaluate the immunostimulating effect of PG, given as a PG additive diet in *Penaeus monodon*, by observing the increment of immunocompetency and disease resistance in shrimps. PG additive diets were 1, 2 and 3% PG in shrimp diet, while a diet without PG was used in the control. Each group of 20 juvenile shrimps in closed-recirculating treated water system was fed PG additive diets or the shrimp diet in control, for 12 weeks. Total haemocytes count and phenoloxidase activity of the shrimps immunity were examined.

Phenoloxidase activity in shrimps fed with 1 - 3% PG and total haemocytes count in shrimps fed with 1 and 2% PG were higher ($P < 0.05$) than those of the controls. The number of surviving shrimps in groups of 12 weeks prefeeding with 1-3% PG increased against the impact of white spot syndrome virus (WSSV) and bacterium *Vibrio harveyi* 1526 pathogens more than their control groups. Relative percent survival (RPS) values in groups fed 0, 1, 2 and 3% PG were 0, 76, 100 and 83% respectively at day 6 against WSSV infection and 0, 31, 36 and 22% respectively at day 4 against *V. harveyi* 1526 infection. Prefeeding of 2% PG showed the highest RPS value for disease resistance of shrimps against both viral and bacterial pathogens. These results suggest that a PG additive diet has the potential to increase immunostimulating activity in the juvenile shrimp, *Penaeus monodon*.

CYTOTOXIC ACTIVITY OF SELECTED PLANTS USED IN ETHNOMEDICINE IN WESTERN NIGERIA

Abimbola Sowemimo, Maryna Van De Venter, Lucinda Baatjies, Trevor Koekemoer

University of Lagos, Nigeria, bimsowemi@yahoo.com, Nelson Mandela Metropolitan University, South Africa, Maryna.VanDeVenter@nmmu.ac.za

Nelson Mandela Metropolitan University, South Africa, Lucinda.baatjies@nmmu.ac.za, Nelson Mandela Metropolitan University, South Africa, Trevor.Koekemoer@nmmu.ac.za

Seventeen plants [*Sapium ellipticum* (Hocsht) Pax (Euphorbiaceae), *Combretum paniculatum* Vent (Combretaceae), *Celosia trigyna* Linn (Amaranthaceae), *Pupalia lapacea* (Linn) Juss (Lythraceae), *Annona muricata* Linn (Annonaceae), *Justica extensa* T. Anders (Acanthaceae), *Hedranthera barteri* (Hook f) Pichon (Apocynaceae), *Alternanthera sessilis* (Linn) D.C (Amaranthaceae), *Ethulia conyzoides* Linn f (Compositae), *Lannea nigritiana* (Sc Elliot) Keay (Anacardiaceae), *Combretum zenkeri* Engl & Diels (Combretaceae), *Combretum molle* R.Br (Combretaceae), *Adenanthera parvonina* Linn (Leguminosae), *Lannea acida* A.Rich (Anacardiaceae), *Cyathula achyranthoides* (H.B & K) Moq (Amaranthaceae), *Drymaria cordata* (Linn) Willd (Caryophyllaceae) and *Cyathula prostata* (Linn) Blume (Amaranthaceae) are used in herbal medicine for the treatment of cancer [1]. The methanolic extracts of these plants were evaluated for cytotoxicity at concentrations of 250 and 500 µg/ml using the *Hela* cell line (cervix tumor cell line) to validate the ethnobotanical uses of the plants and compare their activities. *Sapium* leaves, *C. paniculatum* leaves, *Celosia* leaves, *Annona* leaves, *Drymaria* leaves and *C. prostata* leaves showed 60-80% inhibition, *Hedranthera* leaves 50% inhibition, *Pupalia* leaves, *Justica* leaves, *Alternanthera* leaves, *Ethulia* leaves, *Sapium* stem bark, *Lannea* stem bark, *C. zenkeri* root showed less than 50% inhibition while *C. molle* leaves, *Alternanthera* fruits and *L. acida* stem bark showed no inhibition at all. However, *Sapium* and *Annona* leaves showed activity comparable to the reference compound Cisplatin and both plants had greater cytotoxic activity than *C. paniculatum*, *Celosia*, *Drymaria* and *C. prostata*.

Acknowledgement: The authors would like to thank Profs Coker and Ketiku for providing the *Annona* leaves.

AUTHENTICATION OF PLANTS USED IN TRADITIONAL CHINESE MEDICINE—CHALLENGES AND OPPORTUNITIES

Monique S.J. Simmonds and Christine Leon

Royal Botanic Gardens, Kew Richmond Surrey, TW9 3AB

Plants have played an important role in traditional Chinese medicine (TCM). This talk will provide an overview of how Kew has gone about developing a collection of species of TCM plants that can be used as standards to check the identity and quality of plants entering the trade in Britain. The collection contains samples for DNA based bar -coding, chemical fingerprinting and anatomical analysis as well as traditional taxonomic specimens. The talk will cover the challenges posed by changes in the sale of over-the-counter herbal medicines in Europe and the need for similar collections to cover the wide scope of traditional medicines being traded in Europe.

IN VITRO EVALUATION OF ANTI-AGING ACTIVITIES OF *ARTOCARPUS LAKOOCHA* HEARTWOOD EXTRACT

Parkpoom Tengamnuay, Wora-anong Prugsakij, Adisak Wongkajornsilp

Chulalongkorn University, Thailand, parkpoom.t@chula.ac.th
Chulalongkorn University, Thailand
Mahidol University, Thailand

Artocarpus lakoocha heartwood extract (Family Moraceae) and its main active constituent oxyresveratrol are potent tyrosinase inhibitors. Recently we have shown that the extract lightened the skin in volunteers and possesses several ROS scavenging activities [1, 2]. In this study we evaluated *A. lakoocha* heartwood extract and oxyresveratrol for their anti-aging activities in cultured fibroblasts. Using the MTT assay, the proliferative effect of *A. lakoocha* extract and that of oxyresveratrol was similar to the one of epigallocatechin gallate, french pine bark extract, L-ascorbic acid and Trolox[®] in the same concentration range (25 – 100 µg/ml). All the test antioxidants were also capable of protecting the fibroblasts against cell damages caused by hydrogen peroxide and UV-A radiation. Based on the extent of the LDH release from the fibroblasts, *A. lakoocha*, oxyresveratrol, pine bark and Trolox[®] caused less membrane irritation than L-ascorbic acid and EGCG when compared at the same concentration (100µg/ml). *A. lakoocha* and oxyresveratrol also showed a good anti-collagenase activity, with the respective mean IC50 of 58.8 and 153.1 µg/mL, which were less potent than EGCG (8.0 µg/mL) and pine bark (21.9 µg/mL) but much more potent than L-ascorbic acid (1.331 mg/mL) and Trolox[®] (2.348 mg/mL). Moreover, both *A. lakoocha* and oxyresveratrol could reduce the extent of DNA damages caused by UV-A as detected by flow cytometry. In conclusion, the multi-functional beneficial effects of *A. lakoocha* heartwood extract may have a strong potential for the use in cosmetic and other health-related products.

Acknowledgements: National Research Council of Thailand provided funding.

PRELIMINARY STUDIES ON THE USE OF WATER ACTIVITY (AW) MEASUREMENTS FOR POSSIBLE APPLICATIONS IN THE PLANT EXTRACT INDUSTRY

Astrid van Ginkel, Lina Restrepo Peña, Gema Rodrigo Villar

Fitomon, Spain, astrid@fitomon.com
Lab-Ferrer, Spain, calidad@lab-ferrer.com
Lab-Ferrer, Spain, info@lab-ferrer.com

The objectives of this work were firstly, the screening for aw in liquid and dry herbal extracts (20 and 24 samples respectively), from 5 different manufacturers and secondly, relating aw variations to moisture content changes of dry extracts exposed to different ambientes (three controlled humidity chambers at 98, 76 and 50% RH, at 20°C). Aw was measured with a Dew Point Hygrometer. First results showed that aw values ranged from 0,190 to 0,420 and from 0,780 to 0,999 in dried and fluid extracts respectively and secondly as soon as the dried extracts were exposed to higher RH, a sudden increment of aw was observed while the rise of moisture content was smoother and delayed with respect to aw. The lower the initial aw value of the sample, the steeper was the increment. After 3-4 hours of exposure to 98% RH, aw reached, in some cases, values beyond 0,60 (moulds and yeasts can start developing). Also, glass transition was observed at 76% and 98% RH. This study shows that the exposure of dried herbal extracts to ambient conditions may lead to increment of aw that can affect the quality and shelf-life of the product. It also seems that aw is a more suitable tool than the moisture content for HACCP. Further research is needed to evaluate the possible moisture absorption from the ambiente that can occur when, packaging is not well sealed or/and they are exposed to moist environments, moist manufacturing and mixtures with products of higher aw, among others.

TOWARDS A RAPID QUALITY CONTROL METHOD FOR *HOODIA GORDONII* RAW MATERIAL AND PRODUCTS

Ilze Vermaak, Alvaro Viljoen, Marena Manley

Tshwane University of Technology, South Africa, vermaaki@tut.ac.za

Tshwane University of Technology, South Africa, viljoenam@tut.ac.za

Stellenbosch University, South Africa, mman@sun.ac.za

Hoodia gordonii is a spiny succulent plant belonging to the Apocynaceae family and is indigenous to South Africa and Namibia. It is currently used as the major herbal ingredient in slimming products. Due to its limited geographical distribution and the need for a permit to cultivate and export raw material, the degree of adulteration of commercially available products is very high. Some of these products contain small quantities of real *Hoodia* blended with *Opuntia* species (prickly-pear), sawdust, starch, and other materials used as diluents. There is a definite need for a rapid method of analysis to confirm authenticity of raw materials and to determine whether the alleged active ingredient (P57) is present in various products. Several methods currently used to authenticate and validate *Hoodia* raw material, such as high performance liquid chromatography (HPLC) and microscopy, are laborious and require skilled personnel. Near infrared (NIR) spectroscopy requires minimum sample preparation and does not need highly skilled operators once the spectrometer has been calibrated. NIR spectroscopy was used to determine whether adulterants could be detected in *Hoodia* formulations. Chemometric analysis of the spectral data revealed that common adulterants such as *Aloe* and *Opuntia* species were easily distinguished from *H. gordonii*. High performance thin layer chromatography (HPTLC) provided a fingerprint for *H. gordonii* raw material and was subsequently used to establish that several *Hoodia* products did contain authentic *H. gordonii*. NIR spectroscopy and HPTLC were used successfully to aid in the quality control of *H. gordonii* raw material and products.

HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECT OF *SALVADORA OLEOIDES* IN NORMAL

AND ALLOXAN-INDUCED DIABETIC RATS

J.P. Yadav, Sushila Saini

Department of Biochemistry and Genetics, M.D. University, Ro, India,
yadav1964@rediffmail.com
Department of Biochemistry and Genetics, M.D. University, Ro, India

Diabetes mellitus is a heterogeneous primary disorder of the carbohydrate metabolism with multiple etiological factors that generally involve absolute or relative insulin deficiency or insulin resistance or both. All causes of diabetes ultimately lead to hyperglycemia, which is the landmark of this disease syndrome. *Salvadora oleoides* Decne. (Salvadoraceae family) commonly known, as meetha jal is an oil yielding medicinal and multipurpose tree. It is adaptable to arid and alkaline conditions. Leaves of *Salvadora oleoides* are used to relieve cough, employed in the treatment of enlarged spleens and low fevers. Leaves of *Salvadora oleoides* have been mentioned to possess anti-inflammatory, analgesic and antiulcer activity. The objective of the present study was to evaluate the hypoglycemic and hypolipidemic activity of an ethanolic extract of aerial parts (stem and leaves) of *Salvadora oleoides* in normal and alloxan induced diabetic rats. Diabetes was induced in albino rats by administration of alloxan monohydrate (120 mg/kg i.p.). Normal as well as diabetic albino rats were divided into groups (n=6) receiving different treatments consisting of the vehicle (control), the ethanolic extract (1 g and 2 g/kg b.w) and the standard antidiabetic drug tolbutamide (0.5 g/kg b.w.). Blood samples were withdrawn by cardiac puncture and were analysed for blood glucose and lipid profile on day 0, day 7, day 14, and day 21. The ethanolic extract of *Salvadora oleoides* showed significant reduction (P<0.001) in blood glucose and also produced beneficial effects (P<0.001) on the lipid profile in euglycemic as well as alloxan induced diabetic rats at the end of the treatment period (21st day). It is concluded that the ethanolic extract of *Salvadora oleoides* is effective in controlling blood glucose levels and improves lipid profiles in euglycemic as well as diabetic rats. Hence further study on active principles of *Salvadora oleoides* may provide a better solution for million of diabetics. The lowering effect of TG, TC, LDL, VLDL will lead to the decrease of coronary heart disease (CHD) and an increase in the HDL level, which is known to be a protective factor in CHD.

TOXICITY OF *HYDNORA JOHANNIS BECCARI* (HYDNORACEAE) DRIED ROOTS AND ETHANOL EXTRACT IN RATS

Sakina Yagi, Salah Adam, Yves Chapleur, Dominique Laurain-Mattar

University of Khartoum, Sudan, sakinayagi@yahoo.com
University of Khartoum, Sudan, seiadam2003@hotmail.com Université Henri Poincaré- Nancy 1,
France, Yves.Chapleur@sucre.uhp-nancy.fr
Université Henri Poincaré- Nancy 1, France, Dominique.Laurain-Mattar@pharma.uhp-nancy.fr

Roots of *Hydnora johannis* are used for the treatment of dysentery, diarrhea, cholera, and tonsillitis in Sudan [1]. The high tannin content of the roots may explain the relief provided for the condition due to the astringent and anti-inflammatory effects [2]. The aim of the present work was to evaluate the toxic effects of dried roots and of the ethanol extract of *Hydnora johannis*. Wistar rats were divided into 4 groups: rats in group 1 were controls, group 2 were given increasing concentration of dried roots ad lib in diet; group 3 were given increasing

concentrations of ethanol extract orally; group 4 were given ethanol extract intramuscularly. Histopathological analysis as well as haematological and biochemical tests were carried out. The analysis showed a dose-dependant effect, mainly on the liver and spleen, in the three groups. However, the impact of the dried roots was very mild compared to that of the ethanol extract. Moreover, intramuscular administration of ethanol extract incurred more damage than the oral root treatment. Thus, the difference in the toxic effect in this experiment might be due to the route of administration as well as the extraction method. The amount of tannins could be higher in the ethanol extract whereas dried powder in the diet may yield less of the hydrolysable tannins in addition to the astringent effect of tannins which decrease the rate of absorption.

THEME 6

DEVELOPMENTS IN INDUSTRIAL PROCESSING OF MAPS

CLEAN PRODUCTION OF COMMERCIAL FREEZE-DRIED MANGOSTEEN POWDER FOR MEDICINAL HERB AND NUTRITIONAL HEALTH BENEFITS

Suchada Chaisawadi, Waraporn, Methawiriyasilp

King Mongkut's University of Technology Thonburi, Thailand, suchada@pdti.kmutt.ac.th
King Mongkut's University of Technology Thonburi, Thailand, waraporn@pdti.kmutt.ac.th

Mangosteen (*Garcinia mangostana*) is one of the important medicinal plants in Thailand, which all parts provide a whole range of medicinal properties and high nutritional value. Xanthones, a potent antioxidant in mangosteen have been shown to have extensive biological and pharmacological activities, make it a potential candidate for commercial production. The clean production of freeze-dried mangosteen powder has been designed by using clean technology to serve the need of zero discharge during the process. The wastes from freeze drying process, which are the xanthone-packed pericarp have been considered to utilize as a medicinal herb and nutritional health products. The designed processing in this study consists of freeze drying process and waste utilization process. The freeze-drying process has been done on mangosteen flesh and seed by blending and freeze drying to powder. The waste utilizing process has been divided into two processes, the inner pericarp processing and outer pericarp processing. The inner pericarp processing has been done by scalping the inner pericarp and blending, followed by freeze drying to get the inner pericarp powder and use for nutritional health products. The outer pericarp processing has been done for xanthone extraction by cutting into small pieces, tray drying, grinding into powder and ethanol extraction for medicinal and cosmetic used. The residue of the outer part pericarp and other wastes can be used as biopesticide or fertilizers. The clean production on mangosteen powder processing has been implemented by Fruit Processing Pilot Plant, Industrial Park, KMUTT. The pilot results showed all parts of mangosteen have commercial used and nothing goes to waste. The pilot data showed high productivity along with low environmental impact. The preliminary study on financial analysis showed high feasible for commercial production. Acknowledgements: Thanks to Thai Government Budget Fund 2007 for Financial Supports

EFFECT OF POTASSIUM RATES ON HYDROPONICALLY-GROWN PEPPERMINT (*MENTHA X PIPERITA*)**M.H. El-Masry, M.R. Morales, D. J. Charles, and J. E. Simon**

Dept. Medicinal and Aromatic Plants Research, Horticulture Research Institute, Agric. Research Center, Cairo, Egypt, Mountain State University, Beckley, WV 25801-4511 USA, Frontier Natural Products, Urbana-Champaign, Illinois, USA; New Use Agriculture and Natural Plant Products Program. Rutgers University. Foran Hall. 59 Dudley Road. 08901 New Brunswick, NJ, USA.

Hydroponically grown peppermint (*Mentha x piperita* L) was subject to six rates (25, 150, 217, 300, 450 and 600 g/l) of potassium sulfate (K_2SO_4) to examine the effect on biomass, dry matter and mineral accumulation in plant organs and on leaf total essential oil yield and composition. Potassium level of 300g/l resulted in maximum biomass, dry matter accumulation and total essential oil yield. Essential oil yield was highly correlated with leaf weight, but not with leaf area. Menthone and menthol comprised 72-75 % of the total oil composition consistently across the potassium rates, even when the total oil yields were significantly affected by the potassium rates. Increasing the potassium rate had a significant effect on menthone and menthol concentrations: as the potassium rate increased from 150 to 450 g/l, the percentage of menthone increased (as relative % of total oil), while that of menthol decreased. Other essential oil constituents were significantly affected by the potassium rates but their percentages as part of the total oil were minor. Total nitrogen % and P content (mg/g dried leaves) decreased with each increase in K rate. K content of dried leaves increased with each increase in K increment up to 600 g/l. Ca and Mg content were also decreased with K rate increase, while no changes in Mn, Fe, B, Cu, Zn, Al and Na were observed

INDUSTRIAL EXTRACTION OF WESTERN AUSTRALIAN SANDALWOOD SEED OIL

Dhanushka Hettiarachchi

Wescorp Sandalwood, Australia, danny@wescorp.com.au

Western Australian Sandalwood (*Santalum spicatum*) seed kernels have been used traditionally by indigenous Australians for dietary and medicinal purposes. An elaborate research on the seeds has revealed the abundance of a fixed oil in kernel which consist a rarer fatty acid (xymeninic acid). A pharmacological study of the above seed oil resulted body lipid regulatory and beneficial effects on skin. Sandalwood cultivations are producing considerable amount of seeds which are unused despite of its value, thus we decided to extract seed oil industrially which can be a potential nutraceutical or cosmetic agent. Common methods of cold press and extrusion were found to be unsatisfactory due to the presence of gum material in the kernel. Pre-treatment of kernel material was trialled with physical, chemical and biological means which result in no significant yield. Solvent extraction was successfully developed followed by an economical de-gumming method. Oil of expected quality was yielded in higher percentage but the method was subjected to uncertainty due to developing global trend and legal constrains on solvent extracted plant material for human consumption. Super critical carbon dioxide extraction was experimented as a newer economical method. Above experimented method has resulted higher yields and oil which complies with standards. This less hazardous method produces oil free of contaminants and also makes the exhausted meal clean for any further uses. A recent feasibility study on this method has predicted over 50t production of oil per annum.

GOOD MANUFACTURING PROCEDURES (GMPS) IN THE HERB FOOD AND MEDICINAL PLANT INDUSTRY. ACTUAL SITUATION AND PARTICULARITIES.

Astrid van Ginkel

FITOMON, Spain, astrid@fitomon.com

The Good Agricultural Practices (GAP) and the Good Manufacturing Procedures (GMP) of Medicinal and Aromatic Plants (MAP) apply to growing, primary processing and manufacturing of MAP products and their derivatives, for humans and/or animals (EMA, EC). GMPs are a key aspect of the quality assurance system, and aims to ensure that medicinal products are consistently manufactured and tested according to pre-defined quality standards as required by the health authorisation or product specification guidelines. This minimizes the probability of contamination and adulteration, mix-ups and errors, batch to batch variability and helps enhancing final quality, efficacy and security. In the practice, it is difficult to know when to apply the GMPs in the MAP industry. The aim of this work is to make an exhaustive bibliographic compilation of guidelines and documents related to GMPs and GAPs in the MAP industry, and point the particularities specific of herb products. There are some guidelines and documents from EMA, WHO, FDA, ICH, MHLW, PIC/S, APIC – CEFIC, IPEC-PQG and EHPM. On an international level, documents have been harmonized and formally affected by the ICH and adopted by the EU, MHLW and FDA. Some committees, organisations, federations and councils, also make GMP guidelines. Some aspects are specific of the MAP industry and will lead to special procedures and check lists for GMP and GAP inspections.

PRACTICAL APPLICATION OF CARBON DIOXIDE FOR THE EXTRACTION OF VARIOUS MEDICINAL AND AROMATIC SEEDS

Petras Rimantas Venskutonis

Kaunas University of Technology, Lithuania, rimas.venskutonis@ktu.lt

Medicinal and aromatic seeds were extracted with liquid carbon dioxide (L-CO₂) in order to measure extract yields, the content and composition of essential oils, which are the most important characteristics for the assessment of technological and economical feasibility of commercial applications of the extraction techniques. Preliminary assessment of some biological activities of the extracts, such as radical scavenging capacity and inhibition of microorganisms were also tested. Pilot plant scale extractions were performed using seeds from 9 species: coriander (*Coriandrum sativum*), caraway (*Carum carvi*), dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*), blue fenugreek (*Trigonella coerulea*), sicklefruit fenugreek (*Trigonella foenum-graecum*), amaranth (*Amaranthus lividus retroflexus*), black cumin (*Nigella sativa*) and white mustard (*Sinapis alba*). Different extraction cycles were applied to increase the yield of the extract. Depending on the process time, particle size and extraction cycle the yields varied from 2.3 (sicklefruit fenugreek) to 18 % (black cumin), while the content of the essential oil in extracts was from the traces to 27.5 %. The highest yields were obtained from the seeds containing remarkable amounts of essential oil and lipids. Essential oil composition was analyzed by using gas chromatography and mass spectrometry and the differences between hydrodistilled oils from the whole ground seeds and their extracts were evaluated. It was concluded extraction of some plant seeds with L-CO₂ is economically and technologically feasible process for commercial applications, e.g. producing ingredients for the formulations of different products, particularly flavorings, functional foods and food supplements.

THEME 7

THE ECONOMICS AND MARKETING OF MEDICINAL AND AROMATIC PLANTS

MODELS OF CROP COMMERCIALIZATION AND CROP CLUSTERS

Jim Simon, Keith Shawe, Elton Jeffthas, Erica Renaud, Petrus Langenhoven, Rodolfo Juliani, Ramu Govindasamy and Dan Acquaye

New Use Agriculture and Natural Plant Products Program, School of Environmental and Biological Sciences and Partnership in Food Industry Development for Natural Products, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901-8520, USA
 Agri-Business in Sustainable Natural African Plant Products, ASNAPP-South Africa, University of Stellenbosch, South Africa
 Enza Zaden North America Inc., Vitalis Organic Seeds
 ASNAPP-Ghana, Accra, Ghana

The introduction of new crops into a region, or the cultivation of otherwise wild crafted plants can be a significant benefit to the growers, processors and rural communities. Yet the successful introduction of any new crop requires a purposeful market-driven strategy which requires careful consideration and detailed studies on the plant, the development of appropriate sustainable production systems, harvesting and processing requirements, and marketing factors. Those involved in new crop development have long recognized the difficulties in this initial commercialization phase and the multitude of obstacles that arise. As such, we developed several different models of crop commercialization for use in medicinal and aromatic plant development. The introduction of botanicals need not be pursued as a single enterprise, but as part of a crop rotation scheme, in a multi-cropping or combined agro/forestry system in which the use of crop clusters should be considered. Crop clusters refer to the growing (in fields or forest) of several plants simultaneously to reduce grower risk, enhance the number and nature of products that would become available and to provide more than one crop that could generate income and provide other useful products such as food/fiber/fence posts. Many growers in rural communities farm on a small scale and are resource limited. Given the volatile nature of the marketplace, crop clusters could be a strategic vehicle to minimize their risk while fostering economic opportunities by crop diversification. We developed a selection criteria matrix to identify the most promising crops and plant products and crop clusters.

USE OF *LIPPIA SCABERRIMA* ESSENTIAL OIL TO PROMOTE POSTHARVEST QUALITY OF SUBTROPICAL FRUITS

Sandra Combrinck, Thierry Renier, Wilma du Plooy, Ben Botha

Tshwane University of Technology, South Africa, combrincks@tut.ac.za
 Tshwane University of Technology, South Africa, reniert@tut.ac.za, John Bean Technologies Pty Ltd, South Africa, , Tshwane University of Technology, South Africa, bothabm@tut.ac.za

Lippia scaberrima Sond. is an aromatic shrub occurring in semi-arid, summer-rainfall grassland in Gauteng, the Free State and North West provinces of South Africa. In this study, the essential oil of bulk samples of *L. scaberrima* growing in a natural habitat were evaluated for

their antifungal activity against common commercially important postharvest pathogens of subtropical fruits. The oil was found to exhibit strong in vitro activity against post-harvest spoilage pathogens of mango, avocado and citrus fruit. GC-FID analysis of the oil allowed identification of limonene, 1,8-cineole and R-carvone as the main constituents. Antifungal activity of the oil was attributed to the presence of R-carvone. These results were evaluated by semi-commercial and commercial trials using fruits treated postharvest with coatings amended with essential oil. The control treatments were done using standard commercial fungicide applications, while the experimental treatments all consisted of coatings amended with the various oils and terpenoids, without the inclusion of synthetic fungicides. Effective disease control was obtained from all of the amended treatments. In addition, the overall quality of the fruits was maintained. Results obtained indicate that the essential oil of *L. scaberrima* and selected terpenoids can be used as alternatives to synthetic chemical compounds in the postharvest treatment of fruit. Suitably amended coatings are currently being commercialised.

DIVERSITY AND THE MOST COMMON TRADED SPECIES IN THE SUDANESE MEDICINAL PLANTS

Hassan Khalid, Wail Abdalla

Medicinal & Aromatic Plants Research Institute, Sudan, hsubki@gmail.com
Medicinal & Aromatic Plants Research Institute, Sudan, waillelsadig

The paper covers the most common species used in Sudanese folk medicine in the five floristic belts. In the desert belt the most common species are *Ziziphus spina-christi* L., *Boscia senegalensis* (Pers.) Lam., *Cadaba farinosa* Forssk., *Fagonia cretica*. Species of the semi-desert belt include *Salvadora persica* L., *Maerua crassifolia* Forssk., *Capparis decidua* Forssk., *Acacia senegal* (L.) Willd., *Acacia nubica* Benth., *Ziziphus spina-christi* (L.) *Balanites aegyptiaca* (L.) *Leptadenia pyrotechnica* (Forssk.) DC *Senna alexandrina* Mill. (Cassia senna L.) *Calotropis procera* (Ait.). Thorn Savanna & scrub belt flora include, *Albizzia amara* (Roxb.) Boiv., *Sclerocarya birrea* (A.Rich.) Hochst., *Cadaba glandulosa* Forssk., *Dalbergia melanoxylon* Guill. & Perr., *Acacia seyal* Del., *Calotropis procera* (Ait.) Ait. f. *Acacia senegal* (L.) Willd., *Balanites aegyptiaca* (L.) Del. Species of the deciduous Savanna are *Combretum hartmannianum* Schweinf., *Terminalia brownii* Fresen., *Khaya senegalensis* (Desr.) A.Juss., *Albizzia amara* (Roxb.) Boiv., *Acacia seyal* Del. and the flood region flora cover *Hyphaene thebaica* (L.) Mart., *Acacia polyacantha* Willd. The most common species in trade records (imports and exports) were reported for the last five years (Dec. 2003 – Dec. 2007).

MODELS OF ORGANIC CERTIFICATION IN HERBAL SECTOR OF TRANSITIONAL COUNTRIES OF WESTERN BALKAN (SE EUROPE)

Sulejman Redzic, Hazim Basic, Senka Barudanovic

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina,
sredzic@pmf.unsa.ba
Fac. of Mechanical Engineering, 6 Vilsonovo setaliste St., 7, Bosnia and Herzegovina, , Center
of Ecology & Natural Resources, Fac. of Sci. Univ. Sa, Bosnia and Herzegovina,

Intensive processes of transition in countries of Western Balkan, as well as transition from central economy to market economy have left visible marks on natural resources. Organic certification is aimed toward safety, sustainability and better administration of plants. Evaluation and identification of safety includes the following: application to company in charge for organic certification; visiting places of future exploitation; mapping of territory for gathering medicinal plants and ensuring ecological certificate, issued by ministry in charge, that would guaranty safety of the territory in terms of pollution, use of pesticides, artificial fertilizers, global influences. In administration, it is necessary to conduct permanent education of harvesters and other people employed in herbal sector, to conduct accreditation of people, development of more solid legislation and more effective monitoring and inspections. In order to reach sustainability in use of natural resources, it is necessary to have on disposal the following: list of wild medicinal plants, their biomass, models of reproduction, affiliation with living forms, conservation status, etc. Higher level of sustainability, as well as improvement of overall methodology of organic certification in herbal sector includes evaluation of conditions and bearing capacities of ecosystems on exploitive territory, evaluation of sustainable biomass of plants, evaluation of level of biological and ecological sensitivity of medical plants, establishment of herbal management plant, strengthening human resources and state institutions. Study is demonstrating samples from various bio-geographical areas of western Balkan from Mediterranean to high mountains of Bosnia and Herzegovina.

SIPPO TRADE PROMOTION PROGRAMME OF OSEC FOR MEDICINAL AND AROMATIC PLANTS

Franziska Staubli, Klaus Duerbeck

OSEC/SIPPO, Switzerland, stf@osec.ch
SIPPO Senior Consultant, Germany, k.duerbeck@duerbeck.de

Innumerable medicinal plants grow in the mountains and tropical rain forests of many Asian, African and Latin American countries. Many are barely investigated and their active ingredients are not yet known. They are of interest for the pharmaceutical industry and show great potential for the ever-growing health food market. For emerging markets and countries in transition, medicinal plants represent an opportunity to harvest their national resources in a sustainable manner. It is therefore of utmost importance to harvest, process and transport the medical herbs in a way that preserves the maximum amount of their active ingredients. The increasing demand for wild collected products in the sectors of food, personal health care and medicinal herbs poses major ecological and social challenges. That's why SIPPO initiated the development of a new FairWild standard. We focus on companies from 14 partner countries that offer medicinal herbs, functional or pharmaceutical food products, are ready for the international market, preferably have some export experience, produce according to international quality standards, offer products marketable in Switzerland and the European Union, are motivated and dedicated to deal with new markets, and have a representative in the company who has a sufficient command of the English language. SIPPO Swiss Import Promotion Programme is a mandate from the State Secretariat for Economic Affairs of Switzerland (SECO), carried out by Osec. The programme supports small and medium-sized enterprises (SME) from emerging markets and markets in transition to access the Swiss and European markets.

CHEMICAL ANALYSIS OF OIL AS PART OF MARKETING ESSENTIAL OIL CROPS E.G.

PELARGONIUM VAR ROSE

Karen Swanepoel

University of Zululand, South Africa, karrieza@gmail.com

There is an increasing world demand for the high quality rose geranium essential oil of Pelargonium species which contains geraniol, linalool and citronellol. It is mainly used as, substitutes for the expensive attar of roses in the perfume & flavouring industry and to treat cellulite. The price depends on the quality of the oil and the specific market it is suitable for. If the quality is unknown to the producer, the market is unsure, and the producer may be exploited. The aim was to determine the maximum yield and quality for this cultivar in line with the market requirements. This information and technology is transferred to emerging farmers. The results were obtained from trials and commercial farmers in Mpumlanga, KwaZulu Natal, Eastern and Western Cape as well as small farmers in Gauteng. Chemical analysis of different harvests, were compiled into a collective data base. This can be seen as a sign of the growing industry of essential oils in South Africa by commercial and emerging farmers. Improved agricultural practices and the chemical analysis could make a difference in the marketability of geranium into different markets

VALUE ADDING AND NATURAL PRODUCTS: HOW MUCH DOES INTELLECTUAL PROPERTY MATTER?

Rachel Wynberg, Judith Silveston, Cyril Lombard

University of Cape Town, South Africa, rachel@iafrica.com
PhytoTrade Africa, England, Judith@ipp.demon.co.uk, PhytoTrade Africa, England,
cyril@phytotradeafrica.com

Southern African countries are actively engaged in natural product development but, in common with other developing countries, typically export their biodiversity as raw materials with little or no processing, technological input or other value adding. Intellectual property rights (IPR) protection for innovations relating to biodiversity has been accelerated by the Trade Related Intellectual Property Rights (TRIPS) agreement of the World Trade Organisation and there is concern that this will strengthen the market power of northern innovating firms and raise prices in developing countries. At the same time there is recognition that IPRs can assist developing countries in obtaining access to technological capacity, and provide commercial opportunities for encouraging inward investment. Virtually all species under commercial development in southern Africa have patents associated with their processing and/or use, mostly under foreign ownership. We ask: (1) what is the role of IPRs in stimulating industrial activity in the southern African natural products sector; and (2) does the existence of IPRs stimulate or restrict value adding in countries of origin of biological material and traditional knowledge? Three southern African species currently traded in global markets are analysed: *Harpagophytum* sp. (devil's claw), *Aspalathus linearis* (rooibos) and *Hoodia* spp. We conclude that IPRs seldom restrict value adding, but instead stimulate trade and may provide further commercial opportunities. Low awareness amongst local firms, however, prevents such opportunities from being exploited. Low levels of value adding stem from a complex mix of factors including market access, buyer dominance, a lack of strategic alignment amongst producers, and insufficient technical and

financial capacity.

Acknowledgements: World Intellectual Property Organisation.

THEME 8

NEW DEVELOPMENTS IN LAWS AND REGULATIONS FOR THE USE OF MAPS

THE TRADITIONAL USE AS A REGULATORY CATEGORY – EXPERIENCES IN EUROPE

Estelle Coquillotte, Wieland Peschel

European Medicines Agency, United Kingdom, estelle.coquillotte@emea.europa.eu
The School of Pharmacy, University of London, United Kingdom,
wieland.peschel@pharmacy.ac.uk

The new European legislation introduced with the Directive 2004/24/EC amending Directive 2001/83/EC a harmonised registration scheme for traditional Herbal Medicinal Products (HMPs) at multinational level. With this legislation the Traditional Use (TU) of HMPs gained a new meaning, because the proof of traditions may replace individual product data for efficacy and safety. With the pan-European upgrade from a simply historic category to a legal one, the TU is now part of the regulatory strategy for HMP manufacturers and as such unique in the licensing of pharmaceuticals. We compared the European legal basis for traditional HMPs with the former legal practice by some European countries (France, Germany, Hungary, Spain) and also non-European countries (Australia, Brazil, Canada, India) in terms of six major criteria: (I) self medication character, (II) specified strength and posology, (III) appropriate route of administration, (IV) period of traditional use, (V) sufficient data on safety, and (IV) plausibility of pharmacological effects. Examples (e.g. *Equisetum*, *Solidago*, *Echinacea*). For the acceptance of evidence for Community monographs, the adjustment with monographs of the European Pharmacopoeia, and experience from referral cases are presented. In addition, the different concepts of (1) well-established use medicinal products and (2) foodstuffs with health claims - the major borderlines within European legislation- are discussed. The experiences of European harmonisation process might be useful for Africa and other regions of more diverse traditions when accepting rationally TU in order to strengthen the position of HMPs on the market.

THEME 9

TRADITIONAL MEDICINE AND HEALTH SYSTEMS FOR NEW AND OLD DISEASES

ANTIPLASMODIAL TRITERPENOIDS ISOLATED FROM *CANTHIUM MULTIFLORUM*

M.Traoré, H.L.Ziegler, C.E. Olsen, H. Millogo, J.B. Ouédraogo, I.P. Guissou, O.G. Nacoulma, T. R. Guiguemdé and S.B. Christensen

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Copenhagen, Denmark.

Direction Régionale de Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso

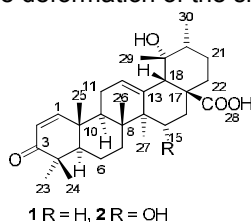
Laboratoire de Parasitologie-Entomologie, Centre Muraz, Bobo-Dioulasso, Burkina Faso.

^dDepartment of Natural Sciences, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark.

Département de Biochimie et de Microbiologie Appliquées, Université de Ouagadougou, Burkina Faso

Institut de Recherche en Science de la Santé, Ouagadougou, Burkina Faso

In Burkina Faso, roots of *Canthium multiflorum* (Thonn.) Hiern (Rubiaceae) are used for the treatment of malaria and hypertension [1]. This investigation was initiated because of the absence of published phytochemical reports on this species and the possibility of the presence of antiplasmodial compounds. A bioactivity guided fractionation of roots of *Canthium multiflorum* led to the isolation of the 2 new ursenoic acid derivatives, 19 α -hydroxy-3-oxo-ursa-1,12-dien-28-oic acid **1** and 3-oxo-15 α ,19 α -dihydroxyursa-1,12-dien-28-oic acid **2** which were tested for antiplasmodial effect and for inducing change of the shape of membranes of erythrocytes. Assay for antiplasmodial activity was performed using chloroquine-sensitive *Plasmodium falciparum* strain 3D7 [2]. Chloroquine was used as a positive control. Investigation of membrane shape effects of pentacyclic triterpenes was performed according to a previous described method (Ziegler *et al.*, 2002) [3]. The structure of the two new compounds was elucidated using MS and NMR spectroscopy. The HR-ESI-MS gave a m/z 467.3161, calcd. for $C_{30}H_{43}O_4$ (M-H)⁻ 467.3167 for **1** and m/z = 507.3089 [M+ Na]⁺ (calcd. for $C_{30}H_{44}NaO_5$:507.3081) for compound **2**. Application of the modified octant rule and comparison of the recorded CD spectrum with those of related pentacyclic triterpenoids with a 1-en-3-one system in the A-ring confirmed the absolute configuration of the two compounds. A moderate antiplasmodial activity (IC_{50} =26 μ g/ml) was observed which was not related to the deformation of the shape of erythrocyte membranes.



1. Nacoulma, O.G. (1996). Plantes médicinales et pratiques médicinales traditionnelles au Burkina Faso: Cas du plateau central. Faculté des Sciences et Techniques, Université de Ouagadougou. 2. Ziegler, H.L., Staerk, D., Christensen, J. et al. (2002b). Antimicrobial Agents and Chemotherapy, 46, 1441–1446. 3. Ziegler, H.L., Jensen, T.H., Christensen, J., Staerk, D. et al. (2002a). Planta Medica, 68, 547–549.

Acknowledgments: This work was supported by Danida. We thank Dorte Brix for technical support.

ETHNOBOTANICAL AND ANTIMALARIAL STUDIES ON PLANTS USED FOR MALARIA MANAGEMENT IN COAST, EASTERN AND NYANZA PROVINCES OF KENYA

Sumesh Chhabra

Kenyatta University, Kenya, scchhabra@yahoo.com

Resistance of *Plasmodium* species to therapeutic agents and the resistance of the vector mosquitoes to insecticides prove that malaria is still the most economically important tropical

disease. Statistics show that about 300 million people are infected with malaria parasite every year. In Africa alone about 2 million people die annually, most of them are children below 5 years of age. With no vaccine likely to be available in the near future, the need for new agents to combat resistant *P. falciparum* is becoming increasingly urgent. Plants continue to be used in the treatment of malaria, either for their anti-parasitic activity or because they possess, or are believed to possess some activities with therapeutic agents for a patient with malaria. An ethnomedical survey of Coast, Eastern and Nyanza Provinces of Kenya revealed the use of 197 plant species for malaria management by the local communities. Different extracts of fifty plants used by these communities were evaluated for the *in vitro* antiplasmodial, *in vivo* antimalarial, cytotoxicity and animal toxicity activities. Phytochemical screening of six of these plants led to the isolation of thirty-six compounds (terpenoids, steroids, flavonoids, naphthalene derivatives and naphthaquinones). Some of these compounds demonstrated interesting bioassay results and suggest that there is potential to generate leads with enhanced antimalarial activity, reduced cytotoxicity and improved bioavailability.

THE POTENTIAL OF SOUTH AFRICAN MEDICINAL ORCHIDS

Mayashree Chinsamy, Jeff Finnie, Johannes van Staden

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa, 202500097@ukzn.ac.za

Orchidaceae is represented by approximately 20 000 species worldwide across a wide range of habitats. They have a broad range of ethnobotanical applications. Southern Africa supports approximately 494 species, of which an estimated 49 feature significantly in South African traditional medicine. We attempted to assemble significant ethnobotanical information available from the literature detailing the uses, preparations and administration of South African orchids in African traditional medicine. In order to address the lack of pharmacological, toxicological and phytochemical knowledge, the use of orchids elsewhere in the world is compared with their uses in South Africa. Antimicrobial and anti-inflammatory activity may be present in South African orchid species: *Ansellia africana* used to treat coughs; *Eulophia* sp. are applied to sore limbs or as poultice to treat pain; *Polystachya ottoniana* are used to soothe pain associated with teething in babies and to treat diarrhoea; and *Satyrium bracteatum* used to treat intestinal worms. Further examples will be discussed. By encouraging the scientific validation of South African medicinal orchid use, conservation efforts would benefit greatly as it may add value to these species. It also provides an incentive for further research initiatives, with an emphasis on essential cultivation techniques.

THE EFFECT OF *MOMORDICA CHARANTIA* (BITTER GOURD) EXTRACT ON THE WOUND HEALING IN STREPTOZOTOCIN- INDUCED DIABETIC RATS

Srijit Das, Teoh Lin, Azian Latiff

Universiti Kebangsaan Malaysia, Malaysia, das_srijit23@rediffmail.com

The protective action of oral *Momordica charantia* (MC) extract on diabetes mellitus (DM)

has been well documented with fewer studies focusing on its topical use. The aim of the present study was to investigate the healing effect of topical MC extract in comparison with the oral route administration. A total of 108 Sprague Dawley rats were used. The animals were subdivided into two main groups: (i) non-diabetic group (n = 54) and (ii) streptozotocin-induced diabetes (n = 54). These groups were further subdivided into three groups: (i) non-treated control group (n = 18), (ii) topically treated group (n = 18), and (iii) orally treated group (n = 18). Wound was inflicted with a 6 mm punch biopsy needle on the skin of the dorsal thoracolumbar region. The animals were sacrificed on the 1st, 5th and 10th day after wound creation. Rate of wound closure and total protein content were estimated. The histological analysis with light and electron microscopy was performed. There was no significant difference in the total protein content, rate of wound closure and histological changes in the non-diabetic groups. Amongst the diabetic groups, the total protein content and rate of wound closure was significantly increased in the topically treated group in comparison to the non-treated and orally treated groups. Histological observation in the topically treated group showed better integrity of the surface epithelium as compared to the non-treated and orally treated groups. The results showed that wounds of streptozotocin-induced DM healed faster in rats treated with topical MC extract as compared to the oral one.

ANTIDIABETIC ACTIVITY OF *DIOSPYROS PEREGRINA* FRUITS AGAINST TYPE II DIABETIC RATS.

Saikat Dewanjee, Anup Maiti, Subhash C. Mandal

Jadavpur University, INDIA, s.dewanjee@yahoo.com
Jadavpur University, INDIA, anup_phytochem@yahoo.com
Jadavpur University, INDIA, subhashmandal@yahoo.com

The aim of this study was to investigate scientifically the oral hypoglycemic effect of *Diospyros peregrina* fruits against neonatal Streptozotocin induced type II diabetic rats. The fruits were extracted successively from petroleum ether, chloroform, ethyl acetate and methanol. The resultant extracts were administered orally at doses of 50 mg/kg b.w. for five consecutive days to type II diabetic rats. Fasting blood glucose levels were estimated on day 0, 1, 3 and 5. Hepatic and renal antioxidant markers namely superoxide dismutase, catalase, reduced glutathione, hydroperoxide, thiobarbituric acid reactive substances profiles; serum lipids and liver glycogen level were estimated after the animals were sacrificed on day 5. The test results were statistically compared with respect to diabetic control group. The ethyl acetate extract exhibited significant hypoglycemic activity ($p < 0.01$) in term of reduction of fasting blood glucose level while other extracts did not display significant effect. The ethyl acetate fraction was also found to revert back significantly the hepatic and renal antioxidant markers levels of diabetic rats near to the normal status. Ethyl acetate extract significantly reduced serum cholesterol ($p < 0.01$) and triglycerides ($p < 0.01$) level whilst improvement of liver glycogen ($p < 0.01$) level was observed. The results were comparable to that of Glibenclamide (1 mg/kg, b. w.) treated group. In this investigation the antioxidant effect of ethyl acetate fraction may ameliorate the antidiabetic effect. The said fraction is flavonoids enriched, which are known phytoantioxidants thus the activity may be due to flavonoids present in the said extract. Acknowledgements: Jadavpur University, India.

STUDIES ON ANTI-INFLAMMATORY EFFECTS OF SOME ACACIA SPECIES

Esameldin Elgorashi, Naoki Wada, Essameldin Warrag, Hiroshi Satoh

Department of Veterinary Pharmacology, Faculty of Agriculture, Tottori University, 4-101 Minami, Koyama, Tottori 680-8553, Japan
Department of Silviculture, Faculty of Forestry, University of Khartoum, Shambat 13314, Sudan

In vitro anti-inflammatory activities of dichloromethane (DCM) and 90% methanol (MeOH) extracts of bark and leaves of three *Acacia* species were investigated using both cyclooxygenase-1 (COX-1) and cyclooxygenase -2 (COX-2) enzymes. The plants investigated were: *A. nilotica*, *A. nubica* and *A. senegal*. The results showed that DCM bark extracts of all species had high COX-2 selective inhibition (IC_{50} values of 0.45, 37 and 17.3 $\mu\text{g/mL}$) compared to COX-1 inhibition (IC_{50} values of 206.3, >250, >250 $\mu\text{g/mL}$) respectively. The DCM bark extracts of the three species were evaluated further *in vivo* in rats with adjuvant-induced arthritis. DCM bark extracts of *A. senegal* significantly reduced the edema when administered at a dose of 300 mg/kg ($p < 0.05$). The extract did not cause lesions in the gastrointestinal mucosa compared to indomethacin which caused severe lesions in the small intestine (ulcer index = $194.3 \pm 2.7 \text{ mm}^2$). On the other hand, DCM bark extracts of *A. nubica* and *A. nilotica* were not active in *in vivo* assay. The results suggest that DCM bark extracts of *A. senegal subsp. senegal* may be useful in the treatment of rheumatoid arthritis.

Acknowledgements: Japanese Society for Promotion of Science (JSPS) for financial support.

CHRONIC TOXICITY, ANTIDIABETIC AND HYPOLIPIDEMIC ACTIVITIES OF *SPONDIAS MOMBIN L.* (ANACARDIACEAE) ON ALLOXAN-INDUCED DIABETIC RATS**Adebayo Gbolade, Adelaja Akinlolu, Matthew Ekor, Adesina Odewabi**

Olabisi Onabanjo University, Sagamu campus, Nigeria, adegbolade@yahoo.com
Olabisi Onabanjo University, Sagamu campus, Nigeria, laja3@yahoo.com, Olabisi Onabanjo University, Sagamu campus, Nigeria, -, Olabisi Onabanjo University Teaching Hospital, Sagamu campus, Nigeria,

S. mombin is traditionally used in Nigerian traditional medicine to treat diabetes in addition to its abortifacient, anthelmintic and other biological activities. Based on preliminary investigation, the stem bark ethanolic extract was investigated for effects on hyperglycaemia, hyperlipidemia, liver and kidney functions, and toxicity to various body organs after chronic daily administration at 150 mg/kg and 400 mg/kg to alloxan-induced diabetic rats for 4 weeks. A significant decrease ($P < 0.05$) in alloxan-induced hyperglycaemia attaining near normalization was accompanied by restoration of pancreatic beta cells, as well as significant diminution ($P < 0.05$) in hyperlipidemia after 4 weeks of extract therapy. Weights of animals significantly increased ($P < 0.05$) after the 3rd and 4th weeks when treated with both extract and chlorpropamide. Reversal of damage to brain and liver was significant ($P < 0.05$) with the extract while the weights of other organs like lung, spleen, heart and kidney did not change significantly relative to diabetic control. Moreover, while the standard antidiabetic drug was significantly hepatotoxic ($P < 0.05$), levels of serum enzymes like alanine aminotransferase and aspartate aminotransferase were unaltered in extract-treated animals. Both the extract and chlorpropamide

significantly ($P < 0.05$) reduced urea level in diabetic rats. Exceptions were lymphocyte and neutrophils. The extract did not show any significant difference in the haematological parameters. These data appear to justify the antidiabetic, hypolipidemic and protective potential for most organs by *S. mombin*, in traditional medicine.

ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACTS OF *SPONDIAS MOMBIN* (ANACARDIACEAE) STEM BARK ON ALLOXAN-INDUCED DIABETIC RATS

Adebayo Gbolade, Matthew Ekor, Adelaja Akinlolu, Marcus Ayoola

Olabisi Onabanjo University, Sagamu campus, Nigeria, adegbolade@yahoo.com
Olabisi Onabanjo University, Sagamu campus, Nigeria, -, Olabisi Onabanjo University, Sagamu campus, Nigeria, laja3@yahoo.com, Olabisi Onabanjo University, Sagamu campus, Nigeria, ayosther@yahoo.com

S. mombin is traditionally used as a purgative, antihelmintic, analgesic, haemostatic, treatment of cough and gonorrhoea, and for diabetes. The present study was, therefore, undertaken to evaluate the antidiabetic activity of ethanolic extract of *S. mombin* stem bark in alloxan-induced diabetic rats. Rats with fasting blood glucose level (FBG) in excess of 150 mg/dl, 18 h after alloxanisation, were treated with 100-250 mg/kg b.w. doses of the extract. Periodic determination of FBG up to 24 h. in an acute study showed delayed onset, but weak hypoglycaemic effect at 24 h. which was less than that of the standard drug, chlorpropamide. Administration of extract at the maintenance dose of 150 mg/kg in a prolonged study revealed sustained and significant increase ($P < 0.05$) in hypoglycaemic activity up to day 7, and this surpassed that of chlorpropamide. The FBG was reduced to a value close to pre-induction value of the untreated normoglycaemic animals and there was restoration of cellular population of the pancreatic beta cells in alloxanised diabetic rats fed with plant extract. The extract did not produce marked changes in body weight of rats throughout the study. The results of this study clearly indicate that *S. mombin* stem bark is a potential source of orally active antidiabetic agents.

ETHNOPHARMACOLOGICAL APPROACH AND LABORATORIES EVALUATION OF TRADITIONAL PHARMACOPOEIAS IN 30 CARIBBEAN BASIN TERRITORIES: TWO PRECONDITIONS TRAMIL FOR THE USE OF LOCAL MEDICINAL PLANTS IN PRIMARY HEALTH CARE.

Lionel Germosén-Robineau

TRAMILnetwork, Dominican Republic, tramilweb@gmail.com

In a spirit of Public Health and applied research, we explain why an original quantitative ethnopharmacological method had to be drawn by TRAMIL to determine the popular traditional uses of medicinal plants in 30 Caribbean Basin territories. Then, the need for networking between regional universities with an aim of validating the significant uses. Finally the strategy of diffusion aiming at the appropriation by the official national systems of Primary Health care of

rational use of local medicinal plants. Moreover we present the Caribbean Herbal Pharmacopoeia e.book.

Acknowledgements: UAG, TRAMIL network.

ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS USED BY IKARE AND IPE PEOPLE OF AKOKO IN ONDO STATE, NIGERIA

Alexander Giwa, Wasiu Odofin, Sunday Aladele, Benjamin Daramola

National Centre for Genetic Resources and Biotechnology PMB, Nigeria,
giwaalexander@yahoo.com

National Centre for Genetic Resources and Biotechnology PMB, Nigeria,
giwaalexander@yahoo.com, National Centre for Genetic Resources and Biotechnology PMB,
Nigeria, sundayaladele@yahoo.com, National Centre for Genetic Resources and Biotechnology
PMB, Nigeria, benjdaramola@yahoo.com

The aim of this study was to investigate the medicinal uses, cultivation and remedies of various indigenous plants, which are commonly used by local people. Ethnobotanical study was carried out in two notable villages in Akoko area of Ondo State (Ikare and Ipe) respectively. The information gathered was obtained through interviewing the herbalist and traditional medicine practitioners. In this study, 68 plant species which are commonly used among the people to cure diseases, were presented. Herbarium specimens were also carefully preserved for detail study. This study helped to preserve the information and skill acquired by processes of trial and error the centuries by these people.

TYPHA CAPENSIS EXTRACTS DECREASE ROS PRODUCTION AND AFFECT HUMAN SPERM FUNCTIONS

Ralf Henkel, Warren Fransman, Uta-Christina Hipler, Gerhard Schreiber

Department of Medical Biosciences, University of the Western Cape, South Africa,
rhenkel@uwc.ac.za

Department of Dermatology, Friedrich Schiller University, Germany

South African traditional healers use about 3,000 higher plants to treat ailments and diseases. As about 25% of prescribed medicines contain plant extracts, an immense need exists to test the effects of these plants and their compounds on male reproductive functions. The objective of this study was to investigate the *in vitro* effects of extracts from *Typha capensis* on sperm motility, viability, production of reactive oxygen species (ROS) and the mitochondrial membrane potential (MMP). Fifty semen samples were washed with Human Tubular Fluid Medium containing 10 mg/ml albumin and a swim-up was performed. To 100 µl swim-up fraction, 1 µg/ml freeze-dried aqueous extract of *Typha* rhizome was added and incubated for 1 hour at 37°C. Parameters were analyzed in the test samples, controls as well as in the pellet. Sperm viability was assessed by means of the Eosin stain. Sperm ROS production was tested by means of dihydroethidine and MMP using the DePspipher kit. To inhibit superoxide production by the extract at different concentrations (0.1 mg/ml; 0.05 mg/ml and 0.01 mg/ml) the Antioxidant Kit

with Pholasin® was used. Statistical analysis was performed using the MedCalc software ver. 9.4.2. Results: Results showed significantly ($P < 0.0001$) decreased motility (81.4% vs. 95.3%), viability (70.8% vs. 77.6%), MMP (49.0% vs. 67.5%) and sperm ROS production (11.3% vs. 14.9%) after incubation with *Typha* extract. The inhibition of ROS production was significantly dose-dependent. Results also indicated a decreased MMP ($P < 0.0001$) and viability ($P < 0.0001$) in the pellet when compared to the swim-up. Conclusions: *Typha capensis* extracts had significant detrimental effects on various sperm functions *in vitro*. It also decreased the cells' ROS production, which could have a negative effect on cellular functions. Moreover, centrifugation not only affects sperm motility and viability but also mitochondrial functionality.

EFFECT OF *PICRORHIZA KURROA* ROOTS ON BLOOD GLUCOSE LEVEL ON ALLOXAN INDUCED DIABETIC RATS

Pramod Hurakadle, Mrityunjaya Patil, Sunil Jalalpure, Fakerappa Manvi

Department of Pharmacognost and Phytochemistry, KLES College, Belgaum-590 010,
Karnataka, India, pramodhj75@rediffmail.com

The polar and nonpolar solvent extracts of *Picrorhiza kurroa* roots were screened at the oral dose of 250 mg/kg body weight for antidiabetic activity using alloxan induced diabetic rats on prolonged treatment studies. Petroleum ether, alcoholic and aqueous extracts of roots of *Picrorhiza kurroa* showed significant ($p < 0.01$) antidiabetic results with prolonged treatment studies. The extracts contained alkaloids, flavonoids and glycosides. The results were comparable with the standard drug glibenclamide and a standard marketed herbal product, which revealed that sterols in petroleum ether extract, flavonoids, alkaloids and glycosides in alcoholic and aqueous extracts were responsible for antidiabetic activity.

EFFECT OF ADMINISTRATION OF AQUEOUS EXTRACTS OF *VERNONIA AMYGDALINA* .DEL LEAVES TO GUINEA PIG DAMS ON MILK PRODUCTION AND CONTRACTION OF THE MAMMARY GLAND AND UTERUS.

Ifeoma I. Ijeh, Kalu K. Igwe, Chukwunonso E. C. C. Ejike

Department of Biochemistry, College of Natural and Applied Sciences. Michael Okpara
University of Agriculture, Umudike, ABIA STATE, NIGERIA, ijehirene@yahoo.com

The effect of aqueous extracts of *Vernonia amygdalina* on litter-weight gain, milk production and uterine and mammary gland contraction amplitudes were investigated using guinea pigs as animal models. The animals were placed in four groups of three animals each with Group 1 as control and groups II-IV receiving 5 mg/kg, 10 mg/kg and 100 mg/kg *V. amygdalina* aqueous extract respectively. Litters of animals in groups I, II, III and IV gained a mean weight (g) of 1.88 ± 0.68 , 3.11 ± 0.10 , 4.20 ± 0.20 and 13.69 ± 1.53 respectively, after five weeks. The differences in the mean weight-gain by the litters in groups II and IV, but not those in group I were significant ($P < 0.05$) when compared to the litters in the control group. All the dams had their highest milk production index (g) at week 2, with animals in groups I, II, III and IV having 11.00 ± 1.00 , 9.00 ± 2.00 , 11.33 ± 5.69 and 14.33 ± 1.53 respectively. The observed

differences in milk production were not however significant ($P < 0.05$). Milk production dropped as the weeks progressed with the lowest for each group recorded at week 4 or 5. At concentrations of 5mg/ml, 10 mg/ml and 100 mg/ml, uterine contraction amplitudes (mm) were 1.22 ± 0.03 , 3.60 ± 0.03 and 9.6 ± 0.24 respectively. The uterine contraction amplitudes for 5 mg/ml and 10 mg/ml, but not 100 mg/ml *V. amygdalina* extracts were significantly ($P < 0.05$) lower than that recorded for equivalent concentrations of the standard drug – ergometrin. The mammary gland contraction amplitudes (mm) recorded for 5mg/ml, 10mg/ml and 100mg/ml *V. amygdalina* extract were 1.2 ± 0.14 , 2.70 ± 0.36 and 6.15 ± 0.13 respectively. The observed values were all significantly ($P < 0.05$) lower than those observed for the equivalent concentrations of ergometrin. Histopathological studies showed no marked toxicity of *V. amygdalina* extract on the cells of the major organs of the experimental rats, even at 100 mg/ml of the aqueous extracts of *V. amygdalina*.

EFFECTS OF AQUEOUS LEAVES EXTRACT OF CASSIA GLAUCA LAM. IN STREPTOZOCIN-INDUCED DIABETIC RATS

Sunil Satyappa Jalalpure, Md Salahuddin

KLES's College of Pharmacy, Belgaum, Karnataka, India, India, jalalpuresunil@rediffmail.com

Cassia glauca Lam. is a glabrous tree belonging to family Caesalpiniacea, found throughout India, tropical Asia and Australia. In folk medicine, leaves of *Cassia glauca* are used for the treatment of diabetes. The aim of this study therefore, was to investigate the antihyperglycemic and antidiabetic activity of aqueous extract of *Cassia glauca* leaves. The various parameters studied included effect on normoglycemic, oral glucose tolerance test, hypoglycemic activity in streptozotocin induced diabetic rats, fasting blood glucose levels, changes in body weight, serum lipid levels, liver glycogen content, serum insulin level and glycated hemoglobin in diabetic control and treatment rats. On oral administration, aqueous extract of *Cassia glauca* leaf at a dose of 500 mg/kg did not show significant effect on blood glucose levels in normoglycemic rats, whereas aqueous extract showed statistically significant ($P < 0.05$) effect by reducing the effect of external glucose load. In chronic model of diabetes, aqueous extract of *Cassia glauca* leaf at a dose of 500 mg/kg and glibenclamide (0.25 mg/kg) were administered for 21 days. At the end of the treatment period there was significant increase in the body weight, liver glycogen, serum insulin level and the HDL cholesterol levels. There was a significant decrease in fasting blood glucose, glycated hemoglobin, total cholesterol and serum triglycerides, thus providing support to the potent antidiabetic property of the plant extract.

TRADITIONAL MEDICINE VALUE IN HIV/AIDS

Kizito Josiah

bethel Aids information research centre, Uganda, kizitodr@lantic.net
Kizito J, Mawanda D, Ntege S, Affiliations: Bethel Aids information and research center, East London, South Africa

The value of traditional medicine in HIV/AIDS is holistic therefore calls for attention and research that may lead to the cure of HIV notwithstanding behaviour and nutrition. An anti viral plant even if it works on herpes simplex is beneficial to HIV/AIDS. *Asparagus africana* has the ability of utilizing oxygen in the body thus promoting deoxidation. The asparagositides in it are anti viral. *Cadiospermum halicacabum* is anti inflammatory, boosts immunity, is a histamine release inhibitor, fights diarrhoea and acts as a reverse transcriptase inhibitor with its cadiospermine in it. Then why not use it in HIV/AIDS? *Gynopsis yardarm* is a vegetable, contains an anti viral diethyltoluamide that makes it useful in HIV/AIDS. Methods: A cross sectional study in four different hospices was done in Eastern Cape for a period of more than one year. Results: A total of 75 patients were followed for over one year. CD4 count increased from 200cells/mms to 450cells/mms. Increment in body weight was noted from 49kgs to 64 kgs. Conclusion: *Hibiscus sabdariffa* is very useful in treatment of HIV/AIDS patients: It has the ability of removing deoxidation and It makes the sick regain euphoriant.

References: 1. Monic Adrians- The medical gardens in the Rwenzori region 2. Jackie segyin-Eynad Home doctor 1994. 3. Medical plants in the tropical countries by German institute for medical mission 4. The Victoria basin researchers (inter university council).

INHIBITION OF WIL-2 NS CELLS PROLIFERATION IS RELATED TO G2/M ARREST AND INDUCTION OF CASPASE-MEDIATED APOPTOSIS

V. G. Mbazima, M. P. Mokgotho, P. Masoko, R. L. Howard and L. J. Mampuru

Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga, 0727 South Africa

Commelina benghalensis L. is an annual or perennial herb with fleshy creeping stems that root readily at the nodes. The plant is used in the eastern and northern parts of South Africa as a crude medicinal agent in the treatment of stomach disorders, skin lumps and/or on cancerous skin outgrowths. We have evaluated CMECB for its possible chemopreventative application *in vitro* by studying its role in the regulation of proliferation, cell division cycle and apoptosis in Wil-2 NS cancer cell line. CMECB inhibited cell proliferation and induced G2/M arrest and apoptosis in Wil-2 NS cells. CMECB-induced apoptosis of Wil-2 NS cells is mediated *via* the modulation of the intrinsic apoptotic pathway. Up-regulation and phosphorylation of p53 resulted in the down regulation of the anti-apoptotic protein Bcl-2. The altered expression of Bcl-2 triggered the release of cytochrome c and activation of caspase-9 followed by activation of effector caspase-3 thus leading to PARP cleavage and apoptosis. In conclusion, CMECB exerts its anti-neoplastic effects by modulating the cell cycle and apoptosis regulatory genes.

Acknowledgements: National Research Foundation (NRF) and the University of Limpopo senate research fund for financial support.

PRELIMINARY INVESTIGATION INTO ANTIMYCOPLASMAL ACTIVITY OF TRADITIONALLY USED NIGERIAN PLANTS

Muraina I.A, McGaw L.J., Picard J. and Eloff J.N.

Phytomedicine Programme, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa (kobus.elloff@up.ac.za)

Mycoplasma species are fastidious organisms that require a specialized medium for their growth, isolation and identification. The use of antibiotics in the treatment of Contagious Bovine Pleuropneumonia (CBPP) caused by these organisms has been discouraged because it promotes the persistence of healthy carrier animals (1). To the best of our knowledge, there is no documented research evidence of the efficacy of medicinal plants on the *Mycoplasma* spp. The aim of the present work was to screen medicinal plants used traditionally for possible antimycoplasmal effects which can be explored for the development of alternative antimycoplasmal agents. Acetone extracts of twenty-one Nigerian medicinal plants were evaluated on isolates of *Mycoplasma mycoides* subsp *mycoides* SC, using a microtitre plate method (2) and a new pH dependant (metabolic inhibition) method described for the first time by the authors. The extracts were also assessed for their cytotoxicity using the colorimetric method (3) and antioxidant properties using the traditional DPPH method and an easy-to-perform quantitative method, which was also described for the first time by the authors. The *Calotropis procera* extract had a good minimum inhibitory concentration of 0.08 mg/ml and minimum cidal concentration of 0.16 mg/ml which was better than the drug commonly used in the field. The extract was less cytotoxic with LC₅₀ of 0.204 mg/ml compared to enrofloxacin with values of 0.017 mg/ml and 0.0062 mg/ml respectively. There were also the presence of alkaloid, saponin, tannins, resins, cardiac glycoside, steroidal rings and flavonoids. It is concluded that the extract of *C. procera* is worth investigating for the development of a potent agent against CBPP which has for long defied solutions by conventional chemotherapy.

Acknowledgement: NVRI, Vom, Nigeria, NRF, South Africa.

[References: 1]. FAO (1967). Report of the meeting of the FAO/OIE/OAU expert panel on contagious bovine pleuropneumonia, Khartoum, Sudan, 12-15 February.

[2] Eloff, J.N. (1998). *Planta Medica*, 64: 711-713.

[3]. Mosmann, T.J (1983). *Journal of Immunol. Methods*, 65, 55-63.

ENDOMETRIAL AND HORMONAL CHANGES IN RATS FOLLOWING ADMINISTRATION OF FENUGREEK EXTRACTED DIOSGENIN

Israa Sulaiman, Amal Nimir, Srijit Das

Universiti Kebangsaan Malaysia, Malaysia, dr_israa70@yahoo.com

Fenugreek (*Trigonella foenum graecum* L.) is an annual plant of the family Leguminosae. The seeds possess significant hypoglycemic, anti-atherosclerotic, anti-inflammatory, anti-ulcerogenic, and anti-neoplastic effects. The most potent component extracted from this herb is diosgenin. A total of 40 healthy female Norway albino rats aged between 7-11 weeks, having regular estrous cycles, were used in this study. Twenty rats assigned to the control group (GI) were administered 4ml of distilled water. The other twenty rats (GII) were given diosgenin (0.024mg/g body weight) suspended in 4ml of distilled water daily for 14 days through oro-gastric tube. Upon completion of 14 days of treatment, a blood sample was taken for hormonal assay and the animals were sacrificed thereafter. There was a significant increase in serum hormones (estrogen, progesterone and prolactin) in the studied group as compared to control one. Histological analysis showed that there was an increase in thickness and hyperactivity of the endometrium, increase in mucin secretion in addition to the increase in eosinophil and metrial glands count. The above changes, which were dose and duration dependant, may

account for diosgenin use as a contraceptive or anti-infertility agent. Acknowledgements: Department of Anatomy, University of Baghdad

PROTECTIVE EFFECT OF *HELYCHRISUM PLICATUM* EXTRACTS ON ANTIOXIDANT DEFENCE STATUS IN CCl₄-INDUCED HEPATOTOXICITY IN RATS

Tatjana Kadifkova Panovska, Svetlana Kulevanova, Mirjana Bogdanova

Institute of Applied Biochemistry, Faculty of Pharmacy, University "Ss. Cyril and methodius",
Macedonia, taka@ff.ukim.edu.mk

Institute of Pharmacognosy, Faculty of Pharmacy, University "Ss. Cyril and methodius",
Vodnjanska 17, Macedonia

Institute of Clinical Biochemistry, Clinical Center, Vodnjan 17, Macedonia

This paper reports a study in which antioxidative and hepatoprotective activity of *H. plicatum* extracts in *in vitro* and *in vivo* conditions were tested. Phytochemical screening (HPLC) of *H. plicatum* from Macedonia revealed the presence of apigenin and naringenin as free aglycones and glycosides of apigenin, naringenin, quercetin and kaempferol in the flowers as well as quercetin and luteolin glycosides and free luteolin in stems and leaves. The antioxidative effect was investigated using three methods: evaluation of the free radical scavenging capacity (DPPH method), inhibition of hydroxyl radical production and protection of beta-carotene-linoleic acid system. The hepatoprotective activity was investigated using CCl₄-induced liver damage model on rats. The extracts under investigation showed radical scavenging activity with IC₅₀ value of 6 to 11 mg/ml. The extracts were capable to react with OH radical with inhibition of its production ranged between 33-58%. The high preventive activity against the bleaching of beta-carotene (15-49% of initial value after 120 minutes) was also observed. The antioxidative activity of the extracts was compared with that of luteolin, quercetin, BHA, BHT and sylimarin. In *in vivo* studies, the biochemical parameters in groups treated with *H. plicatum* extracts at a dose of 25 mg/kg showed significantly different value than that of the CCl₄-treated group. The liver biopsy of all experimental rat groups treated with *H. plicatum* extracts showed significant restoration of normal histomorphological pattern of liver cells. Results of this study suggest that *H. plicatum* represents a natural source with antioxidant and hepatoprotective potential.

VARIATION IN ESSENTIAL OIL COMPOSITION OF *BOSWELLIA CARTERII* BIRDW. AND ITS ANTIMICROBIAL ACTIVITY

Guy Paulin Kamatou, Alvaro Viljoen, Sandy van Vuuren, P. Balwanth, R. Gosai,

Tshwane University of Technology, South Africa, kamatoug@tut.ac.za

Tshwane University of Technology, South Africa, viljoenam@tut.ac.za

University of the Witwatersrand, South Africa, Sandy.Vanvuuren@wits.ac.za

University of the Witwatersrand, South Africa,

Boswellia carterii (*Burseraceae*) is used in traditional medicine, the cosmetic industry and in aromatherapy in many countries all over the world. This plant produces the lucrative commercial oil known as Frankincense oil which has a woody, spicy and haunting smell. Frankincense oil has several pharmacological properties and these include the promotion of the growth of skin cells, healing sores and wounds. Variation in the chemical composition of this oil

has been reported in the literature. This prompted an investigation to study the commercial Frankincense oils from various suppliers. Twenty essential oils were analyzed by gas chromatography coupled to mass spectrometry. Most of the oils were qualitatively similar when looking at the major components. However, there was some significant quantitative variation. The major components that were identified in the oils included α -pinene (2-64%), α -thujene (1-33%); β -pinene (0.3-13%); myrcene (1-9%); sabinene (2-7%); limonene (1-20%); p-cymene (3-17%); β -caryophyllene (0.1-8%). The antimicrobial activity of the oils was investigated on gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and gram-negative bacteria (*Escherichia coli* and *Proteus vulgaris*) as well as on a yeast (*Candida albicans*). Efficacy ranged between 1.5-32 mg/mL, depending on the test organisms studied. The highest sensitivity was observed against *B. cereus*.

A COMPARATIVE STUDY ON THE *IN VITRO* ANTI-DIABETIC PROPERTIES OF FOUR PLANTS FREQUENTLY USED TO TREAT DIABETES IN SOUTH AFRICA.

Trevor Koekemoer, Maryna van de Venter, Saartjie Roux

NMMU, South Africa, tkoekemoer@nmmu.ac.za

Medicinal plants are often used as adjunctive therapy with conventional medications, raising the possibility for synergistic or antagonistic interaction. Since numerous therapeutic targets are exploited to treat diabetes, knowledge on the mechanism by which herbal extracts exert their effect would be beneficial when traditional medicine is combined or used as an alternative to conventional drugs. Ethnobotanical surveys conducted in South Africa indicate that *Artemisia afra*, *Ruta graveolens*, *Sutherlandia frutescens* and *Leonotis* spp. are the most popular plants used to treat diabetes, however their precise mechanism of action is unknown. Various *in vitro* models designed to simulate specific therapeutic targets were used to screen plant extracts for potential anti-diabetic activity. The assays included alpha-glucosidase inhibition, DPPIV inhibition, antioxidant activity, glucose uptake and PPAR-gamma agonist activity. In addition, potential hepatotoxicity was assessed using HepG2/C3A cells. None of the plants showed any significant alpha-glucosidase inhibitory activity nor could they significantly stimulate adipocyte differentiation. The antioxidant capacity of the different plant extracts varied depending on the assay used. *A. afra* and *S. frutescens* had the best metal chelating and oxygen radical scavenging activity while only *R. graveolens* showed H₂O₂ scavenging activity. Both aqueous and ethanolic extracts prepared from *S. frutescens* demonstrated DPPIV inhibition, however the concentration required to achieve 50% inhibition suggests that this activity has little physiological relevance. Except for *A. afra*, none of the plants were toxic to cultured hepatocytes even at a concentration as high as 1 mg/ml. Further studies are required to identify the metabolic targets of these plants.

BIODIVERSITY AND UTILITY OF HERBAL DRUGS IN INDIAN CONTEXT

Chandrakant Kokate

Vice-Chancellor, KLE University, Belgaum, India, chandrakokate@gmail.com

According to Global Biodiversity Assessment estimate of UNEP, the total number of living species across the globe are in the range of 13 to 14 million, of which only 1.75 million species have been scientifically described and documented. Around 20,000 plant species are believed to be used medicinally in third world countries, of which around 8,000 species are systematically utilized traditionally in India. Endowed with a wide diversity of agroclimatic conditions, India is virtually the Botanical Garden of the World. It is one of the top twelve mega diversity countries in the world. Out of eighteen hot spots of biodiversity identified across the globe, India has two located in Eastern Himalaya and Western Ghat regions containing approximately 3500 and 1600 medicinal plants respectively. Under the agreement of G-15 countries, the Department of Biotechnology in India has established three gene-banks for medicinal and aromatic plants which utilize conservation strategies to protect and nurture plants in their native eco-systems (in-situ) or away from their natural habitat (ex-situ). Ayurveda- the Ancient Science of Life – is a time tested traditional system of medicine practiced in India since time immemorial. In past two decades, it has undergone rapid metamorphosis in terms of quality control, safety, efficacy and clinical validations. Herbal medicines derived from Ashwagandha, Punarnava, Brahmi, Isabgol, Tulsi, Turmeric, Neem, Safed Musli, Amla, Shatavari, Garlic, Senna, Tamala, Nutmeg, Shankhapushpi, etc., have revolutionalized modern herbal therapy in India.

INFLUENCE OF *URTICA DIOICA* SEED EXTRACTS ON ACUTE EXPERIMENTAL HEPATOTOXICITY IN VIVO: ANTIOXIDANT AND ANTIHEPATOTOXIC EFFECT

Svetlana Kulevanova, Tatjana Kadifkova Panovska, Mirjana Bogdanova

Institute of Pharmacognosy, Faculty of Pharmacy, University Ss Cyril and Methodius, 1000
Skopje, Macedonia, svku@ff.ukim.edu.mk
Institute of Toxicology, Faculty of Pharmacy, University “, Macedonia
Institute of Clinical Biochemistry, Clinical Center, Vodnjan, Macedonia

Urtica dioica is an annual herb that belongs to the family Urticaceae. It is known in traditional therapy as acute diuretic, natriuretic and hypotensive. Different parts of this plant have also been used in the treatment of hypertension, stimulation of proliferation of human lymphocytes, immunostimulation on neutrophilis, beneficial effect on the prostate tissue and antirheumatic effects. The aim of the present study was to investigate a possible prophylactic effect of *Urtica dioica* seed extracts pre-treatment on prevention of oxidative stress generated by CCl₄ in rats. A dose of 25 mg/kg of *Urtica dioica* extracts was administered i.p. to the test groups of female Wistar rats. The positive control group received CCl₄ and the negative control group received normal saline. In this investigation the level of lipid peroxidation and serum liver enzyme activities were measured. Histopathological examinations were also carried out. The CCl₄ treatment of rats increased the lipid peroxidation and liver enzymes, and also decreased the antioxidant enzyme levels (superoxide dismutase, catalase, glutathione peroxidase and the levels of glutathione). Pre-treatment of the experimental animals with *Urtica dioica* extracts for 7 days decreased the elevated lipid peroxidation and liver enzyme levels and also increased the reduced antioxidant enzyme levels. Histopathological studies provided supportive evidence for the biochemical analysis. The present study has shown that *Urtica dioica* pre-treatment diminishes oxidative stress in the liver generated by applying CCl₄. This finding suggests that *Urtica dioica* has a cell protection effect against oxidative stress in rats.

THE GASTROPROTECTIVE AND ANTIOXIDANT EFFECT OF *BELLIS PERENNIS* L. (ASTERACEAE) EXTRACTS

Leyla Açık, İlhan Gurbuz, Hulya Demir, Fatih Demirci, R. Göktürk

Department of Biology, Faculty of Science and Arts, Gazi University, 06500 Ankara-Turkey,
lacik@gmail.com

Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Ankara, Turkey
Gazi University, Atatürk Vocational Training School, Department of Chemistry, 06500 Ankara,
Turkey

Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470 Eskisehir,
Turkey

Akdeniz University, Science and Arts Faculty, Department of Biology, Antalya, Turkey

Bellis perennis is known as chamomile or papatya" in Anatolia. The plant is used in Turkish folk medicine against tonsillitis, common cold, eczema, eye diseases, and as diuretic, laxative, and diaphoretic [1-3]. In addition, the herb is used for the treatment of peptic ulcer symptoms in various localities [2, 3]. In order to confirm the acclaimed activity on peptic ulcer, aqueous and methanol extracts were prepared from the aerial parts of the plant material and both extracts were tested for their anti-ulcerogenic potential on the ethanol-induced gastric ulcer model using rats. The aqueous and methanol extracts were administered orally at 1100 and 600 mg/kg doses, respectively. Both extracts demonstrated statistically significant anti-ulcerogenic activity ($p < 0.001$). In addition, the aqueous extract of *B. perennis* showed to possess protective effect with 100% ulcer inhibition. On the other hand, the MeOH extract also demonstrated high gastroprotective effect (95% ulcer inhibition). In vivo pharmacological experiments clearly demonstrated that the relevant extracts of *B. perennis* showed significant gastroprotection on this model of ulcerogenesis. Additionally, the antioxidant potential of the extracts was evaluated in vitro by DPPH free radical scavenging assay and total antioxidant capacity methods. The results of this study showed that *B. perennis* extracts is a potential source of natural antioxidants. Chromatographic finger print of these extracts was also documented using LC and LC-MS techniques. Further studies are needed in order to isolate and define the active constituent(s) by bioassay-guided fractionation techniques.

Acknowledgements: This study was financially supported by the Turkish Prime Ministry, State Planning Organization (DPT-1998). References: 1. Baytop, T. (1999). Therapy with medicinal plants in Turkey, past and present. Second Ed., Nobel Tp Kitapevi, Istanbul. 2. Gülay, E.G., Özhatay N., (2006). Turkish J. Pharm. Sci., 3: 73-89. 3. Yazcolu, A., Tuzlac, E. (1995). J. Pharm. Univ. Mar., 11: 333-342

IN VITRO ANTIMYCOBACTERIAL ACTIVITY OF TWO MEDICINAL PLANTS IDENTIFIED FROM NIGER STATE, NIGERIA

Mann, A., K. Ibrahim, A. O. Oyewale, J. O. Amupitan, J. I. Okogun

Department of Science Laboratory Technology, The Federal Polytechnic, Bida, P. M.B 55, Bida,
Niger State, Nigeria

Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research
and Development (NIPRD), P.M.B.21, Garki – Abuja, Nigeria

Department of Chemistry, Ahmadu Bello University Zaria, Kaduna State, Nigeria

Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B. 21, Garki – Abuja, Nigeria
Correspondence author: e-mail: abdumann@yahoo.com Tel: +2348036368173

The global threat of tuberculosis demands for search for alternative antimycobacterial drugs [1]. The crude methanolic extracts of eight Nigerian medicinal plant species used in the treatment of TB and other respiratory diseases were evaluated for activity against a clinical isolate of *Mycobacterium tuberculosis* and attenuated *Mycobacterium bovis* (BCG) using broth microdilution method. The plant parts used were obtained as described by the users from a forest near Baddegi, Niger State, Nigeria. Voucher specimens were deposited in the Herbarium at the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria and National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. Two hundred grams (200g) of each dried plant material was powdered and extracted by maceration with methanol for 72h at room temperature (3 x 250mL). All the crude extracts were filtered and evaporated *in vacuo* (35°C). Each extract was then partitioned with *n*-hexane-MeOH (3 x 250mL, 1: 1) to give *n*-hexane and MeOH solubles as well as all the partitioned extracts for each plant were combined; concentrated and dried *in vacuo*. Both hexane and methanol extracts of each plant species were tested for antimycobacterial activity. Four out of the eight plant extracts exhibited inhibitory activities against *Mycobacterium tuberculosis* at 78 and 1250µg/mL [2]. The hexane fractions obtained after fractionation were the most active fractions for all the plants tested against BCG, having *Anogeissus leiocarpus* and *Terminalia avicennioides* exhibiting the highest activity at 312 and 200µg/mL respectively. Fractions Ta5 and Al4 obtained on further purification exhibited most significant activity (MIC 4.7µg/mL, 7.8µg/mL) respectively. From the results of phytochemical analysis, terpenes and triterpenoid saponins are the most prominent compounds in these fractions and several reports earlier indicated that these metabolites are potential antimycobacterial agents [1]. This class of metabolites presents interesting area for further investigation with special attention on the Combretaceae family from Nigeria flora.

References:

[1] Copp, BR: The Review of Natural Products with antimycobacterial activity. Nat. Prod. Rep., **20**(6): 535-557. (2003). [2] Mann, A., Amupitan, J. O., Oyewale, A.O., Okogun, J. I., Ibrahim, K., Oladosu, P., Lawson, L., Olajide, I., and Nnamdi, A.: Evaluation of in vitro antimycobacterial activity of Nigerian plants used for treatment of respiratory diseases, Afri. J. Biotech., **7** (11): 1630-1636 (2008)

STUDY OF THE ANTIMICROBIAL EFFECTS OF THE STEM BARK EXTRACTS OF *PARKIA BIGLOBOSA* (JACQ.) BENTH AGAINST *STAPHYLOCOCCUS AUREUS*

Hassanata Millogo-Koné

CNRST - IRSS, Burkina Faso, hassmillogo@yahoo.com

Staphylococcus aureus causes many infectious diseases in human. In traditional medicine, the stem bark of *Parkia biglobosa* is used for the treatment of many infections. Extracts of the stem barks were tested against strains of *Staphylococcus aureus* collected from pus, blood, vaginal secretions, sperms and urine and also against a collection strain of *Staphylococcus aureus* as a positive control. To select the most effective extract, tests have been conducted with the decoction, prepared according to the traditional healer recipe, and extracts obtained with different solvents (dichloromethan, 70% aqueous ethanol, ethyl acetate,

water). The results showed that the decoction was active against *Staphylococcus aureus*, thus justifying its use in the traditional medicine. The results also revealed that the 70% hydroalcoholic extract was more active than the decoction and that ethyl acetate extract also exerted strong inhibition against *Staphylococcus aureus*. The dichloromethane extract had no effect against the tested strains. Column chromatography of the ethanolic extract followed by thin layer chromatography led to the isolation of gallic acid. Gallic acid isolated from the ethanolic fraction showed strong inhibition but its effectiveness was lower than what was observed with the synthetic gallic acid. The chemical screening of all these extracts showed that the dichloromethane extract contains mainly sterols, triterpens and coumarins. It also showed that the aqueous, the hydroethanolic and the ethyl acetate extracts contain saponosides, anthracenosides, tannins, flavonoids, reducing compounds but in different proportions, justifying the differences in their respective anti – *Staphylococcus* activities.

FREE RADICAL SCAVENGING ACTIVITY OF TWO MEDICINAL PLANTS USED BY DIABETIC PATIENTS IN BOTSWANA

Daniel Motlhanka, Priscilla Motlhanka

Botswana College of Agriculture, Botswana, [dmotlhan@bca.bw](mailto:dmothlan@bca.bw)
Institute of Health Sciences, Botswana, [dmotlhan@bca.bw](mailto:dmothlan@bca.bw)
Institute of Health Sciences, Nursing Department, Box 985, Gaborone, Botswana.

Water extracts of *Myrnthamnus flabellifolius* (shoot) and *Ozoroa paniculosa* (roots, stem, leaves) and their combinations called “Dankatso” (equal proportion of the above plant extracts) are being used extensively in eastern Botswana traditional medicine. These plants have reputable use as antidiabetic preparations. *M. flabellifolius* is used to alleviate symptoms of diabetes mellitus, stroke and hypertension. *O. paniculosa* is used to alleviate menstrual pain and in asthma (Motlhanka, 2008). The study is part of an ongoing project to search for novel drugs from vast array of medicinal plants from Botswana, which have not been studied extensively (Motlhanka et al., 2005). The antioxidant capacity of the extracts together with their combinations was assessed spectrophotometrically using DPPH free radical scavenging assay. At 25 µg/ml the scavenging power of the extracts were as follows: *M. flabellifolius* alone (66%); *O. paniculosa* root alone (49%); *O. paniculosa* stem alone (81%); *O. paniculosa* leaf alone (79%). At the same concentration (25 µg/ml), combinations: [*M. flabellifolius*+*O. paniculosa* root] (85%); [*M. flabellifolius* + *O. paniculosa* stem] (91%); [*M. flabellifolius* + *O. paniculosa* leaf] (91%). At 100 µg/ml and above there were no significant differences between scavenging potencies for individual extracts and their combinations (“Dankatso”). The scavenging potencies were in magnitudes of 90-91% for individual extracts and their combinations. This study suggests that the presence of antioxidant compounds in water extracts of the studied plants may be responsible for their anti-diabetic properties as advocated in traditional medicine.

Acknowledgements: Traditional healers for supplying the plants.

References: Motlhanka D.M et al. (2005) J. of Pharm. & Pharmacol. Vol: 57 S-7.
Motlhanka, D.M (2008) Pakistan J of Biol. Sci. Vol:11 (5) 805-808

COVARIUM 10 A NEW MEDICINE AGAINST INTESTINAL AMOEBIASIS FROM *CODIAEUM VARIEGATUM* (EUPHORBIACEAE)

Kamini Melanie, Njoya Bilong, Paul Moundipa

Laboratory of Pharmacology and Toxicology, Laboratory of Parasitology, University of Yaounde I, Cameroon, pmoundipa@hotmail.com

During a survey within the Bamum tribe reputed for their attachment to their traditional way of life, 52 medicinal plants were selected for their use to treat liver and intestinal disorders. Among the 52 plants *C. variegatum* displayed high amoebicidal activity *in vitro* in polyxenic culture. We then focussed on developing an improved traditional drug. Extract prepared as described traditionally displayed excellent *in vitro* activity against trophozoites of *Entamoeba histolytica* and the activity was even higher compared to that of the reference drug metronidazole (3.15 µg/ml and 5.97 µg/ml respectively). A finger print of the therapeutic extract revealed the presence of flavonoids; coumarins, and polyphenols compounds. Toxicity studies on mice and rats did not reveal any mortality at acute dose of 5 g/kg and no alteration of the *in vivo* marker liver and kidney functions (aminotransferases, creatinine, mixed function oxidase enzymes). Leaves of the plants were harvested and processed according to traditional advice to manufacture a herbal tea bag called Covarium 10. This drug was used for phase I clinical trial after the approval of the Cameroonian Ethical Committee. Two to three days were sufficient to completely clear the parasite from the intestine following the administration of one tea bag per day, with no secondary clinical effect observed. Biochemical analysis of the blood showed no changes in patients. The results are being patented.

Acknowledgement: Yaounde Hospital, Institute of Tropical Medicine in Hambourg, IFS.

BASELLA ALBA (BASELLACEAE) AND MALE REPRODUCTIVE FUNCTION**P F Moundipa, T K Monsees, N S E Beboy, S Carreau, E A, Nantia**

Lab of Pharmacol and Toxicol, University of Yaounde, Cameroon, pmoundipa@hotmail.com
Dept Medical Biosciences, University of the Western Cape, South Africa,
Laboratoire de Biochimie, University of Caen, France

Hibiscus macranthus and *Basella alba* leaves are mixed in a remedy given, in western Cameroon, to the new king for some weeks during initiation period to sustain his sexual performance. This plant extracts enhanced blood testosterone level in male rats. They were further studied for androgenic activity since they could be used to alleviate male infertility or to treat androgen deficiency in ageing male. Using rat testis slices, methanol extract of both plants significantly induced testosterone synthesis. In primary Leydig cell cultures of bull and rat, extracts from *B. alba* significantly enhanced testosterone levels in a dose dependant manner. *H. macranthus* showed no androgenic activity. Phytochemical screening and HPLC study of *B. alba* extracts revealed presence of terpenoids, limonoides and coumarins. This extract also modulated aromatase gene activity in Leydig cell cultures. In vivo, methanol extract (1 mg/kg) given to adult male rats significantly increased serum testosterone level after 30 days ($p < 0.0005$). This dose was found to be efficient whereas higher dosages of extract were ineffective. No sign of toxicity was detected in liver and kidney during acute or chronic studies.

Acknowledgment: IFS, AUF, Universities of Giessen and Caen (FRG, France)

ANTI-PROLIFERATIVE ACTIVITY OF *COMMELINA BENGHALESIS* A MEDICINAL PLANT AGAINST WIL-2 NS LYMPHOCYTES

Mokgotho M. P., Lebogo K. W., Mbazima V. G., Masoko P., Eloff J. N. and Mampuru L. J.

School of molecular and life sciences, Biochemistry and microbiology and biotechnology department, University of Limpopo, P/bag x1106, Sovenga, 0727, Limpopo Province, South Africa

There are as yet no extremely effective drugs to treat most cancers and many cancer treatments are very expensive. Cancer prevention and treatment using traditional remedies have attracted increasing interest. This study characterizes antiproliferation activity of indigenous traditional medicinal plant which was used for many years to treat skin ulcers and skin lumps in Northern parts of Limpopo Province, RSA. The plant was exhaustively extracted with acetone at room temperature for 10 hours and the resulting crude was sub-fractionated with n-hexane to obtain fraction 1 (F1) and with dichloromethane to obtain fraction 2 (F2). We observed here that both fractions (F1 and F2) inhibited the growth of Wil-2 NS cancer cells. Treatment of Wil-2 NS cells with these fractions (0 – 200µg/ml) resulted in dose- and time-dependent inhibition of cell proliferation. The inhibition of proliferation was associated with apoptosis as determined by Acridine-orange and ethidium bromide dual stain (AES dual stain) and pictures from light microscope. Their morphology related to apoptosis was characterized by cell shrinkage and chromatin condensation. The obtained data suggest that fractions of indigenous medicinal plant have anti-proliferation activity on this cancer cells. A further experiment on whether it is through apoptosis or other factors that fractions exert its direct antiproliferation effects requires further investigations.

ANTIMICROBIAL ACTIVITY OF TRADITIONALLY USED INDIGENOUS MEDICINAL PLANT FROM LIMPOPO PROVINCE**Mokgotho M., Masoko P., Mbazima V., Eloff J.N., Howard R. and Mampuru L.**

Biochemistry, Microbiology and Biotechnology Department, University of Limpopo, Sovenga, South Africa, Limpopo Province, 0727

Plant extracts have been known since antiquity to possess notable biological activity including antioxidant, antibacterial and antifungal properties. *Commelina benghalensis* L has been used traditionally to alleviate diseases and ailments of skin. The antioxidant and antibacterial activities of the plant extracts were evaluated. Four solvents (hexane, DCM, acetone and methanol) were used for preparation of extracts. TLC profiles for all the extracts did not show any compound having antioxidant activity against DPPH radical molecule. Bioautography revealed several compounds having antibacterial activity. The hexane extract had a relatively higher activity compared to other extracts. However, when quantifying the activity, DCM extract showed a better MIC values compared to other extracts.

THE ANTIULCER ACTIVITY OF THE ROOT EXTRACTS OF *TULBAGHIA VIOLACEA***Lerato Ntobaki, Nobuhle Makhanya, Andrew Opoku**

Department of Biochemistry & Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa 3886, RSA, aswe2gether@yahoo.com

A peptic ulcer is the erosion of a circumscribed area of tissue in the wall of the gastrointestinal tract that is accessible to gastric secretions. Although the initiating cause of ulcers is not clear, *Helicobacter pylori*, free radicals, inflammation, excessive stomach acid and pepsin emerge as likely factors. The antiulcer activity of *Tulbaghia violacea* (used by Zulu traditional healers in treating and managing peptic ulcers and other stomach ailments) was investigated. Methanolic extracts (5mg/100ml) of the plant showed little free radical scavenging activity against DPPH (29%) and ABTS (20%) and exhibited a low reducing power. However, the extract significantly inhibited the activities of lipooxygenase, xanthine oxidase, and other lipid peroxidative reactions. Plant extracts exhibited antimicrobial activities against *Staphylococcus aureus*, *Proteus mirabilis* (MIC value < 1.0 mg/ml); *Streptococcus faecalis*, *Enterobacter cloacae*, *Escherichia coli*, *Proteas vulgaris*, and *Helicobacter pylori* (MIC value = 2.0 mg/ml). The extract was able to protect ($p < 0.01$) the stomach lining against indomethacin-induced ulceration. We conclude that *Tulbaghia violacea* root extract has little hydrogen atom donor potential and may not break free radical chain reactions but could act as an anti acid. Its potential in the inhibition of free radical generation, along with its antimicrobial activity justify its use by traditional healers for the treatment of peptic ulcers.

THAI INDIGENOUS MEDICINE TREATMENT FOR KNEE PAIN

D.Onchomchant , P. Pimpisut and P. Deenoi Mae Fah Luang

University, Thasud, Muang district, Chiangrai Province, Thailand, 57110.

A survey of current Thai indigenous medicine treatment for knee pain was carried out in four regions of Thailand, by questionnaires and interviews. The causes of knee pain were classified into two categories: a) physical (injury, degenerative, inflammatory), and b) behavioral (obesity, occupational, and inactivity). The methods used by traditional healers to treat knee pain consisted of 1) aromatic herbal medicine (sesame oil, coconut oil), 2) compounded herbal medicine, 3) traditional Thai massage and/or point-pressure using natural products mainly animal teeth, 4) hot compression, 5) red lime plastering. Most practitioners also performed ritualistic or faith healing. The results of this survey confirm that Thai people continue to use traditional methods to treat knee pain problems. Further studies of the effectiveness and limitations of these methods are warranted so that the application of Thai traditional medicine and local wisdom with the best outcomes may be promoted as an alternative treatment for knee pain. Acknowledgements: Mae Fah Luang University and folk healers in four regions of Thailand.

MEDICINAL PLANTS USED AGAINST VARIOUS AILMENTS BY THE PEOPLE OF "CHAR" AREAS IN RANGPUR DISTRICT, BANGLADESH

Md. Harun-or- Rashid, Rabiea Tanzin, Rownak Jahan, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
masum15bd@yahoo.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
tanzin15bd@yahoo.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
rownak52@hotmail.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
rahamatm@hotmail.com

The "Char" area people live on islands formed in a river. These people have a somewhat different lifestyle and customs apart from the mainstream population. They rely mostly on herbal medicines administered by local traditional medicinal practitioners for cure of their various ailments. The objective of the present study was to conduct an ethno-medicinal survey amongst the traditional medicinal practitioners of the "Char" area people residing on various river islands of the river Jamuna in Rangpur district, Bangladesh. Interviews were conducted using a semi-structured questionnaire. Plant specimens were photographed as well as collected and sent to the Bangladesh National Herbarium for proper identification. A total of 48 plants distributed into 34 families were reported to be used by the traditional medicinal practitioners for cure of diverse ailments. Amongst the families, three plants each belonged to the Amaranthaceae and Solanaceae families, while two plants each belonged to the Acanthaceae, Apocynaceae, Asteraceae, Labiatae, Liliaceae, Lythraceae, Piperaceae, Umbelliferae, Verbenaceae, and Zingiberaceae families. Other families represented by one plant each included Agavaceae, Alliaceae, Amaryllidaceae, Aristolochiaceae, Asclepiadaceae, Bombacaceae, Boraginaceae, Chenopodiaceae, Combretaceae, Euphorbiaceae, Gentianaceae, Leguminosae, Meliaceae, Menispermaceae, Moraceae, Myrtaceae, Papilionaceae, Plumbaginaceae, Poaceae, Ranunculaceae, Rutaceae, and Sterculiaceae families. Cumulatively, the plants were used to treat diverse ailments like rabies, chicken pox, measles, pneumonia, jaundice, blood dysentery, leucorrhoea, hematuria, infertility, cataract, bone fracture, leprosy, skin disorders, debility, helminthiasis, toothache, vitiligo, arthritis, and renal inflammation. The plants can form a useful source of discovery of novel pharmacological constituents for treatment of diverse ailments.

STANDARDISATION OF A STEM BARK EXTRACT OF NAUCLEA POBEGUINII AND ITS *IN VIVO* ANTIPLASMODIAL ACTIVITY

Luc Pieters

University of Antwerp, Belgium, luc.pieters@ua.ac.be

Nauclea pobeguini (Rubiaceae) is widely used in African traditional medicine against malaria-like symptoms. Alkaloids, such as the major compound strictosamide are assumed to be responsible for the activity. An HPLC method was developed and validated for the quantification of strictosamide in an 80% EtOH extract of the stem bark of *N. pobeguini*. The method was validated according to the ICH guidelines. The response of ajmalicine.HCl, used as a secondary standard, was linear in a concentration range from 4.2 to 21.2 µg/mL. The accuracy of the method was validated by means of a recovery experiment (mean recovery of 92.2% (RSD of 9.4%)). The method was shown to be precise with respect to time (RSD of 2.2%, 3 days, n = 6) and concentration (RSD of 2.6%, 3 levels, n = 6). A crude ethanolic extract of the bark, containing 5.6% (w/w) strictosamide, was evaluated *in vivo* in the *Plasmodium berghei* mouse model in a suppressive treatment regimen. The test substance was formulated in PEG400 and orally dosed (PO) at 300 mg/kg twice daily for 5 days. One group received the treatment intraperitoneally (IP) using the same dosing regimen. Chloroquine (10 mg/kg) was used as positive control. Treatment with the crude extract, administered either orally or intraperitoneally,

resulted in moderate depression of parasitaemia during administration period, followed by a quick and full relapse (mean survival time = about 13 days). At termination of the experiment at day 21, a single survivor in the PO group was apparently cured (no parasitaemia), the single survivor in the IP group showed high parasitaemia and was in a moribund state. It can be concluded that the crude extract of *N. pobeguinii* has slight antimalarial potential when administered orally in a suppressive dosing regimen of 2x 5 days at 300 mg/kg. Its action is likely to be static since full relapse occurs quickly after ending the daily dosing.

A SURVEY OF MEDICINAL PLANTS USED BY KAVIRAJES IN THE RAUMARI AREA, KURIGRAM DISTRICT, BANGLADESH

Md. Habibur Rahman, Md. Nasir Uddin, Abdullah Al Farabi, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
habib_mmb@yahoo.com

Raumari area falls within Kurigram district in the north of Bangladesh. There are several villages within the area known as Dantbhanga, Kauniar Char, Batergram, Dhormopur, Horindhora and Shaulmari. The rural populations of these villages lack access to modern medical facilities and have traditionally relied on traditional medicinal practitioners known as Kavirajes for cure of various ailments. The area, being comparatively sparsely populated, is also known for its diversity of medicinal plant species. We conducted an ethnobotanical survey amongst the Kavirajes to gather information on the medicinal plants they use for treatment. Interviews were conducted using a semi-structured questionnaire. The information collected included plant, disease or symptoms for which they are used, medicinal formulations, dosage and side-effects. Each plant as pointed out by the Kaviraj was photographed and specimens sent to the Bangladesh National Herbarium for identification. A total of 27 plants belonging to 22 families were identified as to their frequently being used by most Kavirajes. The plants or plant parts are used to treat coughs, fever, asthma, bronchitis, allergy, body ache, weakness, jaundice, dysentery, diarrhea, acidity, skin disorders, heart disorders, impotency, syphilis, bleeding from nostril, headache, tuberculosis, paralysis, diabetes, coagulation of blood in eyes, elephantiasis, cancer, bone fracture and inflammation of vaginal area. Taken together, the results present some interesting prospects of scientifically validating plant use for the treatment of fatal and chronic diseases like cancer, tuberculosis, paralysis and diabetes.

MEDICINAL PLANTS USED AGAINST VARIOUS AILMENTS BY THE PEOPLE OF "CHAR" AREAS IN SIRAJGONG DISTRICT, BANGLADESH

Md. Habibur Rahman, Khokon Chandra Ghosh, Md. Harun-or- Rashid, Mohammed Rahmatullah

University of Development Alternative, Bangladesh, habib_mmb@yahoo.com

The "Char" area people, live on river islands, have a somewhat different lifestyle and customs compared to the mainstream population. They rely mostly on herbal medicines administered by local traditional medicinal practitioners for cure of their various ailments. The objective of the present study was to conduct an ethno-medicinal survey amongst the traditional

medicinal practitioners of the "Char" area people residing on various river islands in Sirajgong district, Bangladesh. Interviews were conducted using a semi-structured questionnaire. Plant specimens were photographed as well as collected and sent to the Bangladesh National Herbarium for proper identification. A total of 57 plants distributed among 41 families were reported as to being used as remedy for various ailments. These ailments included dental diseases, sexual disorders, cracking of soles of feet, lack of calcium, arthritis, peptic ulcer, constipation, piles, body ache, insomnia, hair loss, hypertension, cardiovascular disorders, mental disorders, leucorrhea, menorrhagia, diabetes, hepatic disorders, jaundice, wounds, colds, fever, tonsillitis, tuberculosis, skin infections, helminthiasis, insect bites, epilepsy, chicken pox, debility, respiratory distress, herpes and asthma. Collectively, these diverse groups of plants can form basis for modern scientific research for identification of lead or novel compounds with diverse pharmacological activities.

ETHNOBOTANICAL SURVEY OF THE MURONG TRIBE INHABITING THE CHITTAGONG HILL TRACTS REGION OF BANGLADESH

Md. Muhit Rana, Md. Shahadat, Hossan Abu Hanif, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
muhit_rana@yahoo.com

The Murongs are a tribal community inhabiting the Chittagong Hill Tracts region of Bangladesh. They rely on their own traditional healers, who are experts in medicinal plants for cure of various ailments. An ethnobotanical survey was carried out amongst the Murong tribe to identify their medicinal plants. Interviews of traditional healers were conducted using a semi-structured questionnaire as to plant or plant parts used as well as medicinal preparation, diseases, dosage, and side-effects, if any. Plants identified by Murong healers were photographed and herbarium specimens were sent to Bangladesh National Herbarium for complete identification. A total of 50 plants were collected out of which 45 plants belonged to 34 families and five plants remain unidentified. The various ailments claimed to be cured by plant or plant parts include body, joint and rheumatic pains, coughs, colds, tonsillitis, bronchitis, sore throat, fevers, vomiting, tooth problem, edema, abscesses, ear infections, wounds, poisonous insect bites, bone fractures, acidity, diarrhea, dysentery, diabetes, cholera, tetanus, intestinal worms, headache, conjunctivitis, urinary tract disorders, leucorrhea, ringworms, eczema, scabies, fungal infections, gonorrhoea, impotency, hepatic disorders, malaria, insanity, and diabetes. Our preliminary data suggest that a number of medicinal plants of the Murong are unique and needs to be scientifically studied for isolation of pharmacologically active constituents or lead compounds.

AN ETHNOBOTANICAL SURVEY OF MEDICINAL PLANTS USED BY THE CHAK TRIBAL COMMUNITY OF BANGLADESH

Md. Muhit Rana, Md. Shahadat Hossan, Abu Hanif, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
muhit_rana@yahoo.com

The Chaks are a small tribal community inhabiting the Chittagong Hill Tracts forest region in southeastern Bangladesh. They are sometimes confused with the Chakmas, who form the

largest tribal group in the region. Unlike the Chakmas, the Chaks have little association with settlers from outside their community. For curing of diseases, they rely on their own traditional medicinal practitioners, who serve as priests and experts on medicinal plants. Since little is known about the medicinal plants of this tribe, we conducted an ethnobotanical survey to learn more about their use of plants, which are quite diverse in the region. Interviews of Chak traditional healers were conducted in their own language and detailed information on usage of plant species to treat various ailments was noted. Plant specimens were photographed, collected and identified at the Bangladesh National Herbarium. A total of 47 plant species belonging to 31 families were collected. The major families included Leguminosae (five plants), Rubiaceae (four plants) and Asteraceae, Convolvulaceae and Labiatae families (three plants each). The diseases for which the various plants or plant parts were used included scabies, eczema, boils, abscess, ringworm, ear disorders, jaundice, stomach pain, intestinal worms, dysentery, bloating, acidity, constipation, throat ache, pneumonia, cough, mucus, tonsillitis, fevers and pains, urinary disorders like burning sensation during urination and frequent urination, as well as rheumatism, menstrual pain, vertigo, vomiting, toothache, wounds, bone fractures, impotency, tumor, cancer, snake, animal and insect bites, malaria, frequent thirsts, edema, kidney stones, allergy and elephantitis.

PLANT GENETIC RESOURCES AND THEIR USE IN TRADITIONAL MEDICINE OF BOSNIA AND HERZEGOVINA (W. BALKAN, SE EUROPE)

Sulejman Redzic

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina,
sredzic@pmf.unsa.ba

Plant genetic resources (PGR) have long been used in traditional phyto-therapy throughout the Balkan Peninsula [1, 2]. PGR use in human phyto-therapy originated from Asia, Northern Africa, Southern and Northern America. They spread to this region through different dynamic processes of anthrop-genesis and ethno-genesis of nations in this area. Previous research showed specific models of use of certain plants [2]. Ethno – botanical interviews of 145 persons (between 45 – 87 years of age) in more than 10 locations in Bosnia and Herzegovina revealed that 145 of cultivated species are used for different purposes in traditional phyto-therapy. Of these, 55 species are used in the Mediterranean and sub-Mediterranean region while only 19 species are used in the mountainous region (>1,500 m above sea). The majority of the species belong to the following families: Brassicaceae, Poaceae, Rosaceae, Fabaceae, Chenopodiaceae and Asteraceae. All plant parts (spines, leaves, fruits, flowers, seeds, rhizomes) are used in the form of infusions (35%), decoctions (25%), “mazzerati” (15%), tinctures (20%), oils, lotions and others (5%). These preparations are used in the treatment of a wide range of mostly chronic diseases such as stomach problems (30%), respiratory diseases (32%), bones and muscle diseases (18%), heart diseases, diabetes, as tranquilizers, treatment of snake and dog bites. The most widely used species are *Abutilon theophrasti*, *Tropaeolum majus*, *Balsamita major*, *Ruta graveolens* and *Avena sativa*.

References: 1. Redzic SS (2007) Coll. Antropol. 31: 869-890

2. Redzic S (2007) Planta Med. 73: 1013. 3. Redzic SJ (2006) Ecol. Food & Nutr. 45: 189-232.

AN ETHNOBOTANICAL SURVEY OF SHAPAHAR AREA, NAOGAON DISTRICT,

BANGLADESH

Md. Hasan Reza, Mohammad Ali, Md. Ariful, Haque Mollik, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
hrmmb1st@yahoo.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh,
alimmb375@gmail.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh
Faculty of Life Sciences, University of Development Alternat, Bangladesh,
rahamatm@hotmail.com

The primarily rural population of Bangladesh relies on traditional medicinal practitioners (Kavirajes) for treatment of their various ailments. Although in general the Kavirajes practice the Ayurvedic system of medicine, each Kaviraj nevertheless has his own special knowledge of indigenous medicinal plants, which they use to treat patients. As such, the use of medicinal plants may vary between different areas and different Kavirajes. We conducted an ethnobotanical survey of medicinal plants used by Kavirajes of Shapahar area at Naogaon district, Bangladesh. A total of 52 plant species were collected, which were used to treat common ailments like diarrhea, colds, coughs, fevers, wounds and helminthic infections as well as a number of other diseases like leprosy, tuberculosis, jaundice, spleen enlargement, rheumatoid arthritis, anemia, alopecia, sexually transmitted diseases, anorexia and hepatitis. These plant species included *Aloe vera*, *Achyranthes aspera*, *Spondias dulcis*, *Alstonia scholaris*, *Catharanthus roseus*, *Hemidesmus indicus*, *Holarrhena antidysenterica*, *Rauwolfia serpentina*, *Alocasia macrorrhizos*, *Areca catechu*, *Cocos nucifera*, *Carica papaya*, *Mesua ferrea*, *Terminalia arjuna*, *Terminalia chebula*, *Coccinia cordifolia*, *Dillenia indica*, *Phyllanthus emblica*, *Acacia catechu*, *Butea monosperma*, *Caesalpinia pulcherrima*, *Mimosa pudica*, *Saraca indica*, *Sesbania grandiflora*, *Tamarindus indica*, *Leonurus sibiricus*, *Mentha spicata*, *Ocimum gratissimum*, *Cinnamomum iners*, *Cinnamomum tamala*, *Cinnamomum verum*, *Asparagus racemosus*, *Lawsonia inermis*, *Punica granatum*, *Hibiscus rosa sinensis*, *Artocarpus heterophyllus*, *Ficus religiosa*, *Moringa oleifera*, *Averrhoa carambola*, *Piper nigrum*, *Cynodon dactylon*, *Zizyphus mauritiana*, *Anthocephalus chinensis*, *Paederia foetida*, *Aegle marmelos*, *Mimusops elengi*, *Smilax zeylanica*, *Datura metel*, *Withania somnifera*, *Aquilaria agallocha*, *Nyctanthes arbor tristis*, and *Curcuma zedoaria*

PHARMACOLOGICAL EVALUATION OF *SPHAERANTHUS INDICUS* LINN. USING ALBINO RATS.**Jeyachandran Robert, Cindrella Louis, Mahesh Ayyavoo**

St. Joseph's College, Tiruchirappalli, India, jeyachandranrobert@gmail.com
National Institute of Technology, Tiruchirappalli, India

Sphaeranthus indicus Linn. is a plant of the Compositae family, which has anti-inflammatory properties. Inflammation due to the infiltration of macrophages and neutrophils is a key event in the mediation of the toxic responses elicited by the notorious hepatotoxicant. Flavonoids and polyphenols isolated from *Sphaeranthus indicus*, owing to their antioxidant, tissue regenerating and free-radical scavenging properties, can cure the oxidative damage

caused by the activation of the drug and its toxic metabolites. The hepatoprotective effect of the bioactive principles isolated from *Sphaeranthus indicus* was evaluated in rats by using serum and tissue markers that signified acetaminophen hepatotoxicity. Serum parameters were evaluated in acetaminophen intoxicated rats to check for liver-specific markers such as AST, ALT, ALP and they were found to be elevated in the serum signifying liver damage. Treatment with the active principles of *Sphaeranthus indicus* significantly lowered these markers and showed regeneration of liver tissue, as revealed by histopathological studies along with the restoration of the activities of the antioxidant enzymes such as glutathione peroxidase (GPx), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase. Enhanced serum bilirubin and diminished protein levels were observed in the intoxicated group, whereas the reverse trend was observed in the treated groups. In addition, as acetaminophen is capable of causing kidney damage by mechanisms identical to those of the liver, the conditions of the kidney was also monitored by analyzing serum urea, uric acid and creatinine levels. The results also highlighted the nephroprotective role of the active principles of *Sphaeranthus indicus*.

THE ANTIMALARIAL AND TOXICITY STUDIES OF SWAZI MEDICINAL PLANTS

Gugu F. Sibandze, Robyn L. van Zyl

Department of Pharmacy and Pharmacology, University of the Witwatersrand, South Africa,
sguguster@gmail.com

Malaria, caused by *Plasmodium falciparum*, is a major cause of morbidity and mortality in sub-Saharan Africa. Many of the standard antimalarial drugs have become ineffective due to the parasite developing resistance against them. Traditional medicine is a source of primary healthcare in most societies in Africa, with about 80 % of the people consulting traditional medical practitioners for healthcare. Traditional healers use various medicinal plants to treat malaria according to its "flu-like" symptoms and fever. In consultation with Swazi traditional healers, fifteen Swazi plants were selected and screened for their antimalarial activity and toxicity profiles. The plants were collected from the Manzini region in Swaziland, air-dried and extracted with dichloromethane: methanol (1:1). Antimalarial activity against *P. falciparum* was determined using the tritiated hypoxanthine incorporation assay. Toxicity profiles of the extracts were tested on human red blood cells and kidney epithelial cells. Of the 33 extracts tested, 5 displayed *in vitro* antimalarial activity with IC₅₀ values less than 20 µg/ml, namely *Terminalia phanerophlebia* (leaves), *Berkheya setifera* (stem/root), *Priva meyeri* (whole plant), *Trichilia emetica* (leaves) and *Breonadia salicina* (bark). The five most active extracts did not cause red blood cell haemolysis at concentrations ten times greater than the IC₅₀. Similarly, *T. phanerophlebia*, *B. setifera* and *B. salicina* were not toxic to the kidney epithelial cells. However, *P. meyeri* and *T. emetica* displayed toxicity (IC₅₀ = 14.633 ± 1.616; 45.945 ± 5.104 µg/ml), respectively. The study supports the traditional use of some of the plants in the treatment of the "flu-like" and fever symptoms of malaria

ANTI-HYPERTENSIVE AND CARDIOVASCULAR EFFECTS OF *TAPINANTHUS DODONEIFOLIUS* (DC. DANSER) (LORANTHACEAE) IN RATS.

Ouedraogo Sylvain, Belemnaba Lazare, Bucher Bernard, Guissou I. Pierre

Health Sciences Research Institute, Burkina Faso, osylvin@yahoo.fr
 Laboratoire de Pharmacologie et Physico-chimie des Interacti, France

The hypotensive and anti-hypertensive effects of aqueous extract from *Tapinanthus dodoneifolius* (AETD) were examined in rats. Intravenous administrations of the plant extract (AETD 10-4 to 310-1 µg/kg iv) caused transient, dose-dependent and significant ($p < 0.05$) reductions in basal arterial pressure of anaesthetised normotensive rats. Further more AETD (310-1 µg/kg) completely abolished adrenalin or phenylephrine evoked-hypertension on rats. The cardiovascular effects were then investigated. AETD did not affect heart rate, but significantly enhanced heart contraction force and relaxation capacity. Cumulative additions of *T. dodoneifolius* aqueous extract (AETD 0.001- 1mg/ml) to the bath fluid induced concentration-dependent relaxation of endothelium-containing normotensive Wistar rat isolated aortic rings pre-contracted with phenylephrine (10-6M). The vasorelaxant effect of AETD on endothelium-containing isolated aortic rings was annulled by removal of the functional endothelium. When Indomethacin failed to inhibit AETD vasodilatory activity, pretreatment of the endothelium-containing vessels with NG-nitro-*L*-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase (NOS) completely abolished this vasodilatory effect. The findings of this experimental animal study suggest that *T. dodoneifolius* aqueous extract possess anti-hypertensive properties; this effect of the plant extract is likely to be mediated, at least in part, through vasorelaxant effect, through nitric oxide synthase activation and subsequent nitric oxide release.

ETHNOMEDICINAL SURVEY OF NOAGAON DISTRICT, BANGLADESH

Farida Yesmin, Md Shahidullah, S.M. Nasir Uddin, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternat, Bangladesh,

korniya830@yahoo.com

Faculty of Life Sciences, University of Development Alternat, Bangladesh

Faculty of Life Sciences, University of Development Alternat, Bangladesh

Faculty of Life Sciences, University of Development Alternat, Bangladesh

Noagaon district falls within the greater Rajshahi Division in the north-western part of Bangladesh. We conducted an ethnomedicinal survey amongst the Kavirajes (traditional medicinal practitioners) of the district to gather information on medicinal plants to treat various ailments. Information on 21 plants distributed into 17 families was collected. The various medicinal plant species (with family name in parenthesis) included *Barleria* sp. (Acanthaceae), *Crinum* sp. (Amaryllidaceae), *Annona squamosa* (Annonaceae), *Holarrhena antidysenterica* (Apocynaceae), *Bombax ceiba* (Bombacaceae), *Canna indica* (Cannaceae), *Wedelia chinensis* (Compositae), *Ipomoea mauritiana* (Convolvulaceae), *Cycas* sp. (Cycadaceae), *Ephedra* sp. (Ephedraceae), *Adenanthera pavonina* (Leguminosae), *Cajanus cajan* (Leguminosae), *Mucuna pruriens* (Leguminosae), *Urena lobata* (Malvaceae), *Cocculus hirsutus* (Menispermaceae), *Stephania japonica* (Menispermaceae), *Piper longum* (Piperaceae), *Glycosmis pentaphylla* (Rutaceae), *Murraya koenigii* (Rutaceae), *Tectona grandis* (Verbenaceae), and *Curcuma zedoaria* (Zingiberaceae). The various ailments treated by whole plant or plant parts were food poisoning, flatulency, dysentery, indigestion, infertility, pain, paralysis, snake bite, debility, bronchitis, jaundice, anemia, skin infections, spermatorrhea, abscess, skin infections, reduced breast milk in lactating mothers, fever, diabetes, and helminthiasis.

**THEME 10
NUTRACEUTICALS****OPTIMIZATION AND BENCHMARKING OF THE ANTIOXIDANT ACTIVITY OF *ATHRIXIA PHYLIKOIDES* EXTRACTS****Dalene De Beer, Elizabeth Joubert**ARC Infruitec-Nietvoorbij, South Africa, dbeerd@arc.agric.zaARC Infruitec-Nietvoorbij, South Africa, joubertl@arc.agric.za

Athrixia phyllicoides (Asteraceae), commonly known as bush tea and used as herbal tea or medicinal drink, is currently being considered for commercialization. The opportunity also exists to exploit bush tea for the food additive and nutraceutical markets. Against this background, the antioxidant activity of bush tea aqueous dried extracts (DEs) was evaluated using several test systems and benchmarked against several commercial DEs from fermented and unfermented rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) teas. The major phenolic compounds in bush tea DEs were tentatively identified. Optimization of the extraction procedure, in terms of solvent, temperature and time, was performed to obtain an extract with high antioxidant activity and content of the major bush tea phenolic compounds. The antioxidant activity of bush tea DEs in free radical scavenging, iron reducing and lipid peroxidation assays was similar to that of commercial honeybush extracts. However, antioxidant activity of bush tea DEs was slightly less than that of commercial fermented rooibos DEs, but substantially lower than that of unfermented rooibos DEs. The major phenolic compounds in bush tea DEs are phenolic acids, especially caffeic acid derivatives. A unique compound, namely 5-hydroxy-6,7,8,3',4',5'-hexamethoxyflavon-3-ol, was also detected in some extracts. The optimized extraction procedure entails extraction with 50% ethanol at 50°C for 20 min using a 50:3 volume to mass ratio. Extraction optimization gave an extract with antioxidant potential similar to fermented commercial rooibos DEs and one of the unfermented rooibos DEs. Further work is needed to identify compounds responsible for antioxidant activity of bush tea DEs.

Acknowledgements: National Department of Agriculture, South Africa, provided funding

VARIATION IN MAJOR POLYPHENOLS OF ROOIBOS (*ASPALATHUS LINEARIS*) AND HONEYBUSH (*CYCLOPIA SUBTERNATA*)**Elizabeth Joubert, Dalene De Beer, Marena Manley**ARC Infruitec-Nietvoorbij, South Africa, joubertL@arc.agric.zaARC Infruitec-Nietvoorbij, South Africa, dbeerd@arc.agric.za, Department of Food Science, Stellenbosch University, South Africa, mman@sun.ac.za

Aspalathus linearis and *Cyclopia subternata*, fynbos plants endemic to South Africa, are commonly known as the respective herbal teas, rooibos and honeybush. Powdered extracts of both plants are also produced for the food and nutraceutical markets. *Cyclopia subternata* is one of the *Cyclopia* species currently of interest, and only one of two under commercial cultivation. The objective of the study was to investigate variation in some of the major polyphenols, and

bioactive compounds, i.e. aspalathin and rutin in rooibos and mangiferin and hesperidin in honeybush in randomly selected seedling plants. Water-acetonitrile extracts of the leaves of rooibos and the shoots (leaves and stems) of honeybush samples were analysed by DAD-HPLC. Authentic standards were used for quantification. Large variation in the dry matter content was obtained for the respective compounds. For both species a high concentration of a compound in a specific sample was not necessarily accompanied by a high concentration of the other compound (eg. aspalathin and rutin). The aspalathin to rutin ratio varied from 6 to 32 with 38% of the samples giving a ratio of 11 to 12 (average values - ratio 13; 8.25% aspalathin; 0.66% rutin). The mangiferin to hesperidin ratio varied from 0.3 to 4.5 with 46% of the samples giving a ratio of 0.6 to 1.0 (average values - ratio 1.4; 0.50% mangiferin; 0.44% hesperidin). The variation in content and ratio of compounds in extracts from seedlings will result in extracts differing in bioactivity. To overcome variation in composition and support standardization of nutraceutical extracts, plant improvement through breeding and selection will be required.

NUTRITIONAL VALUE OF *MORINGA OLEIFERA* LEAVES FROM SENEGAL

H. R. Juliani, Y. Fonseca, M. Diatta, Babou Diouf and J. E. Simon

New Use Agriculture and Natural Plant Products Program, Rutgers University. 59 Dudley Road.
New Brunswick, 08901 NJ.USA

Agri-Business in Sustainable African Natural Plant Products, ASNPP Dakar, Senegal

Moringa (*Moringa oleifera*, Moringaceae) is a tropical plant that grows in a wide range of environmental conditions, ranging from sub-tropical dry to moist, through very dry tropical conditions to moist forest zones. Traditionally, moringa leaves and young pods are used as vegetables to aid digestion and to stimulate appetite, to treat inflammatory swellings, externally as counter-irritants. In children is also used to treat fever, bronchitis and headache. The information on moringa is limited on the nutritional value of moringa grown and collected in many African countries. The objective of this research project was to assess the nutritional value of *M. oleifera* leaves grown and collected in Senegal (Massar, Niaye area). Percentage of moisture, ashes, cleanliness, total proteins, phenols, antioxidants and elemental analysis were performed on dry leaves. Moringa leaves showed low moisture content, lower percentage of total phenols (3-4%), high content of proteins (13-14%) and total minerals (11-13%). The leaves were also high in calcium (2.9-3%), potassium (1%) and iron (50-80mg/100g dry leaves). *M. oleifera* leaves can be a rich source of nutrients and a good nutritional supplemental to undernourished and malnourished populations particularly those vulnerable sectors such as children, pregnant and lactating women and the elderly. According to the elemental composition, total minerals, and antioxidant phenols Moringa can be an affordable avenue to improve health and nutrition in sub-Saharan countries. Given the ease in growing this tree, the plants adaptability to survive and grow on poor soils under harsh conditions, the introduction of this tree into family gardens, yards and communities would help provide an affordable nutritional food. Current work is now focused on the vitamin and specific polyphenol contents of the leaves.

ANTIOXIDANT AND SUPPRESSIVE EFFECTS ON THE SYNTHESIS OF *NITRIC OXIDE* AND *PROSTAGLANDIN E₂* BY THE ETHANOLIC-EXTRACTS OF *CARTHAMUS TINCTORIUS L.* FLOWER

Je-Hyuk Lee, Gun-Hee Kim

Plant Resources Research Institute, Duksung Women's Universi, Korea, leejeh@yahoo.com
Plant Resources Research Institute, Duksung Women's Universi, Korea, ghkim@duksung.ac.kr

Carthamus tinctorius L., belongs to Compositae family, has been used as a folk medicine for the potent tyrosine inhibitory activity and the clinical treatments of osteoporosis and rheumatism in Korea. In this study, we investigated the flower-extracts of *Carthamus tinctorius L.* (CFEs) for an antioxidant activity and the inhibitory influence on the nitrite production and prostaglandin E₂ synthesis, which are involved in the progress of the acute inflammation. The chloroform-extract (CFCE) and n-butanol-extract (CFBE) showed more than 70% scavenging activity of DPPH free radical (100 µg/mL). Additionally, the total ethanol extract (CHEE) had approximately 24% of DPPH radical scavenging activities, but hexane extract (CFHE) had no antioxidant activity. Cytotoxicity on RAW 264.7 macrophage cells was not shown in less than 50 or 250 µg/mL concentration of CFEs. CFEs had inhibited LPS-induced nitrite production in a dose-dependent manner. CFEE (250 µg/mL), CFCE (50 µg/mL), and CFHE (250 µg/mL) had suppressed approximately 70, 64, and more than 98% of LPS-induced nitrite production effectively in RAW 264.7 macrophage cells, respectively. In addition, CFEs did not show the chemical nitric oxide (NO) quenching activity in the cell-free system, but markedly inhibited the inducible nitric oxide synthase (iNOS) mRNA transcription in LPS-stimulated RAW 264.7 cells. In addition, the pretreatment with CFEs attenuated prostaglandin E₂ synthesis and cyclooxygenase-2 (COX-2) gene transcription in LPS-stimulated RAW 264.7 cells. These results suggest that CFEs may exert the potential anti-inflammatory activity through the suppression of inhibitory mediators (NO and PGE₂), caused by inhibition of iNOS and COX-2 gene transcription in RAW 264.7 cells.

EXTRACTION, POTENTIATION AND QUANTITATIVE DETERMINATION OF RESVERATROL FROM SOUTH AFRICAN RED 'CONCORD' GRAPE PUMICE USING ASSAY-GUIDED METHODS.

Chima Igwe, J.N. Eloff, R. van Brummelen

University of Pretoria, South Africa, s27616071@tuks.co.za
University of Pretoria, South Africa, kobus.eloff@up.ac.za, Biomox Pharmaceuticals, South Africa

Resveratrol (trans-3, 5, 4'-trihydroxystilbene) was extracted, potentiated and quantitatively determined from fresh frozen red 'concord' grape pumice using assay-guided methods of column chromatography (CC), thin layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC). Fresh frozen samples of red 'concord' grape pumice were milled in the frozen state and subjected to acid hydrolysis for 30 minutes at 60 °C and resveratrol extracted with 96% absolute ethanol. The extract (10 mg/mL) was filtered and concentrated under vacuum at 40 °C. Samples of the concentrated extract were loaded in two different columns with Diaion HP-20 and Dowex-SD-2 adsorbent resins. Five fractions each were collected using 100% ethanol, ethanol: acetone mixtures (70:30, 60:40, 50:50) and 100% acetone. Each of the fractions was loaded on aluminum back silica gel TLC plates, developed in an Ethylacetate: Methanol: Water chromatographic tank and spotted. The TLC spots showed that the fractions Ethanol: acetone (50:50) and 100% acetone in the Dowex-SD-2 column contained resveratrol and 100% acetone in the column with Diaion HP-20 also contained

resveratrol. These fractions with resveratrol were then analysed using HPLC and the result showed that ethanol: acetone (50:50) fraction contained 0.0222 mg/mL resveratrol, 100% acetone fraction contained 0.465mg/mL resveratrol, for Dowex SD-2 column and for the Diaion HP-20 column, 100% acetone fraction contained 0.221mg/mL resveratrol. The results of the studies were better than the reports of yields quoted in literature.

EVALUATION OF THE METHODS OF PRODUCTION OF SWEETENERS FROM *THAUMATOCOCUS DANIELLI* AND *SYNSEPALUM DULCIFICUM* OF NIGERIAN ORIGIN

Chima Igwe, Aroke Ahmed, Oluwole Olatunji, J.N. Eloff

Federal Institute of Industrial Research, Oshodi, Nigeria, s27616071@tuks.co.za

University of Pretoria, South Africa

Federal Institute of Industrial Research, Oshodi, Nigeria

University of Pretoria, South Africa, kobus.eloff@up.ac.za

Studies were carried out on the most appropriate method(s) and process(es) for the extraction/development and production of sweeteners from local plant materials, namely: *Thaumatococcus danielli* (Thaumatocin) and *Synsepalum dulcificum* (Miraculin) from Nigeria. Investigations were also conducted on the physicochemical characteristics/properties of the sweetener extracts. Almost all the commercially available sweeteners for industrial and domestic use in Nigeria are imported. These commercial sweeteners (sugar, saccharin, acesulfame K, cyclamates, aspartame, etc) aside from non-availability and high cost have their attendant negative health implications. *T. danielli* and *S. dulcificum* are known tropical plants whose fruits contain natural sweetening agents with no characteristic flavour, no proven toxicity to the consumers and used traditionally in most West African countries to sweeten local foods. Aqueous extracts of the fruits of these plants were made and freeze-dried using 3 different methods. The physicochemical properties, namely: visual colour; pH; %Brix; Solubility (at 20 °C); sweetness (10% sucrose); % yield; heat stability; detectable flavour; aftertaste; total solids (%), % crude protein (% NX6:25); % reducing sugars (as fructose); % sucrose; crude fibre; potassium (mg/L); total cyanide (ppm); tannin (qualitative) ninhydrin test; microbial load; Aluminum and Lead were evaluated. The results were creamy; 5.8; 18.0; readily soluble (in water); 3,000; 98; stable up to 600 °C; bland; lingering sweetness; 12.2; 11.1; 0.57; 0.08; 306.3; Nil; positive; bluish colour; less than 1000 cfu/g, *E.coli*: negative in 1g; Not detected and Not detected respectively for *T.danielli*. For *S.dulcificum*, the results were: pale brownish; 3.4; 14.0; readily soluble (in water), 1,500, 90, stable up to 900 °C, bland, non- lingering sweetness; 19.4; 8.7; 10.0; Nil; 0.04; 1161.5; Nil; negative; bluish colour; 780 cfu/g, *E.coli*: negative in 1g; not detected and not detected respectively. The results show that the sweeteners produced from *T.danielli* and *S. dulcificum* using these methods were within specified standards.

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF EDIBLE WEEDS

S. Kumar and D. Kumar

Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, Haryana, India

Chenopodium album and *Vitis trifolia* are widely growing weeds in India. These are nutritious and eaten as food or vegetable. Preliminary phytochemical screening of these plants showed the presence of high amount of phenolics and flavonoids. Subsequent quantification

showed the presence of 0.94% w/w phenolics (calculated as gallic acid) and 0.27 % w/w flavonoids calculated as catechin equivalents per 100 g of fresh mass of *C. album* leaf extract whereas *V. trifolia* was found to contain 1.23 % of gallic acid equivalents of phenolic compounds and total flavonoid content was 0.38 % of catechin equivalent per 100 g of fresh mass. The presence of phenolic compounds prompted us to evaluate its antioxidant activity. Both plants were screened for antioxidant potential by using various *in vitro* methods at different concentration (250–2500 µg/ml). Aqueous leaf extract of *C. album* showed free radical scavenging effect on the DPPH, superoxide anion, hydroxyl radicals, nitric oxide production and hydrogen peroxide radicals as 86.42±2.8, 75.24±3.8, 78.54±3.7, 74.38±4.2 and 74.23±3.5 % respectively at a concentration of 2500 µg/ml. Methanolic fruit extract of *V. trifolia* exhibited significant reducing power and free radical scavenging effect on DPPH, superoxide anion, hydroxyl radicals, nitric oxide production and hydrogen peroxide radicals as 89.15±3.7, 79.71±3.4, 81.27±3.2, 76.83±2.7 and 80.37±4.2% respectively. All these antioxidant activities were concentration dependent which were compared with standard antioxidants such as BHA, α-tocopherol and ascorbic acid.

CONTENTS OF PLANT PIGMENTS IN MEDICINAL PLANTS OF FAMILY LAMIACEAE IN W. BALKANS AS AN INDICATOR OF POTENTIAL ANTIOXIDANT ACTIVITY

Sulejman Redzic, Nezafeta Sejdic, Sasa Pilipovic, Anisa Palic

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina,

sredzic@pmf.unsa.ba

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina, ,
2Institute for Quality Control of Medicines, Sarajevo, 9 Tito, Bosnia and Herzegovina, , Dep. of
Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina

Plant pigments (chlorophyll and carotenoids) are a very important group of primary metabolites. Besides their role in the process of photosynthesis and plant protection from extensive radiation, they have application in pharmaceutical industry, cosmetology and dietetic. Plant pigments also play a significant role in anti-oxidant activity. The aim of this study was the qualitative/quantitative analysis of primary and secondary pigments in selected medicinal species of wild flora in BiH, including endemic species. Plant material was collected during different seasons. It was transported fresh to laboratories where qualitative (paper and thin layer chromatography) and quantitative (spectrophotometric) analysis was carried out. Results showed significant presence of chlorophyll a, chlorophyll b and carotenoids. Ratio between chlorophyll a and chlorophyll b is rarely 3:1, as stated in classical literature but rather close to 3:2 and more, which makes these species even more medicinal and gives them higher potential anti-oxidant capacity.

DNA AND ANTIMICROBIAL FINGERPRINTING OF MEDICINAL ALOE SPECIES FROM THE MASCARENE ISLANDS

J.Govinden-Soulange, S. Khojraty, M. Ranghoo-Sanmukhiya, N. Makunga, H. Kodja

Department of Agricultural and Food Sciences, University of Mauritius, Reduit, Mauritius

Institute of Plant Biotechnology, University of Stellenbosch, Private Bag X1, Stellenbosch 7602,
Republic of South Africa
Department of Plant Physiology; Faculty of Science, Université de la Reunion, Reunion Island

Antimicrobial and molecular studies of three endemic medicinal plants from Mauritius *L. tomentorii* and *L. purpureum* and *Lomatophyllum macrum* from Reunion Island was carried out to differentiate them from *Aloe vera* which belongs to the same family Asphodelaceae. These endemic Aloes are used in the Mauritian and Reunion pharmacopoeia to treat bacterial infections and as antispasmodic.³ Phytochemical screening of the crude extracts revealed the common presence of alkaloids, anthraquinones, coumarins, phenols, saponins and tannins. Anthraquinones and saponins had also been reported as bioactive compounds present in Alooideae and in *Aloe vera*^{1,4}. Antimicrobial activity was tested against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* using the microplate dilution method. Extracts of *Lomatophyllum* species showed more potent antimicrobial activity than *Aloe vera* with MIC ranging 0.003-0.006mg/L as compared to 0.012-0.028mg/L for *Aloe vera*. The antimicrobial properties of *L. tomentorii* and *L. purpureum* were attributed to the presence of alkaloids, coumarins, and saponins which are known to possess antimicrobial attributes. In this study we also used a modified RAPD-PCR technique. to elucidate the genetic closeness of *Lomatophyllum* to *Aloe*. Genomic DNA was extracted using a modification of the method used by Govinden-Soulange *et al* (2007)². RAPD-PCR technique has revealed that *Lomatophyllum* species and *Aloe vera* share some genetic similarities. Consequently we report the genetic diversity of the *Lomatophyllum* species by measuring the genetic distance, which they have with *Aloe vera*. Moreover some biologically active compounds within the *Lomatophyllum* were identified and their possible similarities with *Aloe vera* were unveiled.

Key Words: Biological activities, *Lomatophyllum*, Aloes, genetic distance

References:

5. Atherton, P. (1999). *Aloe vera*: myth or medicine? Positive Health, 20 June/July.
6. Govinden-Soulange J, Ranghoo-Sanmukhiya M, Seeburrun SD. (2007). Tissue culture and RAPD analysis of *Cinnamomum camphora* and *Cinnamomum verum*. Biotechnology. 6(2):239-244
7. Gurib-Fakim, A. Gueho, J., Sewraj, M. and Dulloo, E. (1994). Plantes Medicinales de l'île Rodrigues. Editions de l'Océan Indien, Maurice.
8. Mark, W.C., Anette, Y.D., Anthony, V.C., Gail, R., Paula, J.R. Margaret, A.T. and Lui, E.E. (2000). Phylogenetics of Asphodelaceae (Asparagales): Analysis of Plastid rbcL and trn-L-F DNA Sequences. Annals of Botany, 86: 935-951.

USE OF EDIBLE MUSHROOMS AND LICHENS IN ADDITIONAL NUTRITION OF PEOPLE IN WAR IN BOSNIA AND HERZEGOVINA (W. BALKAN)

Sulejman Redzic, Azra Biscevic, Amira Redzic

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina,
sredzic@pmf.unsa.ba

Faculty of Medicine of University Sarajevo, Bosnia and Herzegovina
Faculty of Medicine University of Sarajevo, Bosnia and Herzegovina, amira_redzic@yahoo.com

All wars, historical and those in modern times, emphasize human nutrition as key issue [1]. Usually, conventional food is insufficient and people are trying to survive by using additional sources in wild plants, animals and mushrooms. Same situation was during the war in Bosnia

and Herzegovina (1992-95). During several seasons in 2000-2006, researches were conducted on use of edible mushrooms and lichens in survival of people in isolated war areas in eastern Bosnia region, where war activities were extremely difficult and followed by killings of civilians, exodus and total blockages. 35 adults were interviewed, all of different sex and age, who spent war period in occupied zones. By using method of ethno-botanical interview and direct field work, determined were 15 species of mushrooms and 5 species of lichens that were used in human nutrition. Most used mushrooms were as follows: *Lactarius piperatus*, *Morchella conica*, *Agaricus campester*, *Boletus edulis*, *Boletus sp.*, *Cantharellus cibarius*, *Russula sp.*, *Tricholoma sp.* and others. Most used lichens were following species: *Evernia prunastri* and *Lobaria pulmonaria* for making mash and lichen flour. Mushrooms were grilled or boiled, and some species were even used raw. Noted were also mushroom poisonings. Even though mushrooms are not most common nutrition ingredient in these areas, people, including soldiers, were forced to use mushrooms and lichens in order to survive. This is a good example of the fact that hunger and fear of hunger and very strong factor in human survival [2, 3].

References: 1.Redzic, S.J. (2006). Ecology of Food and Nutrition, 45, 189-232. 2. Redzic, S. (1999). Pharmacia, 10, 28-41. 3. Redzic, S. (2006b). Proceedings of the 1st IFOAM International Conference on Organic Wild Production, Teslic, BiH, 2006, 117-141.

POTENTIAL USE OF VASCULAR ENDEMIC PLANTS AS A SOURCE OF NEW MEDICINES (WESTERN BALKAN, SE EUROPE)

S. Redzic

Dep.of Biology Fac. of Science University of Sarajevo, 33-35 Zmaja od Bosne, 71 000 Sarajevo,
Bosnia and Herzegovina

The biodiversity of W Balkan includes 7,000 vascular plants. In ethnobotany of this region people use approximately 960 plant species in traditional human and veterinarian phytotherapy and nutrition [1,2]. Except of more common plants there are a lot of endemic and relic vascular plant species as a potentials for the production of new medicines through advanced biotechnology and genetical engineering [3]. Western Balkan area are so rich in endemic species. In our investigation more than 579 plant species have been found on the Dinaric Mts. only [4].

Main aims of this presentation is to found of richness of medicinal wild flora and to determine 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 biotechnology.

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

Among plants that could be potentially significant in terms of the pharmacology and pharmacy it was detected 450 endemic species of Dinaric Mts. and other parts of W Balkan. The most significant new resources are contained within endemic genera: *Pinus* (*Pinaceae*), *Drypis*, *Dianthus*, *Minuartia*, *Saponaria*, *Silene* (*Caryophyllaceae*), *Ranunculus*, *Anemone*, *Pulsatilla*, *Aquilegia*, *Helleborus* (*Ranunculaceae*), *Berteroa*, *Aubretia*, *Malkolmia*, *Alyssum*, *Cardamine* (*Brassicaceae*), *Potentilla*, *Sibireja*, *Geum*, *Dryas* (*Rosaceae*), *Astragalus*, *Genista*, *Oxytropis*, *Anthyllis* (*Fabaceae*) *Athamanta*, *Eryngium*, *Pancicia*, *Peucedanum*, *Seseli*, *Bunium* (*Apiaceae*), *Acinos*, *Micromeria*, *Salvia*, *Satureja*, *Stachys*, *Teucrium*, *Thymus*, *Scutellaria* (*Labiatae*), *Euphrasia*, *Pedicularis*, *Scrophularia* (*Scrophulariaceae*), *Veronica*, *Plantago* (*Plantaginaceae*),

Achillea, *Amphoricarpos*, *Centaurea*, *Crepis*, *Leucathemum*, *Senecio*, *Doronicum* (Asteraceae), *Lilium*, *Chouardia*, *Allium* (Liliaceae) and others. Those plants are potential sources of new metabolites, such as alkaloids, heterozides, saponins, essential oils, tannins, carbohydrates, proteins as well as other secondary and primary metabolites.

References:

- 1.Redzic, S.(2006)Proc.1st IFOAM Intern. Conf. Organic Wild Production, 117-141.
2. Redzic, S.J. (2006)Ecol.Food & Nutr. 45, 3,189-232.
- 3.Redzic, S.(2008) Planta Medica 74(9):1143-1144.
4. Redzic, S. (2007) Planta Medica 73(9): 1013-1013.

USE OF WILD EDIBLE PLANTS IN HUMAN NUTRITION DURING WAR IN BOSNIA AND HERZEGOVINA (W.BALKAN)

Sulejman Redzic, Amira Redzic, Azra Biscevic

Dep. of Biology, Fac. of Sci. Univ. Sarajevo, 33-35 Zmaja od, Bosnia and Herzegovina,

sredzic@pmf.unsa.ba

Faculty of Medicine of University Sarajevo, Bosnia and Herzegovina, amira_redzic@yahoo.com

Faculty of Medicine University of Sarajevo, Bosnia and Herzegovina

During war, especially in territories under siege, one of the biggest problems in survival of people is hunger [1]. Same was in surrounded Sarajevo, in Bosnia and Herzegovina, where inhabitants were kept in total blockage for over 1,400 days continuously. People lacked in all main ingredients, especially in flour, vegetables and fruits. First author of this study lived through all dreadful war occurrences and at the same time worked as an instructor in human survival by using edible wild plants. Parts of those experiences are provided in this study. Beside already achieved results, researches were continued during 1999-2004. By using method of ethno – botanical interview about 135 people of different sex and age were interviewed, in addition to field work, and determined were 124 species of wild plants. Out of 135 species, 240 parts were used (leaves, fruits, over ground parts) in making about 215 different preparations (salads, beverages, vegetable, spices, teas). Most plants had edibility rating 3, then 4 and 5 with distinguished nutritive and caloric values. Most important are the following: *Taraxacum officinale*, *Plantago major*, *Urtica dioica*, *Malva sylvestris*, *Cichorium intybus*, *Arctium lappa*, *Tussilago farfara*, *Sedum telephium*, *Prunus spinosa*, *Cratagus monogyna* and others. During winter, used were fruits of decorative species *Chaenomeles japonica*, *Sempervivum tectorum* and *Sedum hispanica*. As war progressed and hunger had more serious consequences, interest of all people became bigger for everything edible. This study proves that hunger is one of the strongest cohesion factors that unify different groups of people not only based on social, but on ethnical and ideological affiliations [2,3].

References: 1.Redzic, S.J. (2006). Ecology of Food and Nutrition, 45, 189-232. 2. Redzic, S.J. (2006). Ecology of Food and Nutrition, 45, 189-232. 3. Redzic, S. (1999). Pharmacia, 10, 28-41. Need to be returned to authors for further editing and revision.

ANTIOXIDANT CAPACITY IN SOME TRADITIONAL UGANDAN DIETS

Torunn Stangeland

Norwegian University of Life Sciences, Norway, torunn.stangeland@umb.no

Several studies indicate that a plant-based diet protects against the development of both infectious and non-communicable diseases (NCD) like cancer, diabetes and cardiovascular diseases. It is assumed that the antioxidant content in food is an important reason for this protection. Dietary plants contain several hundred antioxidants, many of which are believed to act in a synergistic way to protect the body against oxidative stress. Sub-Saharan Africa is under a double burden of disease, with the classical malnutrition-related and infectious diseases coexisting with the NCD caused by western influenced diet and lifestyle. The objective of this work was to analyse antioxidant activity (AA) in fruits and vegetables from Uganda and investigate whether AA in traditional food is sufficiently high to prevent oxidative stress and thus combat diseases. We used the FRAP (ferric reducing ability of plasma) procedure. The results showed a great variation in AA ranging from 72.3 ± 13.5 (*Syzygium cuminii* seed) to 0.09 ± 0.05 (*Cucurbita maxima* fruit) mmol /100g fresh weight (FW). We estimate serving sizes and determine the total antioxidant capacity (TDAC) per day of three traditional Ugandan diets. The dietary plants with highest AA per serving size are pomegranate (*Punica granatum*), *Canarium schweinfurthii*, guava (*Psidium guajava*), mango (*Mangifera indica*) and tree tomato (*Cyphomandra betacea*) with values ranging from 8.91 to 3.00 mmol/serving. Of the traditional diets the central/eastern (C/E) and the western (W) diets had almost the same AA (9.31-9.78 and 9.75 mmol/day), while the northern (N) diet had an AA of 7.50-8.02 mmol/day.

THEME 11

VETERINARY MEDICINE

BIOSEPARATION AND ACTIVITY OF *KHAYA SENEGALENSIS* FRACTIONS AGAINST INFECTIVE LARVAE OF *HAEMONCHUS CONTORTUS*

Isaiah O. Ademola, B. O. Fagbemi, S. O. Idowu

Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Medicine,
University of Ibadan, Ibadan, Nigeria, ioademola@yahoo.com
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan,
Nigeria

The anthelmintic constituents of *Khaya senegalensis* (Deser.) A. Juss (Meliaceae) bark extract, previously demonstrated to show both in vitro and in vivo activity against gastrointestinal nematodes of sheep were investigated by chromatographic separation of the crude extract and bioassay of fractions. Bioseparation of the crude ethanol (95%) extract was carried out by gradient vacuum liquid chromatographic analysis and thin layer chromatography fingerprinting of eluates. The activities of the fractions were tested by a larval development assay in vitro. The survival rate of infective larvae L3 of sheep nematodes, predominantly *Haemonchus contortus*, was used to assess relative bioactivity. A secondary fraction obtained from further purification by preparative thin layer chromatography of a primary active fraction was also assessed for bioactivity. The compositions of the fractions were determined by qualitative chemical tests. The extracts killed infective larvae of *H. contortus* in a concentration-dependent manner. Best-fit LC50 values are 80.81, 63.73, 44.03 and 63.90 $\mu\text{g/ml}$ for fractions A, B, C, and D, respectively (95% CI). The fractions are composed of saponins (A), saponins and alkaloids (B) saponins, terpenoids, flavonoids, condensed tannins (C), and saponins and tannins (D). Fraction C showed the highest activity of all the fractions ($p < 0.0001$, 1-way ANOVA, Bartlett's test). The

secondary fraction, C1A obtained from fraction C gave best-fit LC50 value of 5.09 µg/ml (95% CI) and was identified to be condensed tannin. The condensed tannins in the bark of *K. senegalensis* are the most potent anthelmintic principles of the extract.

A MIXTURE OF PLANT EXTRACTS CAN BE MORE EFFECTIVE THAN A COMMERCIAL ANTIBACTERIAL FEED ADDITIVE IN POULTRY CHALLENGED WITH *CLOSTRIDIUM PERFRINGENS*

J. N. Eloff

Phytomedicine Programme, University of Pretoria, South Africa. kobus.eloff@up.ac.za

To reduce costs in the production of poultry many birds are kept in close proximity. This leads to infections that reduce productivity and antibiotics are given as prophylactics in the feed. This has led to the development of antimicrobial resistance with some evidence that the resistance may be transferred to human bacterial pathogens. The European Union has banned the use of antibiotic feed additives in animal production. One of the solutions to this problem is using plant extracts. Many plant extracts were tested for activity against poultry bacterial pathogens. The mechanism of activity of plant extracts may also be by stimulation of the immune system of the bird or by reducing inflammation due to the activity of gut microorganisms. Antioxidant activity of plant extracts may therefore play a role. An extract of *Combretum woodii* leaves had good activity against the poultry pathogens and excellent anti-oxidant activity. By simple manipulation of the extracts we could increase the antibacterial and anti-oxidant activity of the crude extract. When it was tested in a small poultry trial, it was relatively toxic to the poultry and in *in vivo* cellular assays. We then investigated the activity of extracts of several plant species that have been used as human herbal medicines against poultry pathogens. From this we selected two that had good complementary activity against different pathogens. We tested different combinations of these extracts and a grape seed extract with good antioxidant activity in a poultry trial. No significant differences were found between positive controls, negative controls and treatments. The good hygienic conditions under which the birds were kept may have led to decreased infection. Some plant extracts gave statistically significantly better results than Zn-Bacitracin, the commercially used antibiotic feed additive in birds challenged by infecting with *Clostridium perfringens* in several performance parameters. The results indicate the potential of using plant extracts to increase animal productivity.

Acknowledgement: This work is based on the theses of Theresa Ntloedibe, Havanah Chikoto and Ilse van Heerden. BioPAD an initiative of the Dept of Science and Technology provided funding.

IN VITRO ANTIVIRAL ACTIVITY OF PROANTHOCYANIDIN A2 AGAINST CANINE DISTEMPER VIRUS

Galligioni V., Dal Pozzo F., Fontana G., Bombardelli E., Baldelli E., Scagliarini A.

Department of Veterinary Public Health and Animal Pathology, University of Veterinary Medicine, Bologna, Italy, violagiabella@yahoo.it
Indena S.p.A., Milan, Italy

Canine distemper virus (CDV) is an enveloped RNA virus, causing a highly contagious disease of domestic and wild canids as well as of other terrestrial and aquatic carnivores. Although the vaccine, outbreaks are still reported worldwide and up to date no effective treatments exist. Proanthocyanidins are flavonoids having well known antioxidant, anti-inflammatory and antimicrobial properties (1,2). In this study the *in vitro* antiviral activity of Proanthocyanidin A2 against CDV and its effects on viral replication were evaluated. The cytotoxicity (CC50) of Proanthocyanidin A2 was tested on growing VERO cells by the MTT assay (3). Extra- and intracellular antiviral activities (IC50) were evaluated by cytopathic effect (CPE) inhibition assay in confluent cells monolayers. In order to investigate the mechanism of action of the compound against CDV, the Time of Addition assay was carried out adding at several times post-infection 150, 100 or 75 µg/ml of Proanthocyanidin A2, followed by virus titration. Proanthocyanidin A2 was not toxic on VERO cells (CC50>200µg/ml), showing only intracellular antiviral activity (IC50=89.81±4 µg/ml) with a selectivity index (the ratio of CC50 and IC50, SI) >2. Analysis by One way ANOVA test demonstrated that Proanthocyanidin A2 decreased the viral growth significantly (P<0.05) up to 16 hours post infection. Proanthocyanidin A2 was active against CDV with a SI greater than Ribavirin, the only compound with demonstrated anti-CDV activity, and it decreased the viral growth in a time- and concentration-dependent way.

References:

1- [Shahat AA](#), [Cos P](#). *et al.*, (2002) [Planta Med.](#) Jun;68(6):539-41; 2- Maldonado PD, Rivero-Cruz I, *et al.*, (2005) *J Agric Food Chem.* Mar 23;53(6):1996-2001; 3- Hussain RF, Nouri AM, Oliver RT. 1993 *J Immunol Methods.* 1993 Mar 15;160(1):89-96;

CINNAMOSMA MACROCARPA AND ITS FRACTIONS ARE ACTIVE *IN VITRO* AGAINST BETANODAVIRUS

Galligioni V., Vaccari F., Galletti E., Matera R., Fontana G., Ciulli S., Scagliarini A.

Department of Veterinary Public Health and Animal Pathology, University of Veterinary Medicine, Bologna, Italy, alessand.scagliarini@unibo.it
Research supervisor, INDENA S.p.A., Milano, Italy.

Betanodavirus is the causative agent of viral encephalopathy and retinopathy (VER). VER is considered one of the most serious viral diseases in aquaculture causing enormous losses in species of high economic value as sea bass and halibut (1). No commercial vaccine is available and the most efficacious countermeasures against VER are the traditional prophylactic methods (2). *Cinnamosma* is widely used in traditional medicine and it's active *in vitro* against several viruses (3). In this study we evaluated the *in vitro* efficacy of the essential oil of *Cinnamosma macrocarpa* against Betanodavirus. The cytotoxicity (CC50) of the oil was determined on SSN-1 cells comparing treated cells with untreated control cells by coulter counter and the antiviral activity (IC50) by the cytopathic effect inhibition assay. The selectivity index (SI) was calculated as the ratio of CC50 and IC50. Due to the efficacy exhibited by *Cinnamosma* oil, 7 fractions obtained from column chromatography with different degree of polarity were tested to identify the fraction of the oil responsible for the antiviral activity. Three fractions showed intracellular antiviral activity with values ranging from 21 µg/ml to 54 µg/ml and cytotoxicity between 100 µg/ml and 200 µg/ml. The SI varied from 3 to 4.8. The most polar extracts were the most effective. *Cinnamosma* oil and its polar fractions were active against the *in vitro* replication of Betanodavirus. Further studies are needed to elucidate the mechanism of

action and to investigate the efficacy of Cinnamosma derivatives *in vivo* as a possible candidate to treat VER disease.

References:

- 1-Samuelsen O., Nerland AH., Jorgensen T., Schroder MB., Svasand T., Bergh O. (2006). Dis. Aquat. Organ. 71(3):239-54.
- 2-Harinantenaina L., Yoshinori A., De Clerq E. (2007). J. Nat. Prod. 70, 277-28

FOLK HERBAL VETERINARY MEDICINES FROM INDIAN DESERT

Shridhar Singh Katewa

Department of Botany, College of Science, Mohanlal Sukhadia, India, sskatewa@yahoo.com

Traditional medicines, especially the folk herbal medicines have recently been receiving heightened interest the world over. The World Health Organization (WHO) in its document on "Health for all by the year 2000" has accepted the role traditional medicine has to play in primary health care [1]. The animals are needed for the well being of the humans and hence the similar application of traditional medicine is now being advocated by food and Agricultural Organization (FAO) for animal treatment [2, 3, 4, 5], as is being done by WHO for human treatment. Indian desert is endowed with xerophytic vegetation and the native people have learnt to utilize these plants to meet the health care needs for millennia. The knowledge is believed to be collectively owned by ancestors and kept under the custody of living old men and women, depending on the community, ethnic, sex, age, caste etc. There is a danger however this method of vesting knowledge in human custodians can be undermined by mortality, thereby losing important information to the future generations. Considering the therapeutic potentials of herbal drugs to be of help in animal treatment and production, the author carried out the Ethnoveterinary survey of Indian Desert on the contribution of such a dynamic folk system of herbal medicine practiced by the hereditary physician belonging to certain ethnic communities. In the present communication, therapeutic uses of 25 plant species against animal diseases mentioning their Botanical identity, family, mode of administration and dosage have been given. This folk wisdom if subjected to scientific scrutiny could benefit the humankind in many ways

HERBAL PRODUCTS FOR PIG HEALTH

Gerdien Kleijer-Ligtenberg, Tedje Van Asseldonk, Maria Groot, Bart Halkes, Gisabeth Binnendijk, Jan-Paul Wagenaar, Johanna (see above)

IEZ (Inst f Ethnobotany & Zoopharmacognosy), Netherlands, info@ethnobotany.nl
IEZ (Inst f Ethnobotany & Zoopharmacognosy), Netherlands, info@ethnobotany.nl
RIKILT, Institute of Food Safety, Wageningen UR, Netherlands, Maria.Groot@WUR.nl
PhytoGeniX, Department of Medicinal Chemistry Utrecht Univer, Netherlands,
S.B.A.Halkes@pharm.uu.nl Gisabeth.Binnendijk@wur.nl; j.wag

On behalf of the Dutch interest group for biological farming (Biologica) a research project was initiated with the goal to increase the availability of herbal food additives of proven quality

and efficacy [1]. This project involved poultry, dairy cows, and pigs, but the results of the latter animals are presented here, only. An inventory identified over 70 herbal preparations available for pig health. On basis of the available data, three products were selected for further clinical experiments. Two products contained (mainly) essential oil of oregano, the third was a mixture of approximately 10 plant (extract)s. For each product approximately 64 organic pigs were fed herbal additives from weaning until slaughter; growers in four pens; fatteners in three pens. Control groups of the same size and housing received feed without these herbs. Study-parameters were growth, feed conversion, mortality, requirement of additional veterinary treatment, meat percentage, back-fat, carcass- and organ-findings. Liver biopsies were evaluated for parameters relating to pig health. Herbal products showed positive effects on growth and/or feed conversion of weaners as a trend ($p < 0,12$). No negative side effects were observed. Slaughter data were promising but not always consistent. To demonstrate health improvement, molecular parameters such as the amount of detoxifying enzymes (CYP450) can be measured. Tests with larger groups of animals are necessary to confirm the reported trends and to obtain significant treatment-effects. The Implementation of these herbal additives in farm management, their optimal dosage-levels, as well as the regulatory aspects of these herbal additives need further consideration.

Acknowledgements: We thank the Dutch ministry of Agriculture, Nature and Food (LNV) for funding and all participating farmers and product suppliers for cooperating. [1] More details on www.fyto-v.nl

PRELIMINARY SCREENING OF PLANT SPECIES THAT MAY BE USEFUL IN CONTROLLING DIARRHOEA IN PRODUCTION LIVESTOCK.

N. P. M. Komape, L. J. McGaw and J. N. Eloff

Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa

There are many causes of diarrhoea in production livestock. Some of these causes are infections with microorganisms such as *Salmonella typhimurium*, *Clostridium perfringens* and *Escherichia coli*. Administration of antibiotic growth promoters (AGPs) as feed additives to these animals helps reduce the occurrence of these microorganisms, and hence counteracts diarrhoea. There are many disadvantages to the use of AGPs, including the development of bacterial resistance with prolonged use. Therefore, there is a need to develop AGPs that are cheaper, of natural origin, effective in preventing diarrhoea in production livestock, and not associated with development of resistance. Leaves of fourteen plant species, *Indigophera cylindrica*, *Rhoicissus* sp., *Ziziphus rivularis*, *Heteropyxis natalensis*, *Syzygium legatti*, *Faidherbia albida*, *Sapium integrerrium*, *Thespesia acutiloba*, *Tamarindus indica*, *Polycias fulva*, *Acridocarpus natalitius*, *Ficus sur*, *Grewia occidentalis* and *Englerophytum magalismontanum* were selected based on their activity against *Escherichia coli* in a broad preliminary screening in the Phytomedicine Laboratory. These plants were extracted using acetone and the plants were further screened for antibacterial activity using bioautographic and MIC methods against *Salmonella typhimurium*, *Clostridium perfringens* and *Escherichia coli*. The total activity of the plants was calculated using the mass extracted and the MIC value obtained. From the results obtained, *H. natalensis* had the best antibacterial activity against the three test microorganisms, followed by *T. indica*. Based on the profiles of the plants from literature searches and other criteria, including their traditional use,

SOUTH AFRICAN ETHNOVETERINARY PLANT EXTRACTS WITH ANTIMICROBIAL AND ANTIVIRAL POTENTIAL

L.J. McGaw, V.P. Bagla, T.A. Mokoka, E.E. Elgorashi, J.N. Eloff

Phytomedicine Programme, University of Pretoria, Private Bag X04 Onderstepoort 0110, South Africa

In South Africa, medicinal plants used for treating various disorders in animals may have useful biological activities, particularly if the cause of the disease is linked to infectious microorganisms. The acetone extracts of leaves of sixteen plant species commonly applied in ethnoveterinary medicine were tested in several *in vitro* bioactivity assays. Antibacterial and antifungal serial microdilution assays were performed against two Gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria, and against *Cryptococcus neoformans* and *Candida albicans*. Antibacterial minimum inhibitory concentrations (MIC) reached as low as 0.039 mg/ml, and 62.5% of extracts inhibited the Gram-positive bacteria. Average antifungal MICs were 0.56 and 0.59 against *C. neoformans* and *C. albicans* respectively. Some extracts showed simultaneous antimicrobial activity and cytotoxicity against Vero cells using a colorimetric cell viability assay while others had higher selective activity. Antiviral assays against the sensitive feline herpesvirus type 1 identified six ineffective plant extracts (37.5%), including *Ziziphus mucronata*, *Rhus lancea* and *Leonotis leonurus*, while three extracts (19%, *Pouzolzia mixta*, *Pterocarpus angolensis* and *Hippobromus pauciflorus*) showed a 1 log reduction in viral growth. A 2 log reduction in viral growth was shown by *Pittosporum viridiflorum* and *Cussonia spicata* extracts (12.5%). A quarter of the extracts, *Combretum caffrum*, *Ricinus communis*, *Schotia brachypetala* and *Sclerocarya birrea*, resulted in a promising 3 log reduction in viral cytopathic effect. We are targeting plant extracts with low cytotoxicity and high biological activity for isolation of active compounds, and development of active standardized extracts.

HAEMATOLOGICAL ASSESSMENT OF BITTER LEAF (*VERNONIA AMYGDALINA*) EFFICIENCY IN REDUCING INFECTIONS IN COCKERELS**R. Y. Olobatoke and J. A. Oloniruha**

Ahmadu Bello University, Kabba College of Agriculture, P. M.B. 205, Kabba, Kogi State, Nigeria

This study was conducted at the Teaching and Research farm of Kabba College of Agriculture, Ahmadu Bello University, Kabba, Nigeria in 2006. Its objective was to assess the antimicrobial effect of bitter leaf (*Vernonia amygdalina*) on performance and blood picture of cockerels. Twenty two-weeks old cockerels were selected and used for the study. They were divided into four groups of five, with each group representing a treatment and each bird in a group constituting a replicate. Air-dried and ground bitter leaf was mixed with commercial (starter and later grower) feed at 0, 5, 7.5 and 10 % inclusion levels. Each group of cockerels was fed with these rations respectively for eight weeks. Weight gain was recorded weekly while percent packed cell volume (PVC) of blood, and total blood leucocyte counts were measured at two-weekly intervals. Data generated were subjected to analysis of variance and differences between treatments mean tested at 5 % probability level. Results of this study revealed that bitter leaf increased significantly the feed conversion efficiency of cockerels when included in

rations with the peak at 7.5% inclusion level. There was however no significant difference in the PVC and average leucocytes counts.

MEDICINAL PLANTS USED TO TREAT CATTLE DISEASES IN NETRAKONA DISTRICT, BANGLADESH

Md. Harun- or-Rashid, Rabiea Tanzin, Rownak Jahan, Mohammed Rahmatullah

University of Development Alternative, Dhaka, Bangladesh, masum15bd@yahoo.com

University of Development Alternative, Dhaka, Bangladesh, tanzin15bd@yahoo.com

University of Development Alternative, Dhaka, Bangladesh, rahmatm@uoda.org

University of Development Alternative, Dhaka, Bangladesh, rahamatm@hotmail.com

Most of the rural population of Bangladesh owns one or two cows or goats, which are used either for agricultural purposes (cows) or for selling to generate extra income (goats). For treatment of cattle diseases, they rely on traditional veterinary medicinal practitioners, who use medicinal plants to treat cattle ailments. We conducted an ethnomedicinal survey amongst the traditional veterinary medicinal practitioners in Netrakona district, Bangladesh to identify plants and cattle ailments for which the plants are used. Information on plants were obtained from the traditional cattle healers and individual specimens identified at the Bangladesh National Herbarium. A total of 46 plants distributed into 33 families were identified. The families included Acanthaceae, Alliaceae, Amaryllidaceae, Anacardiaceae, Araceae, Bromeliaceae, Cannabaceae, Convolvulaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Gentianaceae, Lamiaceae, Leguminosae, Menispermaceae, Moraceae, Musaceae, Myrtaceae, Olaceae, Palmae, Piperaceae, Poaceae, Ranunculaceae, Rhamnaceae, Rubiaceae, Rutaceae, Solanaceae, Ulmaceae, Umbelliferae, Urticaceae, Verbenaceae and Zingiberaceae families. Of the families, the highest number of plants (three plants each) belonged to the Anacardiaceae, Euphorbiaceae, Myrtaceae, and Piperaceae families. The various cattle diseases that were claimed to be cured by the medicinal plants included fever, rigor, leg fractures, wound infections, diarrhea, meningitis, helminthiasis, common cold, enlargement of liver, body ache, asthma, retention of urine, mumps, piles, cataract, loss of hair, glossitis, and lack of milk production.

SECONDARY METABOLITES OF THE SELECTED SPECIES OF MEDICINAL PLANTS IN THE RELATION TO THE PRODUCTIVE PARAMETERS OF LAYING HENS

Janka Poracova, Marta Blascakova, Ivan Salamon, Maria Zahatnanska

Presov University, Faculty of Humanities and Natural Science, Slovakia, poracova@unipo.sk

Presov University, Faculty of Humanities and Natural Science, Slovakia

Presov University, Faculty of Humanities and Natural Science, Slovakia, Presov University,

Faculty of Humanities and Natural Science, Slovakia

The aim of our model experiment was to study the effect of chamomile essential oil (*Matricaria recutita* L.) and dried extract of *Eleutherococcus senticosus* MAXIM. on weight of laying hens Hisex braun and weight of eggs under cage system breeding conditions. In the experiment with chamomile essential oil, 16 hens were divided into two groups – a control group (CGCHA, n = 8) and one experimental group (EGCHA, n = 8); in the experiment with dried extract of *Eleutherococcus senticosus* MAXIM., 20 hens were divided into two groups – a control group (CGEL, n = 10), and one experimental group (EGEL, n = 10). The essential oil of chamomile and dried extract of *Eleutherococcus senticosus* in concentration of 0.1 % was administered to laying hens daily throughout one month. The laying hens were in good health condition throughout the experiment. The weight of laying hens and eggs in experimental group supplemented with chamomile essential oil and dried extract of *Eleutherococcus senticosus* MAXIM. increased insignificantly during 4 weeks of experiment.

ETHNOVETERINARY MEDICINE (EVM) - USING FRESH HERBALS FOR LIVESTOCK HEALTHCARE AT FARMERS' LEVEL

Natesan Punniamurthy

Tamilnadu Veterinary and Animal Sciences University, INDIA, murthyvcri@hotmail.com

EVM practices for mastitis, dermatitis, fowl pox and gastroenteritis are effectively employed in the field. Scientists increasingly recognize that traditional farmers have detailed knowledge of agriculture and animal husbandry. This knowledge is based on experience and observations. Traditional remedies are found to be effective and work for sound scientific reasons. Traditional veterinary practices offer a particularly rich resource for development. Cultivation and conservation of medicinal herbs is assured at the farmers' level assuring participatory efforts. Many poor people derive their only income from harvesting medicinal plants. This income however, is probably declining where natural habitats are disappearing. A strategy that integrates conservation and cultivation of medicinal plants could create long-term employment and income opportunities. EVM seminars are organized for all stake holders including farmers, NGO and veterinarians and efforts are taken to promote the use of fresh herbs for primary health care of livestock. The package is based on the principles of Ethnoveterinary knowledge collected from the field. Efforts are taken to propagate the concept involving the stakeholders at all levels. Preliminary research is undertaken at the laboratory and field levels to validate the recipes. At least three recipes have been demonstrated at the field level and are successfully used by the farmers and veterinarians as well. The clinical conditions are mastitis, nonspecific gastroenteritis and dermatomycosis. Trainings are conducted for farmers, SHG women, field veterinarians and efforts are on to induct the EVM knowledge into the main stream veterinary curriculum.

PLANTS, PLANT EXTRACTS AND PHYTOCHEMICALS FOR MANIPULATING RUMINAL FERMENTATION

J. Wallace

Rowett Research Institute, United Kingdom. john.wallace@rowett.ac.uk

The threat to human health associated with the use of antibiotic and chemical feed additives has prompted legislation in the EU to ban antimicrobial growth promoters (AGP), and has accelerated investigations into plants and their extracts as feed additives. The broad potential of plants and their extracts to replace AGP is illustrated by the progress of an EC Framework 5 project, 'Rumen-up' and its Framework 6 successor, 'Replace'. The Rumen-up project began with a targeted collection of 500 European plants and their extracts, and partners tested their effects on ruminal proteolysis, protozoa, methanogenesis and lactate production. A success rate of about 5% in terms of positive hits illustrated that phytochemicals have great potential as "natural" manipulators of rumen fermentation, to the potential benefit of the farmer and the environment. Some of the positive samples exerted their effect via their essential oils or saponins content. The mode of action of these phytochemicals is at least partially understood. Dietary inclusion of a commercial blend of essential oil compounds significantly decreased NH₃ production from amino acids in ruminal fluid taken from sheep and cattle. This effect was mediated partly by effects on ammonia-producing bacteria. Saponins-containing plants and their extracts suppress the bacteriolytic activity of rumen ciliate protozoa, thereby enhancing total microbial protein flow from the rumen. The Rumen-up project also highlighted potentially useful plants which had a benefit that could not be explained by our present knowledge of the effects of phytochemicals on ruminal microorganisms.

EVALUATION OF THE ACTIVITY OF LEAF EXTRACTS OF TREES THAT HAVE BEEN USED TO TREAT DIARRHOEA IN HUMANS AND ANIMALS

Gabriele Wuerger, L.J. McGaw, Chlodwig Franz, J.N. Eloff

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Medicine Onderstepoort, University of Pretoria, South Africa, gwuerger@hotmail.com
Institute for Applied Botany and Pharmacognosy, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

The use of medicinal plants has been increasing exponentially over the last few years. The rural population of developing countries has always depended greatly on traditional herbal medicine, but also in the first world countries, alternative healing methods have been gaining more and more importance, especially in Europe after the restriction of antibiotic food additives for stock animals by the European Union. In this study 53 tree species were evaluated for their antibacterial activity and their tannin content in order to study the mechanisms of their uses as remedies against diarrhoea in humans and animals. The antibacterial activity was tested against one Gram-positive (*Staphylococcus aureus*) and one Gram-negative (*Escherichia coli*) bacterial strain using the microdilution assay. The assays were done in triplicate and were repeated three times. Furthermore bioautographic assays were done. The compounds were visualised using Thin Layer Chromatography. For the evaluation of the tannin content, the radial diffusion assay was used. Five selected tree species were collected and tested every month for their antibacterial activities and tannin content in the same manner in order to show possible seasonal variations. Those five plants were *Acacia karoo*, *Acacia sieberiana*, *Peltophorum africanum*, *Trichilia emetica* and *Ziziphus mucronata*. Samples of various different trees of the species *Combretum molle* were collected at different locations and analyzed in the same manner to show possible interspecies variation and possible variation due to the localization of the plant. The results of this study will help to understand which mechanism each plant employs to be

used as an effective remedy against diarrhoea.

THEME 12

DIVERSE ASPECTS NOT ACCOMMODATED IN OTHER THEMES

COMPOSITION OF THE ESSENTIAL OILS OF *LAVANDULA STOECHAS* L. SUBSP. *STOECHAS* (BOISS.)

S. Alan, A. Altintas, B. Demirci, F. Koca, K. H. C. Baser

Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, 26470
Eskisehir, Turkey

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskisehir,
Turkey

The genus *Lavandula* (Lamiaceae) represented with 2 species and 3 taxa in the Flora of Turkey (1). *L. stoechas* is used as analgesic, antiseptic, wound-healing, sedative, expectorant, treatment of eczema wounds and as cardiotoxic (2). Aerial parts of *Lavandula stoechas* L. subsp. *stoechas* (Boiss.) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to yield 1.16% oil. The oil was analysed by gas chromatography-mass spectrometry (GC-MS). Forty five compounds representing 97.4% of the essential oil were characterized from *Lavandula stoechas* subsp. *stoechas*. Camphor (45.8%), α -fenchone (31.8%), bornyl acetate (4.2%) and camphene (2.5%) were the main constituents in the oil of *Lavandula stoechas* subsp. *stoechas*.

References:

1. Davis, P.H., Flora of Turkey and the East Aegean Islands, Vol.7, 76-77, University Press. Edinburgh (1982).
2. Baytop, T., Türkiye'de Bitkiler ile Tedavi, İst. Üniv. Yayın No: 3255 Ecz. Fak. Yayın No:40, İstanbul (1984).

COMPOSITION OF THE ESSENTIAL OILS OF *CALAMINTHA NEPETA* (L.) SAVI SUBSP. *NEPETA* AND *CALAMINTHA NEPETA* (L.) SAVI SUBSP. *GLANDULOSA* (REQ.) P.W. BALL

Sevim Alan, Mine Kürkçüolu, K. Hüsnü Can Baser

Anadolu University, Turkey, salan@anadolu.edu.tr
Anadolu University, Turkey, mkurkcuo@anadolu.edu.tr
Anadolu University, Turkey, khcbaser@anadolu.edu.tr

Calamintha Miller (Lamiaceae) is represented in Turkey by 9 species and 13 taxa, 6 being endemic. The rate of endemism in Turkey is over 45%. In the present study, aerial parts of the *Calamintha nepeta* (L.) Savi subsp. *nepeta* were collected from the regions of Tarsus (A), Silifke (B), Bart (C) and *Calamintha nepeta* (L.) Savi subsp. *glandulosa* (Req.) P.W. Ball collected from the regions of the Zonguldak (D) and Manisa (E). Water distilled essential oils from the aerial parts of *Calamintha* species from Turkey were analysed by GC and GC/MS. Trans-piperitone oxide (44.4 %) and piperitenone oxide (11.7 %) were detected as main

constituents in sample A; Pulegone (11.9 %), menthone (11.9 %) and carvacrol (10.0 %) were found as main constituents in sample B; Trans-piperitone oxide (30.9 %), caryophyllene oxide (7.8 %) were detected as main constituents in sample C; Pulegone (19.5 %), menthone (9.7 %), caryophyllene oxide (7.9 %) and trans-piperitone oxide (5.7 %) were detected as main constituents in sample D; Trans-piperitone oxide (34.2 %), limonene (17.3 %) and piperitenone oxide (10.8 %) were found as main constituents in sample E.

COMPOSITION OF THE ESSENTIAL OILS OF *CENTAUREA DIFFUSA* LAM. AND *CENTAUREA ZEYBEKII* WAGENITZ

Ayhan Altintas, Osman Tugay, Betul Demirci, Kemal Husnu Can Baser

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu Un, Turkey,
aaltinta@anadolu.edu.tr

Department of Biology, Faculty of Science and Art, Selcuk Un, Turkey, otugay@selcuk.edu.tr

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu Un, Turkey,
bdemirca@anadolu.edu.tr

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu Un, Turkey,
khcbaser@anadolu.edu.tr

In Turkey, the genus *Centaurea* L. (Asteraceae) is represented by 189 species including 120 endemics, distributed particularly in the Southwest, Central and Eastern parts (1-2). The ratio of endemism is quite high (63.4%). Several members of this genus, such as *C. cyanus* L., *C. behen* L., *C. calcitrapa* L., are used in Anatolian folk medicine (3). Aerial parts of *Centaurea diffusa* Lam. and *Centaurea zeybekii* Wagenitz were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus and the oils trapped in hexane were analysed by gas chromatography-mass spectrometry (GC-MS). Identification of the essential oil components was carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to the series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library) (4-5), and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data (6-8), were used for the identification. Fifty nine and ninety compounds representing 87.1% and 81.6% of the essential oils were characterized from *C. diffusa* and *C. zeybekii*, respectively. eudesmol (45.4%), hexadecanoic acid (4.5%) and hexahydrofarnesyl acetone (4.1%) were the main constituents in the oil of *C. diffusa* and hexanoic acid (9.3%), hexadecanoic acid (8.6%) and nonacosane (6.4%) were the main constituents in the oil of *C. zeybekii*.

ANTIFUNGAL ACTIVITIES OF *LIPPIA* EXTRACTS AND PURIFIED COMPOUNDS AGAINST *PENICILLIUM DIGITATUM*

E. Shikanga Amukohe

Tshwane University of Technology, South Africa, AmukoheES@tut.ac.za

Postharvest decay due to a green mould known as *Penicillium digitatum* is responsible for significant losses in citrus exports from South Africa. Consumer concerns and stricter trade regulations have put pressure on producers to replace synthetic fungicides used commercially to prevent fungal decay with natural products. A strain was found to be resistant to the commercial fungicide Guazatine isolated from Valencia oranges. The objective of this study was to evaluate the abilities of aqueous extracts of Lippia species indigenous to South Africa (*L. javanica*, *L. scaberrima*, *L. rehmannii* and *L. wilmsii*) and isolated compounds (theviridoside, verbascoside and isoverbascoside), to inhibit the growth of this resistant strain. *In vitro* and *in vivo* tests were conducted to confirm the antifungal activity of each extract and compound. All the compounds and extracts showed significant activities *in vitro* against the pathogen. Although verbascoside exhibited low activity at low concentrations (0.4 mg.ml⁻¹), its activity increased drastically at concentrations above 0.6 mg.ml⁻¹. It was also observed that, the activities of *L. rehmannii* and *L. javanica* were the highest *in vitro* among the plant extracts. The *in vivo* results showed that *L. javanica* was the most potent extract at concentrations greater than 0.6 mg.ml⁻¹. Results of the *in vitro* and preliminary *in vivo* studies suggest that employing *L. javanica* aqueous extracts to control *P. digitatum* strains resistant to Quazitin worthy of further investigation. Acknowledgements: NRF for funding

EFFECTS OF A WILD GREEN OAT EXTRACT ON MENTAL PERFORMANCE: A HUMAN CLINICAL STUDY USING SOURCE DENSITY ANALYSIS OF THE HUMAN EEG

Cem Aydogan, Charlotte Schellekens, Carla Wullschlegler

Frutarom Switzerland Ltd., Switzerland, caydogan@ch.frutarom.com
Frutarom Switzerland Ltd., Switzerland
Frutarom Switzerland Ltd., Switzerland

Preparations from green oat have traditionally been used to support mental health and cognitive function [1, 2]. The patented wild green oat extract Neuravena® has been shown to support learning, increase alertness, have stress coping and stimulating abilities and to influence brain activity *in-vivo* [3,4]. Underlying study is a randomized, double-blind, placebo-controlled cross-over clinical trial to confirm Neuravena®'s effects on mental fitness and performance under pressured situations, using source density analysis of the EEG [5] in human volunteers. Twenty healthy men and women ingested one single dose of 2500 mg Neuravena®, or placebo, at start of the trial. Brain activity signals were recorded during 4 hours under relaxed and pressured conditions, using a standardized set of electrodes. Neuravena® was found to change the basic brain activity through a significant decrease of frontal and temporal delta and theta waves as well as an increase in alpha2 wave activity. Changes in evoked short term brain activity during the performance of the concentration stress test showed that Neuravena® significantly increases delta and theta wave activity in the brain regions closely connected to mental performance. The abovementioned changes confirm the positive effects of Neuravena® on cognitive function and are in line with the extract mediating stimulating properties. The intake of Neuravena® helps increase overall mental fitness in an individual's daily life. Moreover, it has

been shown to help improve cognitive function (concentration, learning, alertness) under pressure.

EFFECT OF OLIVE LEAF EXTRACT IN ADULT TWINS WITH MILD HYPERTENSION

Cem Aydogan, Charlotte Schellekens, Tania Moccetti, Andreas Busjahn

Frutarom Switzerland Ltd., Switzerland, caydogan@ch.frutarom.com
Frutarom Switzerland Ltd., Switzerland
Frutarom Switzerland Ltd., Switzerland
HealthTwiSt GmbH, Germany

Introduction: Olive leaves (*Olea europaea L.*) have been used in folk medicine in a variety of indications including hypertension and atherosclerosis. EFLA®943 olive leaf special extract contains the natural active ingredients of olive leaves in concentrated form. Objective: The aim of this observational study was to assess the effect of EFLA®943 as food supplement in the context of dietary measures for adults with mild hypertension. Methods: 20 adult twin pairs with blood pressure values above the optimal level (RRdiast.: 80 mmHg; RRsyst.: 120 mmHg) were enrolled, for whom a non-pharmacological therapy of the diagnosed mild hypertension was indicated. The twin pairs were divided in 2 groups. Non-medicinal measures (dietary recommendations, lifestyle change) were compared between twins to the consumption of a 500 mg EFLA®943 tablet for 8 weeks. For the statistical evaluation of this study, blood pressure, blood sugar and lipids were measured. Additionally, satisfaction and tolerance of the treatment were judged and recorded. Results: In the course of the study, both systolic and diastolic blood pressure values significantly decreased in twins treated with the olive leaf extract. The observed effect was dose-dependent. Among the measured clinical blood parameters, a significant reduction of the LDL-cholesterol values could be shown in EFLA®943-treated twins as well. The supplement was well-tolerated, no side effects were reported. Conclusions: The results of the study support the beneficial of the olive leaf special extract EFLA®943 as dietary supplement in the context of a non-pharmacological approach for the treatment of mild hypertensive states.

THE OPUS STUDY: OSTEOPOROSIS PREVENTION USING SOY

**Cem Aydogan, William Wong, Richard Lewis, Francene Steinberg, Michael Murray
Margaret**

Frutarom Switzerland Ltd., Switzerland, caydogan@ch.frutarom.com
Baylor College of Medicine, USA
U of Georgia, USA
Cramer Paula Amato, Ronald Young, Stephen Barnes etc.

A study was designed to (1) evaluate the effect of soy isoflavones on bone loss as well as to (2) determine the long-term safety of extracted isoflavones in a tablet form. Design: 403 Early postmenopausal women (54.01±3.97 y) were enrolled in an intent-to-treat, multi-site, 2-year follow-up, randomized, double-blind study with 1/3 on placebo, 1/3 on 80 mg/d of isoflavones, and 1/3 on 120 mg/d of isoflavones (SoyLife EXTRA, soy germ isoflavones) along with 1,000 mg/d of CaCO₃ and a daily multivitamin with 400 IU of vitamin D. Bone mineral

content (BMC), bone mineral density (BMD), and serum levels of biochemical bone markers were measured at baseline and annual intervals. The safety indicators included serum electrolytes, lipids, liver and renal functions, complete blood counts, reproductive and thyroid hormones and cancer screening. Results: Baseline characteristics were similar in the three groups. A statistically significant, but small effect on the decrease in whole body BMD was observed with soy isoflavone intake at 120 mg/d vs. placebo after 1 y ($p=0.027$) and 2 y ($p=0.048$) of supplementation. Changes in biochemical safety indicators were neither outside the normal range nor clinically significant. Major adverse effects included one subject with endometrial cancer and one with breast cancer, which were fewer than predicted in this study population. Conclusion: Soy isoflavone supplementation (120 mg/d) appears to be safe and may modestly prevent early menopausal loss of whole body BMD. The benefit was not observed in regional sites with high trabecular bone content.

PHYTOCHEMICAL AND ANTISICKLING ACTIVITIES OF *TERMINALIA CATAPPA* (*COMBRETACEAE*) LEAF EXTRACTS

Babatunde Samuel, Joseph Okogun, Kunle Idowu, A.A. Olaniyi

Phytomedicine Programme, University of Pretoria, South Africa,
bolorundurosamuel@yahoo.com
Nat. Inst. for Pharm Res. & Dev., Nigeria, jokogun@yahoo.com
Fac. of Pharmacy, University of Ibadan, Nigeria, olakunleid@yahoo.com
University of Ibadan, Nigeria, olaniyiaa@yahoo.com

Ethno botanical survey revealed that the dried fallen leaves of *Terminalia catappa* Linn. are used locally in various parts of Nigeria for the management of sickle cell anaemia. This research investigated if *Terminalia catappa* leaf interferes with the basic mechanism of erythrocyte sickling and the group of compounds responsible for its antisickling activity were identified and characterized. Bioactivity guided fractionation of the methanolic extract showed that the highest antisickling activity, in the erythrocyte sickling model, resided in its triterpenoids components (fraction F3, $K = 0.0037 \text{ min}^{-1}$). This fraction drastically reduced the rate of erythrocyte sickling and is very significantly more active than the control and other active fractions F1 and F2 ($P < 0.001$, 1-way ANOVA, Tukey's Multiple Comparison test). The antisickling effect is concentration dependent. Graded dose response showed that 0.75mg/ml is the optimal concentration for *in vitro* bioassay method ($P > 0.05$). Bioactivity guided separation of the extract was carried out on flash chromatography using RP18 and Silica gel stationary phases respectively. Final separation was done by HPLC on Cyanosilica column. Structural elucidation was by use of Nuclear magnetic resonance (NMR) and mass spectrometry (MS). The most active components were established to be two triterpenic acids (1) 2α , 3β , 24-trihydroxy-12-ursen-28-oic acid(2), 2α , 3β , 24-trihydroxy-12, 20(30)-ursa-dien-28-oic acid work shows that there is a scientific basis for the application of this extract in the management of sickle cell anaemia in traditional medicine. Acknowledgements: NIPRD & TRENTO University provided funding

SCREENING FOR ANTIMICROBIAL ACTIVITY OF SOME MEDICINAL PLANTS IN SAUDI ARABIA

Aly. M. M, Bafeel. S. O

Faculty of Science, Biology Department, King Abdul -Aziz University, Saudi Arabia,
sb253@hotmail.com

Our study aimed to evaluate antimicrobial activities of some medicinal plants against some pathogenic yeasts and fungi. The antimicrobial activities of water and organic crude extracts of 6 medicinal plants used

In traditional Saudi Arabia medicine was tested against to and pathogenic yeasts and fungi including strains that were highly resistant to amphotericin B and ketoconazole. Two species of yeasts: *Candida albicans* and *C. tropicalis* and five species of pathogenic fungi: *Aspergillus flavus*, *Alternaria solani*, *Fusarium oxysporum*, *Trichophyton rubrum*, *T. mentagrophyte* and *Microsporium nanum* were used. Of the 6 plants tested, 5 showed antifungal activities against one or more species of fungi and 4 showed excellent activity against *Candida albicans* and *C. tropicalis*. The most active antimicrobial plants were the ethanolic extract of *Azadirachta indica* (neem), *Zingiber officinale* (ginger) *Eucalyptus globules* and *Lawsonia inermis* with minimal inhibitory concentration (MIC) values ranged from 100 -250 $\hat{1}/4$ g/ml. *Rosmarinus officinalis* and *Lepidium sativum* have moderate antifungal activities with MIC of 400 $\hat{1}/4$ g/ml. No toxicity was recorded up to 300 $\hat{1}/4$ g/ml. Moderate toxicity was recorded at 500 $\hat{1}/4$ g/ml for *A. indica* and at 600 $\hat{1}/4$ g/ml for *E. globules*. No antitumor activities were recorded using the Biochemical induction assay (BIA) and *Escherichia coli* as a test organism.

LEARNING FROM TRADITIONAL KNOWLEDGE SYSTEMS TO ENRICH DEVELOPMENT:
WORLD BANK EXPERIENCE

Nicolas Gorjestani

World Bank, U.S.A., ngorjestani@worldbank.org

This lecture will argue that traditional knowledge systems embedded in impoverished communities represent the intellectual capital of the poor. This intellectual capital is a significant resource which could help enrich the development process and contribute to achieving the Millennium Development Goals. For example, in the health area, traditional medicine approaches can help to provide affordable and generally effective health care for more people, especially the poor. The lecture will then explore the lessons of experience of the World Bank's Indigenous Knowledge for the Development Program and the challenges in moving forward and in raising awareness about the important role that traditional knowledge systems can play in the development process. One of these challenges is to change the prevailing mindsets among development practitioners regarding the utilization of the traditional knowledge base of the poor in the development process. Another is to develop approaches to validation of indigenous knowledge that are adapted to the specific nature of such knowledge systems. Finally, there is a need to develop innovative approaches for the protection of community-based knowledge systems.

DEVELOPING A SECTOR BY CREATING COMMUNITY-OWNED ENTERPRISES BASED ON
THE CULTIVATION AND PROCESSING OF ESSENTIAL OILS AND MEDICINAL PLANTS IN
SOUTH AFRICA

Marthinus Horak, Lucky Khumalo, Ebrahim Wadiwala, Bernadette Brown

CSIR, South Africa, rmhorak@csir.co.za
Department Science and Technology, South Africa, Lucky.Khumalo@dst.gov.za
CSIR, South Africa, ewadiwala@csir.co.za
CSIR, South Africa, bbrown@csir.co.za

The Technology Transfer for Social Impact Center of CSIR and the Sustainable Livelihoods Programme of Department of Science and Technology work in partnership to develop the medicinal plant and essential oil sector in South Africa. The approach is to identify and address key technical, marketing and regulatory obstacles that have inhibited the growth of the sector to date. In addition, a portfolio of projects, aim of establishing new enterprises in rural communities based on cultivation and processing of medicinal and essential oil plants, is implemented. The indigenous essential oil species under cultivation include buchu, rose geranium and *Lippia javanica* on a total of 75 ha, currently being expanded to a total of 240 ha. Roman chamomile, German chamomile, peppermint and rose damascena as well as the indigenous species, *Eriosephalus punctulatus* and *E. africanus* are cultivated on pilot sites. Indigenous medicinal plants include *Pelargonium sidoides*, *Sutherlandia frutescens*, *Hoodia gordonii*, *Harpagophytum procumbens* and *Siphonochilus aethiopicus*. The climatic diversity of the country allows the cultivation of a variety of species, but also leads to significant differences in chemical profiles of essential oils produced from the same genetic stock. A desired 1:1 ratio of geraniol (21.3%) and citronellol (21.4%) was obtained for rose geranium oil produced in coastal regions under conditions of water stress. In contrast, certain interior areas being irrigated yielded oil containing geraniol and citronellol in a ratio of 1.0:2.3. In order to develop a model that relates agronomic conditions to oil quantity and quality, data are gathered using twelve telemetric weather stations installed on project sites.

MEDICINAL PLANT BASED PRODUCTS FOR RURAL COMMUNITY DEVELOPMENT: CASE STUDY OF A MICRO-ENTERPRISE IN MEXICO**Lorena Ibargüen**

University of East Anglia, Norwich NR4 7TJ, United Kingdom, I.tinley@uea.ac.uk

Medicinal plants and herbal products are used extensively throughout Mexico, both in the context of traditional medicine, as well as in popular and domestic medicine (Zolla and Mellado 1995). Unfortunately, commercialization has been characterized as inequitable, poorly regulated and based on unsustainable management practices (Hersch 1999). The aim of the research was to identify and understand those factors which may be crucial to enhance the potential of small scale, rural based initiatives in the area of medicinal plant based products in Mexico. The research is based on a case study of a women's micro-enterprise, which produces herbal and cosmetic products in a small rural community in the state of Veracruz, Mexico. Ethnographic methods were used and consisted of open ended interviews, participant observation and a community survey. Structured, and semi structured interviews were conducted with key actors in the policy arena. The study concluded that the herbal product market in Mexico presents serious obstacles for small micro-enterprises. Lack of specialized knowledge, limited access to capital investment, and an inhibiting policy framework for these products are some of the main difficulties. The potential impact of medicinal plant product initiatives on income and community

development in the present conditions is thus very limited. Zolla, C. and V. Mellado (1995). La función de la medicina doméstica en el medio rural mexicano. Las mujeres y la salud. S. González-Montes. Mexico D.F., El Colegio de México. Hersch-Martinez, P. (1999). "La flora medicinal silvestre como mercancía." Antropología Social 35 (Julio/septiembre).

BIOTRANSFORMATION AND BIOLOGICALLY ACTIVE METABOLITES OF (R)-(-)-PHELLANDRENE

Gokalp Iscan, Nese Kirimer, Fatih Demirci, K. Hüsnü Can Baser

Anadolu University, Turkey, giscan@anadolu.edu.tr
Anadolu University, Turkey, nkirimer@anadolu.edu.tr, Anadolu University, Turkey,
fdemirci@anadolu.edu.tr, Anadolu University, Turkey, khcbaser@anadolu.edu.tr

Biotransformation derivatives of natural and aromatic compounds may constitute an important resource for pharmaceutical, fragrance and aroma substances. From this point of view, in the present work, the monoterpene phellandrene was biotransformed by 16 different bacteria, fungi and yeasts. The metabolites of (R)-(-)-phellandrene were 6-hydroxypiperitone, phellandrene epoxide, cis-p-menth-2-en-1-ol, p-mentha-1(7),5-dien-2-ol (syn. yabunikkeol), 5-p-menthen-1,2-diol and carvotanacetone, which were characterized by GC-MS, 1D and 2D NMR techniques after chromatographic purifications. Furthermore, one of the major metabolites which is 5-p-menthen-1,2-diol was subjected to in vitro antibacterial, anticandidal by agar diffusion and microdilution and to TLC bioautographic bioluminescent *Vibrio fischeri* acute toxicity tests. The metabolite showed good inhibitory effects against various pathogenic bacteria (MIC 0.5-0.125 mg/ml) and *Candida* species (MIC 1-0.125 mg/ml) with relatively non-toxic (<10 mg/ml) properties detected by the *V. fischeri* bioluminescence assay. Acknowledgements: This research work is part of the PhD study of G.I. Special thanks are addressed to Prof. Dr. R. G. Berger and Prof. Dr. W.-R. Abraham for providing some of the microorganisms employed in the biotransformation and bioassay studies. Infrastructures of TBAG 107T117 and BAP 060301 projects were used.

COMPOSITION OF THE ESSENTIAL OIL OF *MARRUBIUM ANISODON* C. KOCH

Nese Kirimer, Mine Kürkçüolu, Gencay Akgul, K. Hüsnü Can Baser

Anadolu University, Turkey, nkirimer@anadolu.edu.tr
Anadolu University, Turkey, mkurkcuo@anadolu.edu.tr, Kafkas University, Turkey, , Anadolu
University, Turkey, khcbaser@anadolu.edu.tr

The genus *Marrubium* (Labiatae) is represented by 23 taxa, 13 of which are endemic to Turkey(1). Aerial parts of *Marrubium anisodon* were subjected to hydrodistillation for 3h using a Clevenger-type apparatus. Essential oil of the plant material from Turkey was analysed by GC and GC/MS. Thirty-nine components were characterized in total, representing 87.9 % of the oil. (Z)-farnesene (20.2 %), nonacosane (18.5%) and caryophyllene (13.3 %) were main

constituents.

References: 1. Cullen, J., 1982, *Salvia*, Flora of Turkey and East Aegean Islands; Univ. Press: Edinburgh, P.H. Davis (Ed.), Vol.7, 165-178.

AN ESSENTIAL OIL COMPOSITION OF THE NEEDLES OF SCOTS PINE (*PINUS SYLVESTRIS L.*) GROWING IN THE SOILS OF DIFFERENT TYPE

Eugenija Kupcinskiene, Asta Judzentiene

Kaunas Vytautas Magnus University, Lithuania, e.kupcinskiene@gmail.com
Institute of Chemistry, Lithuania, judzent@ktl.mii.lt

Extracts of pines have diverse physiological and pharmacological actions, its buds, needles and bark are used in phytotherapy. Geographical, seasonal, age-related, inter-population differences of volatile compounds in the needles were defined. Wider surveys of the needle essential oil composition under the different type of soils are still scarce. Despite Scots pine is the prevailing tree in the forests of Lithuania, little is known about its essential oils. The aim of our study was to evaluate the essential oil composition in the needles of Scots pine (*Pinus sylvestris L.*) growing on arenosols (3 sites), luvisols (3 sites) and histosols (3 sites). Current-year and one-year-old needles of 8 pines in each site were sampled in July, 2005. Volatile components of the needles were extracted and analyzed by GC and GC/MS. Seventy one identified component made up 89.1-95.1 % of total oil content. The most predominant fraction was found to be monoterpenes (19.0-40.0 %), with pinene (6.1-26.1 %) and 3-carene (4.9-22.9 %) as the major constituents. Between the pine stands growing on the soils of different type statistically significant changes in percentage concentrations of monoterpenes, sesquiterpenes, oxysesquiterpenes, diterpenes were documented. For the pines growing on histosols and luvisols bigger amounts of shorter chain terpenes and smaller amounts of longer chain terpenes are produced when compared to pines on arenosols. Determined changes in the proportion of components of the essential oils in the needles of the trees growing on different types of soil may significantly modify medicinal value of the needles.

BIOACTIVITY OF *OCIMUM AMERICANUM L.* ESSENTIAL OILS AGAINST FOUR INSECT PESTS OF STORED FOOD COMMODITIES

J.O. Ogendo, A.L. Deng, M. Kostyukovsky, U. Ravid, J.C. Matasyoh, E.O. Omolo, S.T. Kariuki, A.W. Kamau, E. Shaaya

Departments of Crops, Horticulture and Soils; Biological Sciences and Chemistry, Egerton University, P.O. Box 536 Egerton 20115 Kenya (Email: ogendojoshua@yahoo.co.uk)
Departments of Food Science, ARO, The Volcani Center, Bet Dagan 50250, Israel
Department of Aromatic, Medicinal and Spice Crops, ARO, Newe Ya'ar Research Center, Ramat Yishay 30095, Israel

Laboratory studies were conducted to evaluate the fumigant and repellent properties of *Ocimum americanum L.* essential oils against adult *Sitophilus oryzae L.*, *Rhyzopertha dominica F.*, *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis F.* Each essential oil was tested at four (0, 1, 5, 10 µl/L air) and five (0, 0.5, 1.0, 1.5, 2.0 µl oil/ g grain) rates in space fumigation

and choice bioassay studies, respectively, with four replicates per treatment. Corrected percent mortality and PR were arcsine transformed before ANOVA and means separated by Tukey's HSD test [1, 2]. Dose-responses were further subjected to Probit analysis and LC₅₀ values computed [3]. Leaf essential oil at 10 µl/L air 24 h after treatment, caused 88.8, 87.5, 11.3 and 100% adult mortality of *S. oryzae*, *R. dominica*, *T. castaneum* and *C. chinensis*, respectively (LC₅₀ values of 0.38, 0.49, NS and 0.18 µl/L air, respectively). The LC₅₀ values varied with plant part assayed. Leaf essential oil was most efficacious (LC₅₀ values: 0.18-0.49 µl/L air) followed by fruit (1.15-15.07 µl/L air) and stem (3.70-34.68 µl/L air) essential oils, respectively. In the choice bioassay studies, fruit essential oil (at 2.0 µl oil/ g grain and 24 h) produced highest PR values of 81 and 94% against *S. oryzae* and *R. dominica*, respectively. Similarly, the leaf oil repelled 51 and 83% of *T. castaneum* and *C. chinensis*, respectively. Clear intra-species variations in essential oil compositions and inter-insect species differences form the basis of differential bioactivity observed. Leaf oil was high in monoterpenes whereas stem and fruit oil were high in sesquiterpenes. Results show that plant volatiles are potential alternative grain fumigants and further validation studies required for compatibility with tropical agriculture.

References:

[1] Rajendran, S. and Muralidharan, N. Effectiveness of allyl acetate as a fumigant against five stored grain beetle pests. *Pest Management Science* 61: 97-101 (2005). [2] Rozman *et al.* Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. *Journal of Stored Products Research* 43(4): 347-355 (2007). [3] Finney, D.J. Probit Analysis, 3rd ed. Cambridge Univ. Press, London (1971).

BIOMARKERS AMONG TERPENOIDS AND QUANTITY OF EPIPHYTIC MICROFLORA OF ARTEMISIA L. SPECIES NATIVE FOR LITHUANIA

Eugenija Kupcinskiene, Asta Judzentiene, Alvydas Pavilionis

Vytautas Magnus University, Lithuania, e.kupcinskiene@gmail.com
 Institute of Chemistry, Lithuania, judzent@ktl.mii.lt
 Kaunas University of Medicine, Lithuania, mikrobas@kmu.lt

Species belonging to *Artemisia* L. genus are rich in secondary metabolites (flavonoids, essential oils) and have been widely used in pharmacology and food industry. The concentration of the secondary metabolites depends on nutrition and developmental stage of the plant. Little is known about biology of *Artemisia* species (*A. campestris* L., *A. vulgaris* L. and *A. absinthium* L.) naturally growing in Lithuania. The aim of the work included evaluation of biomarkers among terpenoids in the plants, growing under different climatic and environmental conditions. Morphological properties of the plants were evaluated. For the quantity and chemical composition of the essential oils the following methods and techniques were used: gas chromatograph (HP 5890 II) with mass spectrometric detector (HP 5971), HPLC (chromatograph Waters 2690) equipped with VIS-UV detector (Waters 2487) and VIS-UV spectrophotometer (Genesys 5). The main biomarkers among terpenoids were determined (major specific compounds, with amount ³ 10 %): a- ir b -pinenes, sabinene, thujones, 1,8-cineole (for *A. vulgaris*), epoxi-ocimene (for *A. absinthium*), chrysanthenyl acetate (for *A. vulgaris*), trans-caryophyllene, germacrene D, trans-sabinyl acetate (for *A. absinthium*), g-curcumene+acoradiene (for *A. campestris*). Synthesis of the secondary metabolites is influenced by nutrition and related to the quantity of the epiphytic microorganisms. According to the quantity of epiphytic microflora developmental differences were observed, also aboveground organ

specificity was documented. No expressed differences were found between investigated species according to the amount of surface microorganisms. The differences in nitrogen nutrition got the best reflection in the ratio of the amounts of bacteria and fungi.

THE ESSENTIAL OILS OF FOUR ENDEMIC *SALVIA* SPECIES IN TURKEY: *S. DIVARICATA*, *S. ERIOPHORA*, *S. LONGIPEDICELLATA* AND *S. PILIFERA*

Mine Kurkcuoglu, Ahmet Duran, K. Hüsnu Can Baser

Anadolu University, Turkey, mkurkcuo@anadolu.edu.tr
Selçuk University, Konya, ahmetduran22@yahoo.com
Anadolu University, Turkey, khcbaser@anadolu.edu.tr

Salvia (Labiatae) is represented in Turkey by 94 taxa belonging to 89 species with 50 % endemism (1, 2). Water distilled essential oils from the aerial parts of four endemic species of *Salvia* from Turkey were analysed by GC and GC/MS. oil yield (%) and main components (%) were as follows: *Salvia divaricata* 0.87 1,8-cineole (34.4), linalyl acetate (10.3), *Salvia eriophora* 0.80 caryophyllene oxide (13.7), heptacosane (8.9), *Salvia longipedicellata* trace alpha-caryophyllene (47.9), alpha-humulene (11.5), *Salvia pilifera* 0.18 alpha-pinene (9.4), myrcene (5.3), ar-curcumene (5.1)

References: 1. Hedge, I.C., 1982, *Salvia*, Flora of Turkey and East Aegean Islands; Univ. Press: Edinburgh, P.H. Davis (Ed.), Vol.7, 400-461. 2. Ba?er, K.H.C. 2002. Aromatic Biodiversity Among the Flowering plant taxa of Turkey, Pure Appl.Chem., 74 (4):527-545.

THE ACETONE EXTRACT FROM DRIED MATERIALS OF *COMMELINA BENGHALESIS* HAVE ENHANCED EFFECT ON THE CANCEROUS JURKATS T-CELLS

Mokgotho M.P., Masoko P., Mbazima V.G., Sibuyi N., Mampuru L.J.

Biochemistry, Microbiology and Biotechnology Department, University of Limpopo, Sovenga, South Africa, Limpopo Province, 0727

Cancer is one of the major human diseases and causes considerable suffering and economic loss worldwide. Development of safe products for the prevention and treatment of all human cancers is needed. Because of plant safety, there has been growing interest in the beneficial health effects of medicinal plants, with the presence of phenolic antioxidants believed to have the protective mechanisms. Plants have been demonstrated to be a very viable source of clinically relevant anticancer compounds. *Commelina benghalensis* is one of the commonly used medicinal plants in China, Ethiopia and in South Africa. The objective of the study was to examine the antioxidant activity, its phenolic content and the effect it might have on the Jurkats T-cell line using the fresh and the dried extracts. The dried and the fresh stems of *C. benghalensis* were extracted with acetone using a soxhlet method and concentrated to dryness with rotary evaporator. The total phenolic content of the extracts was determined by Folin-Ciocalteu method and antioxidant activity was assayed using DPPH method. The total phenolic contents and antioxidant activity of the extracts as tannic acid equivalents were found to be highest in fresh extract. Trypan blue exclusion dye results demonstrated that the dried acetone extracts had inhibited cell proliferation more. The inhibition was in a dose-dependent manner.

Acknowledgements: NRF and University of Limpopo Research office

THE VARIABILITY OF THE ESSENTIAL OIL COMPOSITION IN THE SAGE COLLECTION OF THE GENE BANK GATERSLEBEN

Lamien-Meda A., Schmiderer C., Lohwasser U., Börner A., Franz, Ch., Novak J.

University of Veterinary Medicine, Institute for Applied Botany and Pharmacognosy, Department of Farm Animal and Public Health in Veterinary Medicine, Veterinärplatz 1, A-1210, Vienna, Austria. Aline_meda@hotmail.com, Aline.Lamien-Meda@vu-wien.ac.at
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany. lohwasse@ipk-gatersleben.de

Genebanks are valuable resources for ex-situ conservation and breeding of economic plants and wild plant relatives. Analysing plant secondary compounds of such collections delivers useful information about the variability (heterogeneity) in the collection and enables plant breeders to pre-select accessions when breeding for specific plant secondary compounds like essential oil content and/or composition. For garden sage (*Salvia officinalis* L.), 10 individual plants of each of the 19 accessions available in the genebank were analysed for their essential oil content and composition. The essential oil content was in the range of 0.8% to 2.4%. The essential oil composition was comparable to results already published with the exception of two accessions, which were very low in α -thujone (3%) and β -thujone (<1%), representing a new chemotype in *Salvia officinalis*.

PLANT ANTIFUNGAL EXTRACTS ACTIVE AGAINST PLANT PATHOGENIC FUNGI

Salome Mahlo, Lyndy McGaw, J. N. Eloff

University of Pretoria, South Africa, mamokone12@yahoo.com
University of Pretoria, South Africa, lyndy.mcgaw@up.ac.za
University of Pretoria, South Africa, kobus.eloff@up.ac.za

The antifungal activity of six plant leaf extracts (*Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum*, *Olinia ventosa*, *Vangueria infausta* and *Xylothea kraussiana*) prepared using four extractants (acetone, methanol, hexane and dichloromethane) was investigated. For selection of the plants, preliminary studies showed that out of 350 plant species screened for antimicrobial activity, these plants exhibited the best activities against two animal fungal pathogens, *Cryptococcus neoformans* and *Candida albicans*. A serial microdilution method was used to determine minimum inhibitory concentrations (MICs) of the extracts against seven plant pathogenic fungi (*Aspergillus niger*, *A. parasiticus*, *Colletotrichum gloeosporioides*, *Penicillium janthinellum*, *P. expansum*, *Trichoderma harzianum* and *Fusarium oxysporum*). All plant extracts were active against the tested microorganisms. *B. buceras* extracts had the best antifungal activity against four of the fungi, with MIC values as low as 0.02 mg/ml and 0.08 mg/ml against *Penicillium expansum*, *P. janthinellum*, *Trichoderma harzianum* and *Fusarium oxysporum*, *P. janthinellum*, *T. harzianum* and *F. oxysporum* were the most sensitive fungal species, with the same average MIC values of 0.28 mg/ml, and the remaining four fungi were more resistant to the extracts tested, with MIC values above 1 mg/ml. The number of active compounds in the plant extracts was determined using bioautography with the above-mentioned fungal pathogens.

Breonadia salicina and *O. ventosa* were the most promising plant species, showing at least three antifungal compounds while the other plants showed none in this technique. Bioactivity-guided fractionation of *B. salicina* leaf extract led to the isolation of three active compounds, the structures of which are being elucidated

GLORIOSA SUPERBA: REMEDY OR POISON?

Alfred Maroyi and L.J.G. (Jos) van der Maesen

Botany Department, Rhodes University, PO Box 94, Grahamstown 6140, South Africa
Biosystematic Group, Nationaal Herbarium Nederland, Wageningen University Branch,
Generaal Foulkesweg 37,
6703 BL Wageningen, The Netherlands

The tropical African and Asian plant species, *Gloriosa superba* L. (Colchicaceae) is not only a notorious human and livestock poison, but is also widely used as herbal medicine. The plant's medicinal uses and poisonous properties were collated over its distributional range. *Gloriosa* has caused illnesses and even fatalities in humans and animals due to both intentional and accidental poisoning. Its tubers have been used for centuries for homicide, suicide and inducing abortion. On the other hand, *Gloriosa* is frequently used against digestive, respiratory, cardiovascular troubles and as a sedative medicine. Traditional healers seem to be aware of its toxicity as the amounts they prescribe are such that toxic symptoms are minimized. Using larger dosages usually result in poisoning. Its poisonous properties are due to colchicine and gloriosine, the tropolon alkaloids regarded as the biological hallmark of *Gloriosa*. These alkaloids act slowly, the first toxic symptoms appearing many hours after administration of the species. Death occurs at the earliest in 12 hours from circulatory collapse, respiratory or cardiac arrest [1]. The tropolon alkaloids are unusually rapidly absorbed after oral administration, strongly bound to plasma and cell protein, eliminated through the gall-bladder and reabsorbed through the intestines which explains the long-lasting effect of a single dose or the accumulation of smaller colchicine doses and may also explain the severe gastrointestinal side effects [1]. With such potentially dangerous substances, care in the use of *Gloriosa* as medicine is essential to avoid complications of overdose.

References:

[1] Goodman, L.S. and Gilman, A.: The Pharmacological Basis of Therapeutics. Fifth Edition. McMillan Publishing Co. Inc. / Bailliere Tindall, New York / London 350-352 (1975)

PROSPECTS FOR USE OF ALIEN INVASIVE WEED EXTRACTS AGAINST FUNGAL PHYTOPATHOGENS.

Moraba Meela, Ladislaus Mdee, J. N. Eloff

University of Pretoria, South Africa, Moraba.Meela@mrc.ac.za
University of Pretoria, South Africa, MdeeLK@yahoo.com
University of Pretoria, South Africa, kobus.eloff@up.ac.za

Plant pathogens, in particular plant fungal pathogens, cause a major threat to food security. There are about 5 000 fungal species that destroy economically valuable crops. Plant pathogenic fungi attack most crops in the field and also post-harvest, therefore decreasing their production and their shelf life. The most important method of protecting plants against fungal attack is the use of synthetic fungicides, however the development of resistance towards the use of these fungicides is of great concern. This has spurred on research for search for safe, efficacious and environmentally friendly fungicides. Invasive weeds over time have shown resistance towards fungal pathogens. The possibility that these weeds may contain active principles to resist fungal attack should be considered. Previous work investigating *Melianthus comosus* indicated excellent antifungal activity, but problems in cultivating the species prompted our investigation of readily available invasive weeds. After seven invasive species (*Chromolaena odorata*, *Ipomoea alba*, *Tecoma stans*, *Passiflora suberosa*, *Passiflora subpeltata*, *Aristolochia sp.*, *Solanum seaforthianum*) were screened against 10 plant fungal pathogens, *Rhizoctonia solani*, *Fusarium oxysporium*, *Penicillium janthinellum*, *P. expansum*, *Aspergillus parasiticus*, *Colletotrichum gloeosporioides*, *Trichoderma harzianum*, *Pythium ultimum* and *Phytophthora nicotiana*. Acetone extracts of *Tecoma stans* had promising antifungal activity with an average minimal inhibitory concentration (MIC) value against all the fungi as low as 0.55 mg/ml. Bioassay-guided fractionation of the leaves of the *Tecoma stans* dichloromethane (DCM) extract resulted in the isolation of one major compound, oleanolic acid. This compound had good antifungal activity with an average MIC value as low as 0.13 mg/ml against the 10 plant pathogenic fungi and clear bands against all tested fungi on bioautograms, indicating fungal growth inhibition. Cytotoxicity of the isolated compound was investigated using the Vero monkey cell line and gave an LC50 value of 0.1296 mg/ml. This study shows that weeds may be a source of active antifungal extracts or compounds active against phytopathogenic fungi.

Acknowledgements: University of Pretoria (UP), National Research Foundation (NRF).

ANTI-TRYPANOSOMAL ACTIVITY OF THE ETHANOLIC EXTRACT OF *BUCHHOLZIA CORIACEA* SEEDS

N.E. Nweze, L.B. Fakae and I.U. Asuzu

Department of Veterinary Medicine, University of Nigeria, Nsukka
Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka
Correspondence e-mail: Nwakaego_ernestina@yahoo.com

The seeds of *Buchholzia coriacea* Engler (Capparaceae) are folklorically used in Eastern Nigeria, to treat feverish conditions. The seeds are commonly called wonderful colas. They are chopped up and soaked overnight in the local gin. The infusion is drunk for the cure of such ailments as malaria in humans. Malaria and animal trypanosomiasis are two important protozoan diseases which are endemic in Nigeria. The crude ethanolic extract of *Buchholzia coriacea* seeds was evaluated for anti-trypanosomal activity in mice experimentally infected with *Trypanosoma brucei brucei*. Parasitaemia was monitored using the rapid matching technique as well as the microscopic examination of the buffy coat. Packed cell volume of the blood was monitored using the microhaematocrit tube. In the acute toxicity test no deaths were recorded.

Signs of dizziness were observed at 2000 mg/kg/ip. The extract reduced the number of trypanosomes in the blood. The reduction was dose dependent. At 1000 mg/kg there was a complete clearance of the trypanosomes in the blood 13 days post-infection (PI) and after 3 days of consecutive treatment. Treatment was stopped and by the 15th day PI there was a relapse of infection. Reductions in packed cell volumes (PCV) followed infection. But as treatment was instituted, increase in PCV was observed. The increase was dose-dependent and may have resulted from the effect of treatment as there was a concurrent reduction in parasitaemia. This finding suggests that the ethanolic extract of *Buchholzia coriacea* seed has anti – trypanosomal activity in mice experimentally infected with *T. brucei brucei*.

APPLICATION OF WATER COLOUR PAINT INCORPORATED WITH ESSENTIAL OILS AGAINST *TRAMETES VERSICOLOR* ON RUBBERWOOD

Matan Narumol

Walailak University , THAILAND, nnarumol@yahoo.com

Antifungal activities of water colour paint incorporated with cinnamon oil, clove oil, anise oil, citronella oil, orange oil, tangerine oil, turmeric oil, guava leave oil, nutmeg oil and lime oil against a white-rot decay fungus (*Trametes versicolor*) identified from rubberwood were investigated. The agar dilution method was employed to determine the minimal inhibitory concentration (MIC) by mixing water colour paint with essential oil at ratios 1:1, 1:2 and 1:4. It was found that cinnamon oil, clove oil and anise oil were the strongest inhibitors with the MICs of 1:4. Decay fungi test of *T. versicolor* on treated rubberwood (spray with water colour paint incorporated with essential oils at ratio 1:1, 1:2, 1:4) was then conducted according to the ASTM D1413-05b. After 12 weeks of exposure at 25°C and 100%RH, the average weight loss of each rubberwood sample was determined. The results indicated that rubberwood treated with water colour paint incorporated with cinnamon oil, clove oil and anise oil at ratio 1:4 were classified as “highly resistant”. These findings suggested that water colour paint incorporated with essential oil has good potential for protecting rubberwood products from the attack of *T. versicolor*.

EVALUATION OF THE REPRODUCTIVE EFFECTS OF *CNESTIS FERRUGINEA* (DE CANDOLLE) ROOT EXTRACT IN MALE RATS

Funsho Olayemi, Yinusa Raji, Olajire Adegoke, Matthew Oyeyemi

University of Ibadan, Nigeria, funsho_olayemi@yahoo.com

University of Ibadan, Nigeria, yraji2yahoo.com

University of Ibadan, Nigeria, funsho_olayemi@yahoo.com

University of Ibadan, Nigeria, funsho_olayemi@yahoo.com

Cnestis ferruginea is a shrub used as antibacterial and antimalarial agent in Nigeria.

Many antimalarial medicinal plants have reproductive effects. We evaluated the possible reproductive effects of the methanolic extract and purified fractions from the root in rats. Phytochemistry, column chromatography, thin layer chromatography and nuclear magnetic resonance spectroscopy of the root extracts were carried out. The methanolic extract was administered orally at 500, 1000 and 2000mgkg-1bw to rats weighing 160-220g in the test groups and distilled water to those in the control group for 5, 30 and 60 days. Effects of the extract and purified fractions on male fertility were assessed by sperm function analyses, testicular weight, mating experiment and histological examination of testes. Student's t-test and ANOVA were used to test for levels of significance. There was no lethality by the extract up to 2000mgkg-1bw. Phytochemistry revealed the presence of alkaloids. Acute and prolonged oral administration of methanolic extract caused reduction in sperm count, viability, motility, morphology, testosterone concentration and weight of testes. Each of the six chromatographic fractions (1-6) from the extract administered orally (100, 1000 and 2000µgkg-1bw for 60 days) caused significant reduction in sperm counts, motility, viability, morphology and testosterone values. The extract caused reversible degeneration of testis. However, fractions 3 and 4, which contain quinolizidine alkaloid, caused the highest reduction in fertility. There was recovery from the reproductive effects of the extract after 60 days of withdrawal. The results suggested that *Cnestis ferruginea* possesses reversible male antifertility effects which are due to quinolizidine alkaloid.

ACCURATE QUANTIFICATION OF PURE OR IMPURE SUBSTANCES IN THE MICRO- TO MILLIGRAM RANGE BY ¹H-NMR

Jan Schripsema

Universidade Estadual do Norte Fluminense, Brazil, jan@uenf.br

Frequently the accurate quantification of micro- to milligram quantities is needed, e.g. for the preparation of standard solutions for GC or HPLC quantification, or for biological assays. Weighing these quantities is imprecise and dilutions should be prepared, which is often not possible due to unavailability of larger quantities. Another factor to be considered is that the reference compounds are often impure. Therefore, the weighing of reference standards can lead to large systematic errors in the assays. ¹H-NMR is, in principle, very suitable for the accurate quantification of specific compounds. In the present study, small quantities of cyanobacterial toxins, to be used as standards for GC and HPLC analysis, were quantified, and also a series of novel iridoid glucosides, isolated from *Pentas lanceolata*, was quantified prior to the determination of the specific rotation of the compounds. Two cyanobacterial toxins: Anatoxin-a and [D-Leu1]-Microcystin LR have been quantified using ¹H-NMR. To obtain an accurate quantification to both samples an accurate quantity of pyridine was added as internal standard. Relative to the signals of pyridine, it was possible to determine the precise quantity of the toxins, using specific signals, which did not have any overlap with other signals. In the case of anatoxin-a it was possible to obtain an accurate quantification of a 22.4 microgram quantity using a standard 400 MHz spectrometer. In the case of the iridoid glucosides the major advantage was the insensitivity to impurities in the samples Acknowledgements: FAPERJ, CNPq.

LAVANDULA PEDUNCULATA (MILLER) CAV.: TRICHOMES, ESSENTIAL OILS AND *IN VITRO* CULTURE

Mónica Zuzarte, Maria José Gonçalves, Augusto Dinis Carlos

Department of Botany University of Coimbra, Portugal, mzuzarte@hotmail.com

The morphology and histochemistry of *Lavandula pedunculata* glandular trichomes as well as the chemical composition and intraespecific variability of its essential oils were investigated. The antifungal activity of the essential oils and their major compounds was evaluated against different pathogenic fungi. Moreover, *in vitro* cultures of axillary meristems were established and the influence of different concentrations of benzyladenine (BA) in the culture medium was analysed. SEM analysis showed the presence of three types of glandular trichomes: peltate, capitate type I and capitate type II, as well as a "mixed" type. Histochemical tests revealed a lipidic and terpenic secretion for all types of glandular trichomes, excluding the capitate type I. The essential oils of 43 samples of *L. pedunculata* from the north and centre of Portugal were isolated by water distillation followed by GC and GC/MS analysis. Quantitative differences were found, particularly in the amounts of 1,8-cineole (2.4 – 55.5 %), fenchone (1.3 – 59.7 %) and camphor (3.6 – 48.0 %). A significant antifungal activity was found against the dermatophyte strains. The essential oil with high contents of camphor was the most active with MIC and MLC values ranging from 0.32 – 0.64 µL/mL, justifying therefore, the use of these oils against dermatophytosis. *In vitro* cultures of axillary buds of *L. pedunculata* were established in MS medium supplemented with different concentrations of benzyladenine (BA). The production of axillary shoots was favored by the use of 0.25 mg/L of BA and the rooting was spontaneous. The *in vitro* plantlets showed an indument and an essential oil composition very similar to that of respective field-growing plants, and may be used for the same purposes as wild type plants.

QUANTITATIVE STRUCTURE – ACTIVITY RELATIONSHIP OF SUBSTITUTED GLUCOSYLFLAVONE AND ITS ANALOGUES BY MOLECULAR ORBITAL AND LINEAR FREE ENERGY APPROACHES.

Cindrella Louis, Jeyachandran Robert

National Institute of Technology, Tiruchirappalli 620015, INDIA, cind@nitt.edu
St. Joseph's College, Tiruchirappalli 620 002, INDIA, jeyachandranrobert@gmail.com

Glucosylflavone (5-hydroxy7-methoxy6c-glucosylflavone) the active principle from a natural source (*Sphaeranthus indicus* Linn) has been extracted and its medicinal activity assessed. An attempt has been made in this study to evaluate the structure – activity relationship of the active principle of this important medicinal plant and its structural analogues based on Molecular Orbital and Linear free energy approaches. The structure plays an important role both in the cases of structurally specific and non-specific drugs. As the characteristics of structurally non-specific drugs depend on the equilibrium conditions, the concentration of the drugs in cell lipids becomes an important criterion in deciding about the efficacy of the drugs. The partition coefficient of the active principle and its analogues indicate that the methoxy,

ethoxy and propoxy substituents increase thereby hinting at a lower requirement of the propoxy substituted derivative at a lower concentration. Higher -oxy- derivatives did not bring about the proportionate advantage. The molar refractivities and the Connolly Accessible area of the active principle and the analogues highlight the structure- activity correlation of the models studied and their synthesis and pharmacological evaluation. As the binding of the drugs to protein enforce steric constraints, structural tailoring needs to consider the steric energy of molecular models. The steric energy of the analogues are computed using GAUSSIAN03 at B3LYP 6-31G wave function and compared with that of the active principle. The molar refractivities of the active principle and the molecular models range between 111.4 to 120 cm³/mol highlighting the distinct correlation between the structure and the biological activity.

VARIABILITY OF ROSMARINIC ACID, CARNOSIC ACID AND ANTIOXIDANT ACTIVITY IN THE SAGE COLLECTION OF THE GENE BANK IN GATERSLEBEN

Lamien-Meda A., Nell N., Lohwasser U., Börner A., Franz C., Novak J.

University of Veterinary Medicine, Institute for Applied Botany and Pharmacognosy, Department of Farm Animal and Public Health in Veterinary Medicine, Veterinärplatz 1, A-1210, Vienna, Austria. Aline_meda@hotmail.com, Aline.Lamien-Meda@vu-wien.ac.at
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, 06466 Gatersleben, Germany. lohwasse@ipk-gatersleben.de

Salvia officinalis L. (common sage) is a well-known medicinal plant with a variable essential oil composition, and an interesting anti-oxidative potential which is due predominantly to two main substances and their derivatives, rosmarinic and carnosic acid. In this study the rosmarinic acid, caffeic acid and some anti-oxidative parameters (total flavonoids, total phenolics, DPPH, FRAP) of 19 accessions of sage were evaluated in the sage collection of the genebank in Gatersleben (Germany). The aerial parts of 10 individual plants of each accession cultivated in 2006, were collected two times (2007 and 2008, from the same experimental field) at the beginning of the flowering period, and used for this study. The rosmarinic acid content was 6-46 mg/g in 2007 and 9-25 mg/g in 2008, and correlated well with all the other antioxidant parameters e.g. total flavonoid, total phenolics, DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric reduction antioxidant power) antioxidant activities. The carnosic acid evaluated only with 2008 sample was varying from 0.5 to 4.3 mg/g and had low significant correlation with the antioxidant parameters. The results showed altogether the high potential of *S. officinalis* which is already in use as a natural antioxidant, due to its main phenolic compound, rosmarinic acid and carnosic acid. The present study showed a high variability between and also within accessions through the studied parameters, which could be exploited by plant breeders in sage genotype selection.