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BOOK OF ABSTRACTS

CONTENT

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B001 The effect of metal salts and metal complexes with HDEHP on the separation of carbohydrates by TLC method on Diol-silica plates by the use of anhydrous mobile phase

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The present work is concerned with the optimisation of chromatographic conditions for TLC separation of sugars, which occur in nature. The effects of metals (Sr, Ca, Zn, Ni, Cu, Ag) introduced to chromatographic system in the form of salts (1) or complexes with HDEHP and different temperatures (-5ºC, 4ºC, 20ºC, 40ºC, 60ºC) of development have been examined. The analysis of main classes of sugars, mono-, di-, and oligosaccharides (30 compounds) has been performed on Diol-plates by use of non-aqueous mobile phase and single development in the presence of metal ions. Thin layer chromatography was performed on Diol-plates (10 cm x 20 cm), E. Merck (Darmstadt, Germany). The impregnation by dipping was conducted in a glass vessel by immersion of the plates in the 0.2 M salt solution or appropriate complex solution for 1.5 hour. Conditions of the impregnation were established by use of AAS method due to Pye Unicam-SP 192 (Cambridge, UK) single-beam atomic absorption spectrometer (2). The plates were developed in a horizontal DS-chamber (Chromdes, Lublin, Poland) at ambient temperature and in a horizontal DS-chamber adapted for temperature control (patent pending) (3). The carbohydrates in D-form were dissolved (2 mg/mL) in acetone-water (3: 1). Spots were detected by spraying the plates with 0.1% naphthoresorcin in ethanol-20% H_2SO_4 (1:1) and drying for 10 min at 80ºC. Molecular modelling of metal complexes with Diol-plates was performed on dual processor PC graphic station with use of PC Spartan Pro v.1.06 software. Complexes were optimized with semi empirical PM3 method. Obtained retention values are not dependent on the concentration of HDEHP in mobile phase. The concentration of the additive used, however, influences the shape of the spots, which is the best for 2%-3% HDEHP in acetone. Differentiation of selectivity is possible in the case of using HDEHP in complex with metal ions. The best selectivity was obtained on Diol-plates with use of HDEHP-Sr (II) in acetone as mobile phase. Compact spots were also achieved at higher temperatures. This phenomenon is connected with increasing sample solubility.

References: 1. Flieger J. and Szumilo H. (2001) J. Planar Chromatogr. 14: 338-342. **2.** Flieger J., Szumilo H. et al. (2002) J. Planar Chromatogr. (in press). **3.** Dzido T. (2001) J. Planar Chromatogr. 14: 237-245.

B002 New interferometric detector for biomolecular interaction analysis

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Direct optical methods for detecting biological interactions have gained wide acceptance in the recent years (1). They have already become competitive in terms of sensitivity with traditional methods, which use radioactive, enzyme, and fluorescence labels for detecting molecules involved in the biochemical reactions, e.g. potential new drugs derived from nature. In contrast to conventional methods, the newly introduced technology allows a label-free detection in a real-time mode and is therefore a valuable tool for biomolecular interaction analysis (BIA).

During BIA measurement, sample constituents of interest interact with the surface of the bio-layer (Figure 1). This leads to a change in the thickness of this layer, resulting in a change in the phase-difference between the interfering waves (laser light 850 nm). This change causes a shift of the maxima and minima in the interference spectrum, which is used for the measurement of the increase in the thickness of the bio-layer. The newly developed method is highly valuable for a screen on new bioactive compounds, especially those derived from nature.

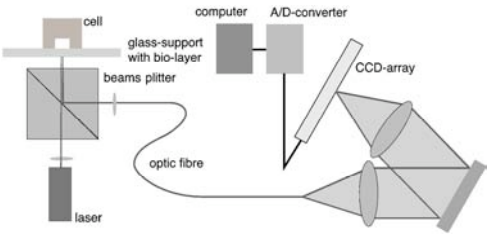

Figure 1. Scheme of the interferometric detector (2).

References: 1. Haake H.M. et al. (2000) Fresenius Journal of Analytical Chemistry 366 (6-7): 576-585. **2.** Nikitin P.I. et al. (2000) Quantum Electronics 30 (12): 1099-1104.

B003 Innovative analytical system for screening on lectins

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Lectins are proteins or glycoproteins from plants or animals, which are able to bind specifically sugar-residues of cell walls or membranes. This reaction changes the physiology of the cell wall and influences the metabolism of the cell. Some lectins of plants stimulate the immune system by unspecific activation of T cells or influence cell division; others cause agglutination of cells (e.g., erythrocytes) and are therefore from therapeutic interest (1).

In a new approach, biomolecular interaction analysis (BIA) was utilized for a screening program on lectins. In a first step, a lectin-binding sugar was covalently immobilized on a surface of thin glass plate (100 µm). Then, the test solution was divided in several parts and different mono-saccharides were added to each part. Individual samples were analysed by BIA and characteristics of the binding-domains were specified. Alternatively, glasses coated with different types of sugars may be used. In dependence of the added monosaccharide, a more or less stable binding to the sugar-surface of the glass-support was monitored. Additionally, the method can be used for a bio-guided fractionation of nature-derived extracts. As an example, BIA analysis was tested for the production of a recombinant lectin in *E. coli*.

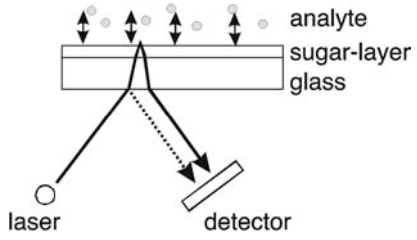

Figure. Scheme of the interferometric detector (2) for the analysis of sugar-lectin interactions. The reflected light of the sugar-layer is analysed by a CCD-array.

References: 1. Tsokos M. et al. (2002) Virchows Archiv-An International Journal of Pathology 440 (2): 181-186. **2.** Nikitin P.I. et al. (2000) Quantum Electronics 30 (12): 1099-1104.

B004 Recombinant fluorescent proteins for testing of affinity modules in phytochemical analysis

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To examine the quality and content of bioactive compounds in herbal medical products, modern affinity techniques, e. g. those based on affinity modules, can be used. These modules consist of polymeric material coated with biomolecules (e.g., antibodies) which specifically interact with compounds of interest. To characterise the quality of modules, a powerful tool for testing based on recombinant DsRed was developed. DsRed is a recently discovered flourescent protein from a corallimorpharian of the *Discosoma* genus exhibiting an intrin-sic and unique red fluorescence. Further on, DsRed is of impressive brightness and stability against pH changes, de-naturants, photobleaching, and does not require any cofactors for fluorescence (1).

After functionalising the polymer-surfaces of modules by plasma-treatment, a spacer was introduced which displayed a metal-chelating group at its outer end (Figure 1). In the next step, Ni-ions were trapped and bound to recombinant His-tagged DsRed. The modified DsRed was overexpressed in *E. coli* for the first time. A nearly quantitative immobilisation of this model protein was obtained for solutions containing 5 - 30 µg of His-DsRed, related to one affinity module with a weight of 50 mg. Immobilisation of His-DsRed could be performed within 120 min. Affinity modules allowing coupling of proteins as monolayers as well as those carrying an polypropylene-glycol gel structure at the inner surface were tested. The latter one showed a significantly better binding capacity and shorter immobilization times.

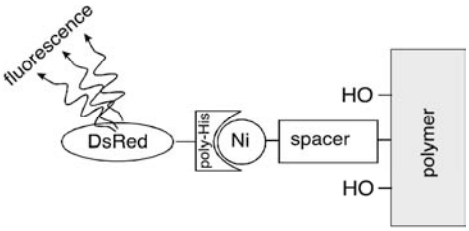

Figure 1. Immobilization of DsRed on polymeric surfaces.

References: 1. Baird, G.S. and Tsien, R.Y. (2000) Biochem. 97, 11984-11989.

B005 Polymer based affinity modules for modern phytochemical analysis

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Most extracts derived from nature are highly complex mixtures of various compounds. But quality, efficacy and also toxicity of those extracts used for pharmaceutical purposes are often related to a small number of substances. Using newly developed affinity modules, analysis of compounds of interest can be performed out of a crude extract. These modules are based on polymeric materials and carry an immobilized bio-component, which is responsible for the specific recognition of a target molecule. In recent years, several immobilisation techniques for enzymes, antibodies, glycosides and other molecules were published (1), but these methods refer to expensive base-materials (*e.g.*, polymeric carbohy-drates, silica-materials). The aim of investigations described here was the development of inexpensive and multifunc-tional affinity modules for a rapid analysis of single compounds.

Poyethylene and polypropylene were used as porous polymeric materials. After activation of the surface, a spacer was attached followed by covalent coupling of a specific affinity-group (2,3). This affinity-group is able to interact with defined molecules like lectins, avidin or metal-chelating proteins (Figure 1). The latter molecules are coupled to the biologic recognition elements as antibodies, antigens or DNA, which have already been developed. The modules can be used for phytochemical analysis, detection of microorganisms in pharmaceutical preparations, and DNA-analysis. Systems for detection of pyrrolizidine alkaloids and detection of microbial contaminants (*e.g. Salmonella*) are under current testing.

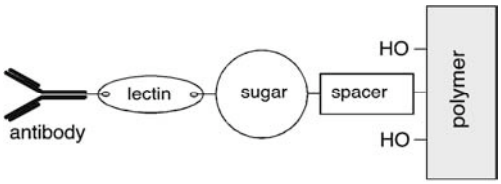

Figure 1. Coupling of an antibody via lectin-sugar interactions.

References: 1. Srere, P.A. and Uyeda, K. (1976) Meth. Enzymol. 44, 11-19. **2.** Keusgen, M. et al. (2001) Biotechnol. Bioeng. 72, 530-540. **3.** Milka, P. et al. (2000) Biotechnol. Bioeng. 69 (2000) 344-348.

B006 Comparison of photometric and HPLC-ELSD analytical methods for *Tribulus terrestris*

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The aerial parts of *Tribulus terrestris* have been used to manufacture the Tribestan product which has been used to treat male and female sexual disfunctions, this action is attributed to the steroidal saponins protodioscin and protogracillin. A photometric method of analysis (1) has been used by this manufacturer and adopted by other manufacturers in Bulgaria. The saponins have very poor UV absorption and are unable to be determined by HPLC-DAD, a recent report has outlined the determination of steroidal saponins by RP-HPLC with ELSD detection (2). A comparison of the photometric method and the HPLC-ELSD shows that the photometric method is unable to accurately measure the level of protodioscin and related compounds in even an ideal Tribulus preparation. The poor specificity of the photometric method leads to increasingly more inaccurate results once the sample constituents vary. A wide variation in the saponin distribution of drug samples of different geographical and plant part has been found and has shown that the photometric method responds to a range of compounds, not just protodioscin, samples with no protodioscin or related saponins still respond to this method. In samples with no protodioscin the absorption spectrum is markedly different to that obtained with protodioscin.

With the ready availability of reference standard materials of high purity and confidence, adoption of the HPLC-ELSD as the preferred method of analysis of *Tribulus terrestris* products is strongly recommended.

References: 1. Gjulemetowa, R. et al, (1982) Pharmazie, 37: 296. **2.** Ganzera, M. et al, (2001) J. Pharm. Sci., 90: 1752-1758.

B007 Detection of aristolochic acid in Chinese phytomedicines and dietary supplements used as slimming regimens

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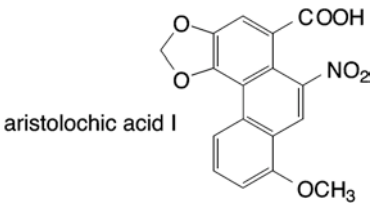
During the last decade, numerous cases of intoxication, resulting mostly in end stage renal failure, have been reported after consumption of slimming regimens prepared from Chinese plants. These intoxications were associated with species from the *Aristolochia* genus, such as *Aristolochia fangchi*, known to contain very nephrotoxic and carcinogenic compounds named aristolochic acids (1). A thin layer chromatography assay was developed for a preliminary identification of aristolochic acid I in complex mixtures. Using a new method based on direct on-line coupling between HPLC and UV-DAD/MS (2), aristolochic acid I was detected and, when possible, quantified in forty-two herbal preparations sold on the Swiss market. Four of them were found to contain aristolochic acid I and two were suspected to contain aristolochic acid derivatives. Immediate removal of these products from the Swiss market was called for.



aristolochic acid I

Acknowledgements: The authors would like to thank the Swiss National Science Foundation for financial support (grant n° 2000-063670.00 to Prof. K. Hostettmann).

References: 1. Vanherweghem J.L. et al. (1993) Lancet; 341: 387-391. **2.** Ioset, J-R. et al. Planta Med. (in press)

B008 A new alkaloid from *Heliotropium ovalifolium*

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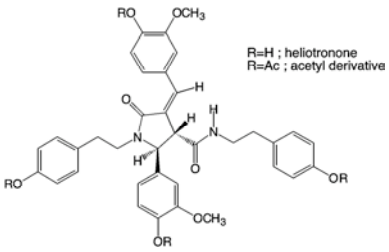
Since hypothesis of a plant intoxication was suggested to explain the flaccid trunk paralysis in free-ranging elephants on the southern shore of Lake Kariba in Zimbabwe (1), we focused our investigations on the flora of Fothergill Island. After observation of the elephant feeding habit, *Heliotropium ovalifolium* Forsk. (Boraginaceae) was suspected to be responsible for the floppy trunk syndrome. Two antifungal benzoquinones, heliotropinones A and B, have already been described from the dichloromethane extract of *H. ovalifolium* aerial parts (2). A new complex alkaloid named heliotrononе have been isolated from the same extract. Its structure was elucidated by spectrometric methods including ESI-HR, EI, D/CI mass spectrometry, ^{1}H, ^{13}C and 2D NMR experiments.



Acknowledgements: The authors would like to thank the Swiss National Science Foundation for financial support (grant n° 2000-063670.00 to Prof. K. Hostettmann).

References: 1. Kock, N.D. et al. (1994) Journal of Wildlife Diseases 30: 432-435. **2.** Guntern, A. et al. (2001) Phytochemistry 58: 631-635.

B009 Quantitative evaluation of coumarins from HPLC data without reference substances

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HPLC is the most common method used for the analysis and quantification of coumarins in plant extracts. The detection is usually made by UV at 254 nm or 310 nm (1, 2) or by multiple wavelength absorption with a diode-array detector (DAD) (3). Quantification is achieved using reference substances if available, which is often not the case. Due to the high variability of the specific absorptions ($A_{1\%,\ 1cm}$) of the different coumarins, normalisation (peak area percentages) is not exact enough. Therefore, in the course of our chemotaxonomic investigations of coumarins in Apiaceae fruits an evaluation method was worked out providing a formula which can be applied independently of reference substances and which allows the quantitative calculation of all known and unknown coumarins within plant extracts from one HPLC run. For the separation of the coumarins HPLC (RP-18, methanol-acetonitrile-water, gradient) was used. DAD detection and a sufficient peak separation are the prerequisites for the successful application of this simple method of calculation. The formula is as follows:

$$c\ (mg/ml) = (F / w) \cdot (A_{max}/A_{250}) \cdot (1 / v) \cdot 1.989 \cdot 10^{-5}$$

c (mg/ml) = content of a coumarin in the test solution; F = peak area [mAu · sec]; w = peak width [min]; A_{max} = highest absorption within the spectrum [mAu]; A_{254} = absorption at 250 nm within the spectrum [mAu]; v= volume of injection [µL].

The conversion factor (1.989×10^{-5}) was calculated from HPLC data of the furocoumarin xanthotoxin and is based on the assumption that the ratio of the highest absorption of a compound and its absorption at wavelength 250 nm is a constant and therefore independent of the concentration of the compound in the extract. The paper presents the causality and the deduction of the above-mentioned formula. Furthermore an example will be given calculating the composition of the coumarin fraction of *Heracleum mantegazzianum* fruits with 6 furocoumarins.

References: 1. C.A.J. Erdelmeier et al. (1985) J. Chromatography 346: 456-460. **2.** M.A. Hawryl et al. (2000) J. Chromatography A 88: 75-81. **3.** H. Vuorela et al. (1989) Planta Med: 55, 181-184.

B010 Monoclonal antibodies against oleanolic acid – a tool for a novel strategy in herbal drug screening

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Recently we reported on generation and characterisation of monoclonal antibodies against furanic labdane diterpenes (1). The methods were now adapted to produce monoclonal antibodies against oleanolic acid. This compound is a basic structural part of saponins in numerous medicinal plants which are known to exhibit a great variety of pharmacological effects. Total estimation of saponins is though difficult due to the limitations of detection methods, which normally refer to biological effects or analysis of a single compound.

In order to create an antigen suitable for production of antibodies oleanolic acid had to be coupled to a protein carrier. Thus, oleanolic acid was conjugated with BSA or thyroglobulin either directly or via succinic acid. The oleanolic acid-protein conjugates were used for immunisation of Balb/c mice. According to the methods developed by Köhler and Milstein (2) hybridoma cells were established. Cell-lines producing monoclonal antibodies were selected by ELISA.

Up to now four immunisations were performed. Screening of primary hybridoma cell lines resulted in selection of more than forty cell lines for further characterisation. Three cell lines were characterised in detail using a set of more than twenty triterpenes which were tested in competitive ELISA. For example, specificity of monoclonal antibodies produced by cell-line 10F10 is directed towards structural features of rings A and B. It was shown that the monoclonal antibodies are suitable to recognise the target structure also in crude extracts of herbal drugs.

One application of these monoclonal antibodies will be a target-structure orientated screening of plants from the Brazilian Atlantic Forest. Using this screening system the detection of plants rich in triterpenes will not depend on indirect physical effects, analysis can be performed directly in the fields and even less stable compounds will be detected.

References: 1. Brand, K. et al. (2001) GA annual meeting, Erlangen, Germany. **2.** Köhler, G. and Milstein, C. (1975) Nature 256, 495-497.

B011 Evaluation of chemical stability and skin irritation of lawsone methyl ether in oral base

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Lawsone methyl ether (2-methoxy-1,4-naphthoquinone) was first isolated from the dried flowers of *Impatiens bal - samina* L. (1). Its activities against *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum gypseum*, *Epider - mophyton floccosum* and *Candida albicans* have been reported. The values of both minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of the naphthoquinone against *Trichophyton* and *Microsporum* were 2.50 µg/ml whereas values for both *Epidermophyton* and *Candida* were 1.25 µg/ml (2). Because of the antifungal activity of lawsone methyl ether, attempts were made to formulate an oral anticandidiasis preparation from semisynthesized lawsone methyl ether. Its acute toxicity, chemical stability and skin irritating property were also evaluated. In this study, lawsone methyl ether was semisynthesized by methylation of lawsone. It exhibited low acute toxicity with LD_{50} of 70.7 mg/kg upon intraperitoneal administration in mice. An oral preparation of 0.5% lawsone methyl ether in sodium carboxymethyl cellulose oral base, appeared to be stable under heating-cooling cycle test. Lawsone methyl ether in oral base did not cause any skin irritation under primary skin irritation test and cumulative skin irritation test. In contrast, the solution of lawsone methyl ether, potassium salt produced erythema with some papulosquamous in the cumulative skin irritation test.

Acknowledgements: Faculty of Pharmaceutical Sciences, Prince of Songkla University.

References: 1. Little, J.E. et al. (1948) J. Biol. Chem. 174: 335-342. **2.** Phadungcharoen, T. et al. (1988) Thai J. Pharm. Sci. 13: 117-126.

B012 Stability testing on typical flavonoid containing drugs

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Stability of herbal drug compounds is essential to guarantee a constant quality of herbal drugs and related finished products during the storage period. Up to now, only few investigations on the genuin herbal drug material exist. In most cases no data about stability of the pharmacological active components or marker substances are documented in the respective monographs.

The aim of the presented work is to examine possible changes in the flavonoid HPLC-fingerprint of the most common flavonoid containing herbal drugs, in order to provide detailed information about quality of the respective herbal drug material during long term and stress testing periods.

For long term testing, herbal drugs are stored at constant conditions of 25°C/60% rH (climatic zone II) according to the ICH-regulations over a 2 year period (1). To accelerate possible changes of the flavonoid pattern, birch leaves were exposed to increased temperatures of 70°C and 100°C. Typical stress conditions, in accordance with the ICH guideline (40°C/75% rH) are also tested (1).

During the storage period, the stability of flavonoids measured as the total flavonoid content by the current pharmacopoeial method (acid hydrolysis of flavonoid glycosides and photometric determination of an Al-chelate complex) is compared with HPLC-fingerprint chromatograms (2). Methanolic extracts of herbal drugs are directly injected in a HPLC system.

It is interesting to note that during long term testing, no significant changes in the flavonoid pattern can be detected. However, samples of birch leaves, stored at high temperatures, showed a decrease of most flavonoids and the total flavonoid content and an increase of the aglycone quercetine. Similar results were obtained for storage at 40°C/75% rH. It can be concluded, that under usual storage conditions, stability of these flavonoids is guaranteed over at least a 2 year period. Only extreme temperatures or humidity cause a significant reduction of the flavonoid content.

References: 1. EMEA (2001) ICH-Guideline: Note for guidance on quality of herbal medicinal products. **2.** Ph. Eur. Suppl. (1998) Monograph “Birch leaves”.

B013 Stability testing on senna and valerian dry extracts

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Quality criteria for the marketing authorisation of herbal medicinal products are permanently increasing. This includes the proof of sufficient stability of the respective plant material or its galenic formulation.
The presented work investigates the changes in quality of different plant extracts under the influence of environmental factors, such as increased temperature and humidity.
The materials included in this study were different methanolic and ethanolic dry extracts, according to the actual pharmacopoea and the corresponding herbal drug of valerian root, sennae pods and sennae leaves. The design of the stability testing was based on the ICH guidelines for stability testing of new drug substances and products (1), with the appropriate storage conditions of 25°C/60% RH, 30°C/60% RH and 40°C/75% RH.
TLC and HPLC fingerprint methods were utilized for investigating the changes in the chemical composition of extracts and herbal drugs. Due to the low content of some critical substances, SPE was used to separate and concentrate the found substances. The identification of the obtained products was confirmed by UV-spectra (HPLC-DAD) and mass spectrometry (LC-MS).
Distinct qualitative and quantitative changes in the fingerprint chromatograms were recognized for nearly all extracts examined under storage conditions of 40°C/ 75% RH. Depending on storage conditions, packaging form and additives, changes in powder conditions and hygroscopicity were recognized. Modifications of the physical parameters correlate with the changes in the fingerprint chromatograms of the extracts and the respective herbal drugs.

References: 1. Committee for proprietary medicinal products, CPMP/QWP/556/96, Note for guidance on stability testing of existing active substances and related finished products, 10/ 98.

B014 Quality control of *Eleutherococcus senticosus* roots: validation of an HPLC-method

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An HPLC method for the determination of eleutherosides B and E was developed based on published methods (1-3) and optimised. The extraction procedure, the extraction solvent and time and need for repeating the extraction till exhaustion were investigated. In the final method the powdered drug is heated on a water bath (60°C) for 30 min in 50% methanol. After cooling down, the solution is filtered into a round-bottomed flask. The residue is treated in the same way for a second and third time. The resulting filtrate is evaporated under reduced pressure until about 10 ml is left in the flask. This residue is quantitatively transferred into a volumetric flask and further diluted. A reversed phase HPLC system was used to evaluate the samples: column: RP-18; mobile phase: a gradient going in several stages from 90% phosphoric acid, water (0.5: 99.5) to 90% acetonitrile; detector: 220 nm.
Because eleutherosides B and E are not commonly commercially available ferulic acid was chosen as external standard. The correction factors for the response of ferulic acid against both eleutherosides were determined and validated (linearity and precision).
The method was fully validated, i.e. the linearity, the precision (repeatablility and intermediate precision on different days and at different concentration levels) and the accuracy (recovery) of the method were investigated and statistically evaluated.
This method, published in Pharmeuropa (4) is currently investigated by the European Pharmacopoeia Commission and open for remarks.

Acknowledgements: National Fund for Scientific Research – Flanders.

References: 1. Wagner, H. et al. (1982) Planta Med. 44: 193-8. **2.** Bladt, S et al. (1990) Dtsch. Apoth. Ztg. 130: 1499-508. **3.** Yat, P. et al. (1998) Phytochem. Anal. 9: 291-295. **4.** Eleutherococcus, PA/PH/Exp.13A/T (01)52ANP (2002) Pharmeuropa 14 (1): 104-5.

B015 Characterization of the protein-core of an arabinogalactanprotein from the roots of *Echinacea pallida* by a new gaschromatographic method

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Preparations of roots of *Echinacea pallida* are well known as an herbal immunostimulant. Pharmacological studies have shown that glycoproteins are considered as an active principle (1).
From an aqueous extract of the roots of *E. pallida* we isolated a fraction with a molecular weight of over 30000 Dalton and purified arabinogalactan-type glycoproteins (AGPs) by precipitations with the β-glucosyl Yariv reagent. Structural investigations of this purified AGP revealed a high carbohydrate moiety (about 90%) (2). Also of interest was the qualitative and quantitative composition of the small protein part (<10%). Amino acid composition was analyzed by gas chromatography using a new method (3). After a specific short derivatization-procedure analysis of more than twenty amino acids is possible within about fifteen minutes.

References: 1. Lohmann-Matthes ML and Wagner H (1989) Z. Phytother. 10: 52-59. **2.** Classen B. and Blaschek W. (2000) Carbohydr. Res. 327(4): 497-504. **3.** Phenomenex (2001), EZ: faast® Protein Hydrolysate Kit manual.

B016 Specificity of polyclonal antibodies directed against an arabinogalactan-protein from pressed juice of *Echinacea purpurea*

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Up to now, standardization of Echinacea preparations, which are used as nonspecific immunostimulants, mostly relates to low molecular weight compounds (1). Only once, a method for detection of a polysaccharide/glycoprotein fraction from an extract of *Echinacea purpurea* has been described (2). From pressed juice of *E. purpurea*, we isolated a high molecular weight arabinogalactan-protein (3) with complement stimulating activity (4). With the aim of standardization of Echinacea preparations on AGP, polyclonal antibodies were raised in rabbits.
To characterize the epitope of the antigen, partial acid hydrolysis of the AGP was carried out. The hydrolysis led to loss of arabinose residues at the periphery of the molecule and resulted in loss of reactivity with the antibodies. This reveals that the antibodies are directed against the carbohydrate moiety of the molecule and not against the protein backbone.
To test the specificy of the antibodies, several AGPs from other plants were tested in a Sandwich-ELISA for cross-reactivities. Although there are great similarities in the sugar composition of different AGPs, there were no cross reactivities to AGPs from cell culture of *Echinacea purpurea*, from roots of *E. pallida* and from gum arabic, and only little cross reactivity to an AGP from the aerial parts of *Rudbeckia hirta*. Interestingly, the cross reactivity to an AGP from roots of *Baptisia tinctoria* increased after reduction of the uronic acid residues of this AGP.
There may be two reasons for this specificity of the antibodies:
1) a particular structure of the terminal sugar residues
2) a specific glycosylation pattern, due to a characteristic amino acid sequence of our *Echinacea purpurea* AGP.

References: 1. Bauer, R. in Prendergast, N.L. et al (1998) Plants for food and medicine, Royal Botanic Gardens, Kew. **2.** Egert, D., Beuscher, N. (1992) Planta Med. 58, 163-165. **3.** Classen, B. et al. (2000) Carbohydr. Res. 327, 497-504. **4.** Alban, S. et al., Planta Med., submitted.

B017 Analytical characterisation of different green teas from world market and investigations on extratability of phenols and alkaloids

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Green tea preparations are featuring a strongly increasing market potential for their antioxidative, chemopreventive, antibacterial and stimulatory effects. As pharmacological active components the flavan-3-ols, the oligomeric procyanidins and the xanthins are known. To establish an overview on the quality of green teas sold on the market an HPLC-method (C-18 column, detection 274 nm, water-MeOH gradient) was established and validated for the simultaneous quantification of the 3 xanthin alkaloids beside catechin, catechingallate, epicatechin, epicatechingallate, epigallocatechin, epigallocatechingallate, gallocatechin, gallocatechingallate and gallic acid. 49 commercialy available green teas were analyzed and those compounds quantified. The tannin content, determined as the sum of catechins, epicatechins, catechingallates, and epicatechingallates varied between 8 and 19 %, while the alkaloid content with coffein being the dominant alkaloid was in a range between 1,7 to 4,7%. Decaffeinated green teas showed strongly reduced alkaloid content, but also a significant reduction of tannins (range 6,7 to 9,2%), indicating that the extraction process is not specific for the alkaloid fraction. Statistical group analysis indicated significant reduction of xanthin and catechin content in older teas. No significant differences were observed between green teas produced in tropical and temperate climatic zones. In these experiments slightly reduced – but not significant different – contents of xanthins and tannins were detected. No significant differences were evaluated between green teas produced as "bio-ecological"-teas with special agricultural limits. No significant differences were observed between green teas commercialized in pharmaceutical specialized trade and food trade. The main differences between green teas are shown to be the way of preparation of the aqueous extract: the longer the extraction time and the higher the stirring of the extracts the higher the respective xanthin and tannin content. Only when 30 min extraction time was chosen a decrease of phenolics was observed. The higher the water hardness, the lower was the tannin content in the extracts.

B018 Polymeric proanthocyanidins from the bark of *Hamamelis virginiana* L.

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Several pharmacological activities have been reported for polymeric proanthocyanidins from the bark of *Hamamelis virginiana* L. Up to now, no study on the exact composition of the polymeric compounds was done. We here present a detailed phytochemical characterization of polymeric proanthocyanidins from the bark of *Hamamelis virginiana* L. The polymers have been isolated from an acetone-water extract. After extraction with petroleum ether and ethyl acetate, water soluble compounds were separated on Sephadex LH-20. The fraction eluted with ethanol contained dimeric to oligomeric proanthocyanidins and high amounts of carbohydrates. The fractions eluted with methanol and acetone-water contained polymeric proanthocyanidins, which were further separated on Sephadex LH-20. The polymers were characterized as follows:

Determination of chain extension units and chain terminating units by complete acid-catalyzed degradation with benzyl mercaptane. Gallic acid, catechin, gallocatechin, epicatechin-4-benzylthioether, 3-*O*-galloyl-epicatechin-4-benzylthioether, epigallocatechin-4-benzylthioether and 3-*O*-galloyl-epigallocatechin-4-benzylthioether were isolated and identified by ^{1}H-NMR-spectroscopy.

Determination of the molecular weight was done by 2 methods: GPC of the peracetates and HPLC-analysis after complete thiolytic degradation; determination of interflavonoid-linkages by partial thiolytic degradation. Proanthocyanidin B1 and B3 were identified. In conclusion, the proanthocyanidin polymers can be described as follows: they are mixed procyanidin-prodelphinidin-polymers. A di- and trihydroxylation of the B-ring occurs at a ratio of about 1: 1. The polymers are completely galloylated at position 3, except the chain terminating unit which is catechin (95%) or gallocatechin (5%). The stereochemistry within the chain is 2,3-*cis*. Interflavonoid-linkages seem to be predominantly 4β→8-bonds, there are hints that 4→6-bonds also occur. Degrees of polymerisation vary from 17 to 29 monomeric units which corresponds to molecular weights of 12900 to 22100.

B019 A simple HPLC-UV procedure for the assay of ginkgolic acids in *Ginkgo biloba* extracts

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Extracts from the leaves of *Ginkgo biloba* L. belong to the most widely used phytotherapeutics. Some alkylphenols (anacardic or ginkgolic acids, cardanols and cardols) have been identified as potentially hazardous constituents in ginkgo extracts (1). Ginkgolic acids found in ginkgo leaves mainly contain C13, C15 and C17 alkenyl chains.

These compounds, besides strong allergenic properties, possess possibly mutagenic and carcinogenic activity and do not contribute to the therapeutic action of ginkgo extracts. Accordingly a requirement for minimum concentrations of these constituents has been included in the monographs of UE and US Pharmacopoeias by establishing a limit value of maximally 5 ppm.

The typical analytical procedure to quantify these constituents involves an enrichment by liquid-liquid extraction of the aqueous *G. biloba* solution with ethyl acetate or aliphatic hydrocarbons (e.g. hexane), concentration of the organic layer and analysis by reversed phase chromatography. This procedure is time consuming and lacks in reproducibility.

Aim of this study is to develop an HPLC-UV method which, without solvent extraction, allows a simple and precise determination of ginkgolic acids. The identification of the ginkgolic acids and related alcohols, and the specificity of the new method were assessed by means of HPLC-APCI-MS and HPLC-DAD studies.

References: 1. Jaggy, H. and Koch, E. (1997) Pharmazie 52: 735-738.

B020 Ontogenetic changes of cysteine sulphoxides in *Allium ursinum* L.

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Leafs of ramson (*Allium ursinum* L., Alliaceae), a wild-growing *Allium*-species of Europe and Northern Asia, are widely used in traditional medicine and as spice. According to garlic (*Allium sativum* L.), the best-known representative of the genus *Allium*, ramson contains a high amount of several cysteine sulphoxides as well as the enzyme alliinase (Figure 1). Volatile sulphur-containing compounds (being formed by the contact of alliinase and cysteine sulphoxides in disrupted-plant material) create the characteristic flavour of *Allium* species and are suggested to have various therapeutical effects. Consequently, high amounts of cysteine sulphoxides have a direct impact on the quality of ransom as a phyto-pharmacon and spice (1, 2).

Cysteine sulphoxides were detected in all investigated plant parts (bulbs, germs, leafs). *A. ursinum* contains methiin and alliin as the main constituents in all parts of the plant and the pattern of these compounds differs from that of garlic, which contains significantly less methiin, but higher levels of alliin. Additionally, traces of isoalliin and propiin were found. Over the vegetation period, the total content of cysteine sulphoxides in leafs decreased from 0.4 % to < 0.1 %. Significant differences were also found for the inner and outer part of the bulb. The entire bulb showed highest levels in early spring (0.4 %) also followed by a rapid decrease down to 0.1 %. Thus, the date of harvest strongly affects the quality of this phyto-pharmacon and its flavouring properties.

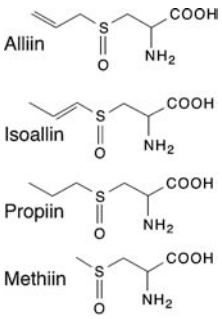

Figure 1. Typical cysteine sulphoxides of *Allium* species like methiin, alliin, isoalliin, and propiin.

References: 1. Krest, I. et al. (2000) J. Agric. Food. Chem. 48: 3753-3760. **2.** Krest I. (2000), Entwicklung und Optimierung eines Alliin-Biosensors, Dissertation Univ. Bonn, Germany.

B021 Isolation and characterization of an alliinase of the basidiomycete *Marasmius alliaceus*

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Species of the genus *Marasmius* are saprophytic basidiomycetes typically growing in woody areas of Europe. *Marasmius alliaceus* (Jacq. ex Fr.) Fr., Tricholomataceae, as well as some other members of the genus *Marasmius*, is reported to have a characteristic smell reminding to garlic. Volatile sulphur-containing compounds, particularly dimethyl-polysulphides according to those found in various *Allium* species, had already been described (1). These compounds are typically formed by the contact of cysteine sulphoxides with the enzyme alliinase followed by secondary chemical reactions (Figure 1). This formation was already investigated in detail for many Alliaceae, but not for Tricholomataceae. Sulphur compounds resulting from this enzymatic digestion are reported to have antimicrobial and various therapeutical effects.

Alliinase activity of *M. alliaceus* was investigated in the recent study. The enzyme was isolated from dried *Marasmius* material. Alliinase activity was found as high as this had been reported for wild *Allium* species (2). Several cysteine sulphoxides were tested as substrates. In contrast to alliinase from garlic (*Allium sativum* L.), the now described enzyme showed highest activity towards isoalliin.

The strong garlic-like smell leads to the assumption, that also high levels of cysteine sulphoxides are present in cells. In order to carry out further analytical studies, *M. alliaceus* was transferred to agar plates and cultivation was carried on under controlled conditions. Further on, *M. alliaceus* might be tested on biological activity.

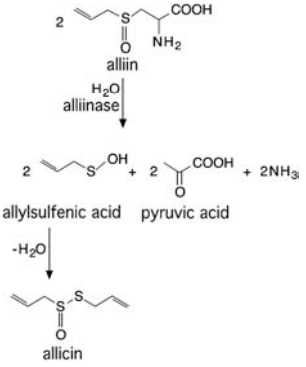

Figure 1. Enzymatic cleavage of the cysteine sulphoxide alliin.

References: 1. Rapior, S. et al. (1997) J. Agric. Food Chem. 45: 820-825. **2.** Krest, I. et al. (2000) J. Agric. Food Chem. 48: 3753-3760.

B022 Detection of allergenic urushiols in *Ginkgo biloba* leaves

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Ginkgo biloba is, like members of the Anacardiaceae family, a plant well known for its content of long chain alkylphenols with allergenic potential. Ginkgo leaves are widely used to prepare extracts for the treatment of peripheral and cerebral circulatory disorders as well as dementia of different aetiology. Since alkylphenols represent a substantial risk factor for adverse drug reactions, suitable techniques for elimination of these compounds from the standardised ginkgo extract EGb 761 have been developed. In order to demonstrate the effectiveness and reliability of this production process, we have now performed a comprehensive qualitative and quantitative analysis of the alkylphenols in ginkgo leaves and followed their fate during manufacturing of EGb 761. For this purpose reference compounds were synthesised or isolated from plant sources. Detection of alkylphenols was performed by silylation GC-MS. Our results confirm that ginkgolic acids constitute the major group of long chain alkylphenols in ginkgo leaves (approx. 2%). Cardanols (3-alkylphenols) were found at a concentration of about 0.1%. Surprisingly, we now observed that ginkgo also contains isourushiols and urushiols (about 20 – 30 ppm), which are by far the most important known contact allergenic compounds from plant sources. Analysis of samples collected at different stages during the patented production process demonstrated that all alkylphenols are removed in parallel. In the final product the content of every alkylphenol was generally below the detection limit of 0.05 ppm. Therefore, it can be concluded that proofing the absence of the predominant and easily quantifiable ginkgolic acids provides a reliable means for control of the pharmaceutical quality of the final product.

Acknowledgements: The author wishes to thank E. Koch for critical advice in preparing the manuscript, K. Klessing for provision of synthetic ginkgolic acids and cardanols, and H. Schneider for excellent technical assistance and measurement of GC/MS spectra.

B023 Qualitative and quantitative investigations on St. John's Wort by HPTLC

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Today St. John's Wort, *Hypericum perforatum*, is one of the most important herbal drugs on the market. Because its mechanism of physiological activity is still somewhat uncertain and controversial, quality control of drug, extract and medicinal products is difficult. HPTLC is widely used for fingerprint identification and stability tests of extracts of *Hypericum*. Several official and non-official methods have been published, all of which should be suitable for identification of the drug and detection of hypericin. However, all methods yield significantly different results and most of them do not allow to distinguish hypericin and pseudohypericin with certainty. For convenient detection and quantitation of hyperforin, another active principle of *Hypericum*, so far no suitable TLC method is available.

This poster describes work concerning a thorough comparison of methods for fingerprint identification of St. John's Wort from pharmacopoeias and the scientific literature with respect to performance, reproducibility and stability of the analyte in the chromatographic system. One system, ethyl acetate - dichloromethane - acetic acid - formic acid - water (100:25:10:10:11), was chosen, further optimized and evaluated for possible quantitative determination of hypericin and pseudohypericin using silica gel and silica gel DIOL as stationary phase.

Furthermore a new HPTLC method for detection and quantitative determination of hyperforin in raw material and Herbal Medicinal Products is presented. The method was validated according to ICH guidelines.

B024 Comparative study of the hypericins and hyperforin contents of commercial Saint John's Wort preparations in relation to the recommended intake dosage

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St. John's Wort (*Hypericum perforatum*) preparations are widely used as an effective herbal medicine for the treatment of anxiety and mild depressive disorders. The anti-depressive effect of this phytomedicine involves multiple bioactive constituents and several neurochemical systems. The main active compounds are: the phloroglucinols hyperforin and adhyperforin, responsibles for the inhibition of serotonin, norepinefrine and dopamine re-uptake (1), the naphthodianthrones hypericin and pseudohypericin, affecting the dopamine turnover (2), and the flavonoids, that interact with the benzodiazepine binding sites of the $GABA_A$ receptor (3). However, unfavourable effects of these active compounds have also been reported (4). It seems then, that aiming at an adequate prescription, the contents of these active compounds in formulations may provide a more relevant information than the amount of the herbal drug or the amount of a dried extract.

This work describes the study of 17 commercial *Hypericum perforatum* preparations (capsules and tablets) aiming at the quantification of the major active compounds. The commercial preparations have been purchased in pharmacies and in herbal-dietetic food stores from different European countries. The analysis of all samples includes solvent extraction, sonication and chromatographic analysis. Basically, this methodology follows the chromatographic procedure described in the literature for the routine analysis of St. John's Wort preparations (5). The obtained results show significant differences in the contents of hyperforin, hypericin and flavonoids depending on the specific commercial preparation. It was also observed that, in some preparations, the recommended dosage do not strictly correspond with the contents of these active compounds. This fact results in very different recommended intake of hypericins and hyperforin per day ranging from 0.20 to 25 mg and from 0.35 to 55 mg, respectively.

Acknowledgements: Financial support from the University of Alcalá (ref: UAH 2002/062) is greatly acknowledged.

References: 1. Jensen et al. (2001) Life Sci. 68: 1593-1605. **2.** Butterweck et al. (2002) Brain Res.930: 21-29. **3.** Baureithel et al. (1997) Pharm. Acta Helvet. 72: 153-1574. **4.** Di Carlo et al. (2001) TiPS 22: 292-297. **5.** Li et al. (2001) J. Chromat. B 765: 99-105.

B025 Analysis of non-polar and polar compound classes of *Piper methysticum* Forst. by Coordination Ion Spray – Mass Spectrometry (CIS-MS)

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For the on-line characterisation of plant extracts LC/ESI-MS is a widely used tool. However, the disadvantage of this method is the poor sensitivity of non-polar components in several plant extracts. Coordination Ion Spray – MS (CIS – MS) helps to overcome this lack of sensitivity, the characteristic weakness of electrospray.

CIS-MS is a new ionisation method in which positively and negatively charged complexes are formed by the addition of a suitable central atom to the analytes, and these complexes can be detected by mass spectrometry. Since both polar and non-polar organic compounds can form coordination compounds with an appropriate central atom, this form of ionisation is highly versatile (1-3).

For on-line LC/CIS-MS, the solution containing ions of a high complex binding affinity with analytes is added by sheath-flow technique. Various metal ions such as silver, copper, nickel, alkaline and earth alkaline metals have already been used for positive ion CIS-MS. Furthermore, central atoms with an electron deficiency such as boron can be used for the formation of negatively charged coordination compounds. CIS is not only an excellent tool in coupling HPLC and CEC with MS by a coaxial sheath-flow interface combining both applicability to a wide variety of compound classes with high sensitivity, but also offers additional structural information when used in combination with MS/MS.

These advantages are demonstrated in our extensive analysis of *Piper methysticum* Forst. (Kava-Kava; Piperaceae). We show the excellent complexation properties of the several kavapyrones leading to a more precise peak identification than LC/ESI-MS.

References: 1. Bayer, E. et al. (1999) Angew. Chem. Int. Ed. 38: 992. **2.** Rentel, C. et al. (1999) Electrophoresis 20: 2329. **3.** Rentel C. et al. (1998) Anal. Chem. 70: 4394.

B026 Rapid quantification of kavapyrones and water content in Kava-Kava extracts by Near-Infrared Reflectance Spectroscopy (NIRS)

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Kava-Kava (*Piper methysticum* Forst, Piperaceae) has been used for hundreds of years as an intoxicating beverage in ceremonial rites and in traditional medicine by Pacific Islanders. In Europe preparations of the plant are used for the treatment of anxiety, restlessness und uptightness. The kavapyrones are known to be the active components and are used for standardization. Several HPLC methods for the determination of the six major components (methysticin, dihydomethysticin, kavain, dihydrokavain, yangonin, demethoxyyangonin) have been published (1-3). Although these methods meet all the regulatory criteria of qualitative and quantitative determination, they are time and solvent consuming.

In our work we show that NIRS is a versatile alternative method for characterising Kava-Kava extracts. It is a rapid, non-destructive and cost-effective method allowing simultaneous determination of the components by multivariate analysis (4). A quantitative NIRS method was established for the determination of kavain, total kavapyrones and water. Reference measurements were performed by HPLC and Karl Fischer titration.

Using Partial Least Squares (PLS) regression, a multivariate calibration was done for the water content, kavain and total kavapyrones using PLS2. Satisfactory calibration statistics were obtained for kavain with a root mean square error of calibration (RMSEC) of 0.05 and a root mean square error of prediction (RMSEP) of 0.06 at a concentration range from 4.5 to 7% in the dry extracts. For total kavapyrones we obtained an RMSEC of 0.08 and an RMSEP of 0.09 at a concentration range from 28.8 to 31.3% and for water we obtained an RMSEC of 0.03 and an RMSEP of 0.03 at a concentration range from 1.5 to 4%. The study emphasizes the potential of NIRS as a rapid and highly effective alternative method to conventional quantitative analysis of plant extracts.

References: 1. Ganzera, M., Khan, I.A. (1999) Chromatographia 50: 649-653. **2.** Boonen, G. et al. (1997) J. Chromatogr. B 702: 240-244. **3.** Gracza, L. et al. (1980) J. Chromatogr. 193: 486-490. **4.** Molt, K. et al. (1997) Pharmazie 52: 931-937.

B027 Identification and quantification of main components of *Chamomilla recutita* (L.) Rauschert oily extracts

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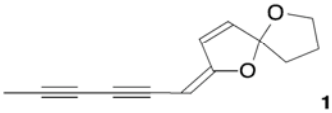
The composition of *Chamomilla recutita* (L.) (i) extracts obtained by polar solvents (water, ethanol) is well investigated. They contain flavonoids, coumarins, sesquiterpene lactones as precursors of chamazulene, etc. However, extracts of (i) obtained by non-polar natural plant fixed oils (soya, olive, etc.) are significantly enriched by more hydrophobic components.
The main constituents of the volatile fraction of (i) oily extracts were identified by their mass spectra and GC retention indices on standard non-polar phases. They are two isomeric (*E*) and (*Z*)-2-[2,4-hexa-di-yn-yl]-1,6-dioxa-spiro-[4.4]-non-3-enes (**1**, $C_{13}H_{12}O_2$, MW 200, trivial name "en-yn-dicycloethers") known since the beginning of 1960s. Non-volatile fraction of (i) oily extracts contains minor quantities of coumarins and flavonoids, but the major component which has been isolated by preparative HPLC and characterized by mass, UV-spectra and LC retention indices was identified as posthumulone (**2**, $C_{19}H_{26}O_5$, MW 334),:



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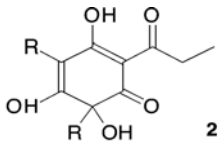

2 R = prenyl

The presence of compounds of humulone and lupulone series (so-called bitter α– and β-acids) is considered as typical only for hop extracts and has never been reported previously for chamomile, where they prevail over other compounds only in extracts obtained by non-polar solvents like natural plant fixed oils.

Acknowledgements: This work was supported by Moscow pharmaceutical factory.

B028 Substances in *Echinacea pallida* root; variation due to extraction procedures

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The genus *Echinacea* is a member of the daisy family (Asteraceae). The three most common species are purple coneflower (*Echinacea purpurea*), narrowed-leaved purple coneflower (*E. angustifolia*) and pale purple coneflower (*E. pallida*). All three have a long history of medicinal use, both in the United States and Europe. They are commercially important sources of phytopharmaceuticals and other medicinal preparations, and are widely used for self-medification of mild respiratory infections. Ethanolic or aqueous extracts and liquors obtained by pressing are derived from the root (radix) as well from the herbal parts (herba) of the plants. The chemistry of some of the *Echinacea* species is well documented (1). The extracts contain varying concentrations of flavonoids, essential oils, polysaccharides, derivatives of caffeic acid, polyacetylenes, alkamides and alkaloids, and several components give stimulation of the non specific immune system.
The aim of the investigation presented, is to show the relationship between content of low and high molecular weight substances in different extracts of *E. pallida* root.
The low molecular weight substances were analysed by TLC and the high molecular weight (polysaccharides) by methanolysis followed by trimethylsilylation and GC. To investigate the biological activity, tests for radical scavenging and complement fixing ability were used. The fractions capability to precipitate Yariv antigen, were also tested. The different extraction procedures gave varying amounts of derivatives of caffeic acid, including echinacosid. These compounds were radical scavengers, but were inactive in the complement fixing test. The different extraction procedures also gave variations in the carbohydrate content and composition. Several of the high molecular weight fractions showed high biological activity in the complement test system.

References: 1. R. Bauer (1996), Z. Ärtzl. Fortbild 90: 111-115.

B029 Total metabolite profiling of *Matricaria recutita* L by high field ^{1}H NMR spectroscopy - the effect of origin and extraction methods

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Phytomedicine has been used for many centuries, and its use continues to increase. Quality control of phytomedicines is currently under intense scrutiny in the commercial sector, by academic researchers, and by regulatory authorities. QC methods can be subjective, and revision of the techniques currently used is required in order to meet the increasing demands of accuracy and reproducibility. Variations in both the origin and the preparation methods of phytomedicines can potentially contribute to inconsistent quality of the final products, even from batch to batch. It is therefore desirable to establish an analytical tool for profiling plant extracts which addresses the totality of the chemical profile, thus providing a means for controlling the quality of a phytomedicine without reference to active molecules or sometimes arbitrarily chosen marker compounds. High-resolution ^{1}H NMR spectroscopy combined with chemometric analysis offers an innovative way to analyse metabolic changes. Here we have demonstrated an application using a combination of ^{1}H NMR and chemometric methods and applied them to German chamomile (*Matricaria recutita* L.). The ^{1}H-NMR profiles of chamomile from three different origins, each prepared with three different extraction methods were recorded at 600 MHz. Data were processed and analysed using principal components analysis. The major differences due to the origins and extraction methods can be identified. NMR analysis of the whole extract showed Egyptian chamomile had higher glutamate and proline contents together with a lower sugar content compared to Slovakian and Hungarian sources. PCA score plots also showed separations between the both the extraction and drying process of the samples, thus facilitating the ability to highlight sampling handling issues

B030 Quantitative analysis of steroidal sapogenins in various fenugreek extracts (T*rigonella foenum-grae - cum* L.) by GC-MS

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Steroidal sapogenins, especially diosgenin, are widely used as precursors for semi-synthesis of numerous pharmaceutical drugs, such as contraceptive hormones, anti-inflammatory agents or steroidal diuretics (1,2). Fenugreek (*Trigonella foenum-graecum* L.) has been reported to contain up to 2.2 % (dry weight) of these compounds, mainly diosgenin (3), and thus represents a potentially useful commercial source.

Due to their low volatility, steroidal compounds usually require derivatisation before GC analysis. But due to irregular derivatisation process, this procedure may considerably complicate the interpretation of chromatograms, especially in the case of plant extracts.

In this contribution, we present a quantitative GC-MS method, using the SIM mode without derivatisation. This method allowed to identify and quantify unambiguously three steroidal sapogenins, namely diosgenin (D), smilagenin (S) and tigogenin (T). Analysis were performed using a HP-5 MS column (30 m x 0.25 mm x 0.25 µm), with helium as carrier gas at a flow-rate of 1 mL/min. The injector (splitless, 1 µL) was heated at 280 °C. The temperature program was initial 190°C (1.5 min), from 190 to 310 °C at 7 °C/min and hold at 310 °C for 10 min. For each compound, a quantification ion was selected, as well as two confirmation ions. Target ions were m/z = 271 for diosgenin, and m/z = 273 for both smilagenin and tigogenin. Confirmation ions were m/z = 300 and 342 for diosgenin and 302 and 287 for both smilagenin and tigogenin.

The developed method allowed a sensitive and selective determination of the compounds in seed, leaf and root extracts of fenugreek obtained by microwave-assisted extraction. Typical measured concentrations were for seeds: 0.150 % D, 0.072 % S and 0.068 % T; for leaves: 0.048 % D, 0.038 % S and 0.031 % T; for roots: 0.023 % D, no S detected and 0.006 % T.

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B031 Influence of plant matrix on microwave-assisted extraction process. The case of diosgenin extracted from fenugreek (*Trigonella foenum-graecum* L.).

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Diosgenin, a steroidal sapogenin occurring in fenugreek (*Trigonella foenum-graecum* L., Fabaceae) is distributed in all plant organs.The effect of experimental parameters on microwave-assisted extraction process of this compound was studied.

Due to the number of variables involved in the extraction process, a chemometric approach was selected, which allowed to study the effect of each parameter as well as their interactions. The same experimental scheme was applied to the different plant parts, including air-dried leaves, seeds, fresh leaves and air-dried roots. The three selected parameters were the extraction time, the composition of the extracting solvent (mixture of water and 2-propanol) and the microwave power applied. The amount of solid sample (granulometry < 220 µm), as well as the solvent volume were fixed. Quantification of diosgenin was made by GC-MS.

It was demonstrated that diosgenin extraction from seeds was mainly influenced by the microwave power applied. Optimal conditions for seeds were 30 min, 58 % water, 40 W. In the case of leaves, a microwave power of 40 W during 8 min, with a high proportion of water in the extracting mixture (58 %) was found beneficial. On the other hand, roots required drastic conditions, i.e. long extraction time (24 min), high water proportion (60 %) and high power setting (40 W), probably because of the lignification of this matrix.

Finally, on the basis of a second-order model, optimal conditions were determined for each matrix. The prediction quality of the models was experimentally verified by performing extractions in triplicate for each matrix at the determined optimal conditions. Comparison was made between the experimental and the predicted responses, and results were found to be identical.

In contrast to classical univariate methodology, chemometrics allowed a significant gain in terms of time and reliability with a limited number of experiments.

B032 Headspace solid-phase microextraction combined with gas chromatography-mass spectrometry: a new method for tracing cannabis profiles

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This work describes the application of headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) to the analysis of different cannabis materials. Without any preliminary sample preparation prior to the analysis, the whole plant material (marijuana) and resin (hashish) from different origins were directly submitted to HS-SPME in order to obtain a chromatographic profile of cannabinoids. Target analytes were the three major cannabinoids, cannabidiol (CBD), Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabinol (CBN). The efficiency in cannabinoid sampling of some commercially available microextraction fibers (100 µm PDMS, PDMS/DVB, CAR/PDMS, 1 cm length DVB/CAR/PDMS) was evaluated after optimization of experimental parameters. Sampling was thus performed at 80°C for 2 hours and the fiber was then desorbed at 280°C for 12 minutes. The 100 µm PDMS fiber was found to be the most effective for this application. Under these conditions it was also possible to detect other cannabinoids in some samples. Method repeatability was finally evaluated and provided acceptable results.

This simple, rapid and repeatable method gives characterizing chromatographic profiles and can be applied to identify the quality and the origin of cannabis samples.

B033 Influence of the extraction solvent on the spectrum of extracted substances of *Valeriana officinalis* L. and *Humulus lupulus* L.

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The choice of the extraction solvent determines the spectrum of extracted substances. In the other side the choice of the lead substances for the quality control should be in congruence with the chosen extraction conditions. Extractions of valerian and hop were prepared with aqueous solvents containing 0% - 100% methanol respectively ethanol. For valerian the amounts of valerenic acids and of the free amino acids arginine, glutamine and γ-aminobutyric acid (GABA) were determined by HPLC (1,2). The presence of valepotriates was checked with TLC (3). For hop the amounts of bitter acids, xanthohumol and the free amino acids asparagine and GABA were investigated with HPLC (2,4).

For valerian the results implicate the choice of valerenic acids as lead substances for extracts, produced with a solvent containing more than 40% methanol respectively ethanol. Amino acids could be a possible alternative for aqueous extracts (solvent contains 0 - 60% methanol respectively ethanol).

For hop the bitter acids and xanthohumol as lead substances are only suitable for extracts, produced with a solvent containing more than 60% methanol respectively ethanol. For more polar extracts the amino acids are better suited as lead substances.

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B034 Detection of amino acids via TLC as a rapid method for the screening of hop and valerian

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The quality control of pharmaceutical herbal drugs and products via free amino acids was suggested as a possible alternative within the scope of a dissertation (1). This suggestion was followed for a combination product of hop and valerian. Reasons have been difficulties regarding the quality control of hop: In the PhEur monograph of *Humulus lupulus* L. the proof of identity is done via bitter acids, but a quantitative test is not given (2). Effective substances or the effective principle are not enlightened up to now. The bitter acids, with importance borrowed from the beer production, proved not to be stable for a longer time. This is also the case for other substances like the flavonoid xanthohumol (3).

A possible alternative in the quality control of hop could be the determination of the amino acid asparagine, quantified with HPLC (1). For valerian arginine and glutamine could be used as lead substances for the quality control via amino acids (1). The presence of γ-aminobutyric acid (GABA) in drugs is of general interest because of its possible pharmacological effect as inhibitory neurotransmitter (4). Because there are only few data about free amino acids in drugs, a rapid and simple method was needed to have a faster test than HPLC to analyse a high number of samples.

A TLC method was developed for the simultaneous detection of asparagine, arginine, glutamine and GABA. An aqueous extract will be separated on silica gel with a solvent mixture of 3.5 ethyl acetate : 1.0 acetone : 3.5 methanol : 2.0 aqueous sodium hydroxide (0.3 N) followed by the detection with ninhydrin. The semiquantitative results were confirmed by HPLC. This method can also be used as a rapid check for the selection of hop and valerian batches with high amounts of the desired amino acids.

Acknowledgements: This work was financially supported by Zeller AG, Romanshorn, Switzerland and GlaxoSmithKline, Parsippany, USA.

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B035 TLC detection of the iridoid glucoside aucubin and the phenylethanoid acteoside in Plantaginis lanceolatae folium

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The crude drug Plantaginis lanceolatae folium is described in the DAB (1) and a proposal for a monograph for the European Pharmacopoeia (Ph.Eur.) has been published (2). Characteristic constituents in the crude drug are the iridoids aucubin and catalpol and the phenylethanoid acteoside (3). Aucubin has a liver protective activity (4). Significant antimicrobial activity of the aglycone part of aucubin and catalpol has been found and could nicely be demonstrated using a thin-layer chromatographic bioassay (5). The phenylethanoid acteoside is most likely accountable for an anti-oedemic activity (3). Whereas for the TLC-identification in the DAB monograph (1) aucubin is used as a marker, for the Ph.Eur. (2) it is proposed to use acteoside.

Here we present an easy TLC detection method for both aucubin and acteoside. Using the solvent system, proposed for the Ph.Eur. (2), the iridoid glucoside **aucubin** can be detected on the TLC plate by only heating at about 120°C for 5-10 min. In day light aucubin is visible as a blue zone and in ultraviolet light at 365 nm as a red-brown fluorescent zone. For the detection, **formic acid** (present in the mobile phase) is essential. The detection is very specific; the iridoid glucosides catalpol, harpagoside, gentiopicroside give no such a coloration. The phenylethanoid **acteoside** can also be detected on the TLC plate by only heating. In day light acteoside is visable as a yellow zone, and in ultraviolet light at 365 nm as a blue fluorescent zone. The blue fluorescent zone in ultraviolet light at 365 nm can also be seen without heating. In the new Ph.Eur. monography of Plantaginis lanceolatae folium (6), this new TLC detection method has been adopted.

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B036 Occurrence of phenolic substances in artichoke residues

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The common disposal of the artichoke wastes proceeding from the tinning industry process is as organic mass, animal feed or fuel. In order to assess a new usage of this residue, its phenolic composition was tested. The found compounds exhibit activity as free radical scavengers and quenchers of reactive oxygen species (ROS) (1-3).

The phenolic fraction from an artichoke waste extract supplied by Euromed S.A. (Mollet del Vallés, Spain) was purified by a liquid-liquid partioniting. A clean-up of the sample using Sephadex LH-20 was carried out to eliminate interferences. The different fractions were monitored by thin layer chromatography performed in a mixture of ethyl acetate / water / acetic acid and visualyzed under UV (254 and 360 nm). The similar fractions were joined to gave six final fractions. Liquid chromatography coupled to ionspray mass spectrometry in tandem mode (LC-DAD-MS/MS) with negative ion detection was used to identify a variety of phenolic compounds. Mass spectrometric studies were carried out using an API 3000 triple quadrupole mass spectrometer (Applied Biosystems) equipped with a Turbolonspray source. A C_{18} Luna (Phenomenex) column 50 x 2.1 mm i.d. 3.5 µm particle size was employed in a linear gradient profile with water and acetonitrile both containing 0.1% formic acid. Different approaches were used for the positive identification of a given compound: comparison of retention time and product ion scan spectrum with those of a standard and also the use of other MS modes such as the neutral loss scan to rapid test the presence of glycosides in artichoke samples. As a result of this study, gallic, chlorogenic, protocatechuic, quinic and caffeic acids and their quinic derivatives (caffeoylquinic compounds), esculin, cynarine, scolimoside, catechin, epicatechin and their gallate derivates, luteolin and luteolin-glycosides, naringenin, apigenin, apigenin-glycosides and diglycosides, quercetin and quercetin-glycosides, rutin and kaempferol-3-rutinoside, were identified.

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B037 Identification of phenolic compounds in apple wastes

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The phenolic composition of wastes proceeding from the apple juice industry were studied to give a new usage to this residues as a potencial source of natural antioxidants. Phenolic compounds found in this residues have the structural requirements of free radical scavengers and have potential as food antioxidants (1,2).

An apple residue dry extract produced by Euromed S.A. (Mollet del Vallés, Spain) was evaluated. The extract was cleaned by a liquid-liquid extraction followed by a chromatographic process using Sephadex LH-20. The different fractions were monitored by thin layer chromatography performed in a mixture of ethyl acetate / water / acetic acid and visualyzed under UV (254 and 360 nm). The similar fractions were joined to gave nine final fractions. Liquid chromatography coupled to ionspray mass spectrometry in tandem mode (LC-MS/MS) with negative ion detection was used to identify a variety of phenolic compounds. LC was performed with an Agilent series 1100 separation module equipped with a PDA detector. An API 3000 triple quadrupole mass spectrometer (Applied Biosystems) equipped with a Turbolonspray source was used. A C_{18} Luna (Phenomenex) column 50 x 2.1 mm i.d. 3.5 µm particle size was employed in a linear gradient profile with water and acetonitrile both containing 0.1% formic acid. Positive identification of a given compound was performed on the basis of their retention time and mass spectra in product ion scan or MRM (multiple reaction monitoring) mode compared with those of a standard. In this way, caftaric, chlorogenic, *p*-coumaric and ascorbic acid, catechin and their dimers (procyanidins), quercetin, quercetin-3-rutinoside, quercetin-3-arabinoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, kaempferol, kaempferol-3-glucoside, kaempferol-3-rhamnoside, mircetin-3-glucoside, phloridzin and phloretin xyloglucoside were present in apple waste.

Acknowledgements: CRAFT-Project FAIR-CT-98-9517 financed by the EC. Nufri and Euromed S.A. is acknowledged by supplying the apple waste and extract respectively.

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B038 The occurences of petasine in the leaves of *Petasites paradoxus*

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The sesquiterpene esters petasine, neopetasine and isopetasine are the active principles of *Petasites hybridus* G., M. et Sch. (butter bur, Asteraceae), petasine chemovariety, and may be used in phytopharmaceutical preparations for their spasmolytic effects on the smooth musculature and pain relieving activity in the case of migraine (1,2). These compounds are present in the rhizomes and in lower concentrations in the leaves (3). Recently efforts have been made to use the leaves instead of the rhizome because they are more easier to produce and harvest when the plants are grown in fields (4).

P. paradoxus (Retz.) Baumg. is a further species of the genus *Petasites* growing in Central Europe. It can be differentiated from *P. hybridus* by the shape of the leaves and the structure of the rhizome. *P. paradoxus* occurs in the lime stone Alps predominately on moist lime stone debris. Plant material of this species has been collected in summer 2000 and 2001 in Upper Austria and Styria respectively. The plant parts (leaves, leaf stalks and rhizomes) were extracted with dichloromethane and the extracts were analysed by GC/MS on a nonpolar column (5).

In the leaf extracts of plants from both origins petasine was the main compound followed by neopetasine and isopetasine. Together they made up more than 50-60 % of the fraction extracted. The leaf stalks or the rhizomes did not contain or were very low in these sesquiterpenes. The rhizomes however contained at least three further, not identified sesquiterpenes.

As the concentrations of the petasines in the leaves of *P. paradoxus* were in the same range as in the leaves of *P. hybridus*, leaves of *P. paradoxus* might be an alternative source for the petasines.

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B039 Optimization of furanocoumarins HPLC separation occurring in *Heracleum sibiricum* L. by Drylab software

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Furanocoumarins commonly occurring in Apiaceae family have been in the leucodermy and psoriasis therapy for many years(1). For this reason the plant materials containing psoralen derivatives – the source of these compounds - are the subject of investigations. One of the species containing number of these substances is *Heracleum sibiricum* L. commonly growing in central Europe (2).

The chromatographic separation of furanocoumarins is a difficult methodological problem because of their closely related structures (e.g. positional isomers) and similar retention behaviour. For this reason the furanocoumarins separation has to be performed by computer aided optimization. The aim of our work was the search of possibilities for the separation of the *H. sibiricum* L. extract by RP-HPLC using the gradient elution methods. The gradient profile was found by use of Drylab for Windows computer program. The retention data from two gradient separations (5-100% of methanol or tetrahydrofuran or acetonitril in water during 20 min and 5-100% methanol or tetrahydrofuran or acetonitril in water during 60 min) were set in the Drylab program to found the optimal gradient profile. The experiments were performed using LC-20 Shimadzu liquid chromatograph equipped with detector SPD-10 A-UV/Vis (λ=254 nm), pump LC-10 AT, column oven CTO-10 AS and controlled by SCL-10 A program. Stainless steel column Supelcosil LC-18, length 150 mm, Ø 4,6 mm (Supelco, USA) packed with 5mm particles was used in the experiments.

The complete separation by RP-HPLC using the gradient elution with acetonitril/water of seven coumarins: bergapten, ksantotoxin, izopimpinellin, heraclenin, imperatorin, byakangelicol and byakangelicin, components of *H. sibiricum* L., was obtained for the first time.

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B040 Characterization of betacyanins from *Hylocereus polyrhizus* (Weber) Britton & Rose

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Cactus fruits and red beet are food sources rich in betalains (1). While the betalain profile of red beet is well known, data on the pigment pattern of cacti are comparatively limited. Very recently, the pigment pattern of the red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose has been reported for the first time (2, 3). In the present study, methods for betacyanin characterization and colour evaluation for *H. polyrhizus* pigments are described.

Removal of both mucilages and sugars permitted gentle pigment concentration. Alkaline, acid and enzymatic hydrolysis of betacyanins were used for their characterization. Betacyanins, betacyanidins and organic acids released through hydrolysis were monitored using HPLC-DAD. By electrospray ionization mass spectrometry assignment of eight betacyanins was possible. CIEL*a*b* values were recorded at pH 6.5 in McIlvaine buffer.

By β-glucosidase activity, three pigments were cleaved, thus indicating their glycosidic nature. Acid hydrolysis yielded betanidin and isobetanidin, whereas alkaline treatment resulted in malonic and 3-hydroxy-3-methyl-glutaric acids together with betanin and isobetanin. Therefore, all *H. polyrhizus* pigments were ascribed to the betanin-type. Further characterization was performed by HPLC-DAD-MS analyses. All betacyanins showed identical visible maxima, but lacked absorption indicative of cinnamic acid substitution at 310 nm. Bougainvillein r-I, betanin and isobetanin were found to constitute the non-acylated betacyanins of *H. polyrhizus*, whereas phyllocactin, hylocerenin, their corresponding C_{15} isomers together with a hitherto unidentified betacyanin were acylglycosides amounting to 75% of total pigments. The colour of *H. polyrhizus* was characterized by high chroma and a purple tonality.

H. polyrhizus is a promising source for colouring food, drugs and cosmetics. Besides their attractive appearance, betacyanins exert antioxidant activities with a high affinity to biological membranes (4). Therefore, cactus fruits are especially valuable for future food and health applications.

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B041 Fatty acids composition in fruit of wild rose species

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Rose hips have been used both in treatment and in food industry for many years, mainly because of their high content of vitamins, especially vitamin C. The fruit usually represent a waste material during production of pharmaceutical and nourishing medicaments. In the meantime they are an underestimated source of valuable oil containing unsaturated fatty acids, which are essential for correct functioning of human organism. The data mentioned in references show that content of oils and fatty acids in some of the rose species were partly investigated, however there is lack of complex comparing examinations in this field (1, 2, 3).

In the study comparison of the amount and composition of fatty acids, especially unsaturated ones from 11 rose species growing commonly in Poland was established for evaluating their pharmaceutical properties. The oil was extracted with n-hexane. The obtained oil samples were methylated and fatty acid methyl esters were analyzed using GLC (4, 5). A Hewlett-Packard Model 6890 chromatograph equipped with flame ionization detector was used. Results were quantified by measurement of peak areas. The content of oil in rose fruit of particular species ranged from 6,2% to 12,9%. The highest amount of oil (>10%) was stated in the fruit of *R. rugosa, R. subcanina* and *R. canina*. The composition of oils was similar in investigated species. 17 components were identified. An average composition was estimated as follows: linoleic acid $C_{18:2\ 9,1\ 2}$ (44.4-55.7%), α-linolenic acid $C_{18:3\ 9,12,15}$ (18.6-31.4%), oleinic acid $C_{18:1\ 9}$ (13.5-20.3%), palmitic acid $C_{16:0}$ (2.3-3.3%), stearic acid $C_{18:0}$ (1-2.5%), octadecenic acid $C_{18:1\ 11}$ (0.38-0.72%), eicosanoic acid $C_{20:1}$ (0.3-0.7%), eicosanodienoic acid $C_{20:\ 2}$ (0-0.16%), erucik acid $C_{22:1}$ (0.03-0.17%) and minor fatty acids. The identification of the compounds was performed by comparison of their retention times and mass spectra with data of the authentic samples and references. All of the investigated oils showed a high quantity of essential unsaturated fatty acids ranging from 71% to 78%, which was considered promising for pharmaceutical purposes.

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B042 Epicuticular wax profile for the leaves of *Newbouldia laevis* Seem. and its possible ecological and medicinal significance

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Newbouldia laevis Seem. (Bignoniaceae) is a small tree of the tropical rain forest and Savannah zones of Western Africa that is widely used by the native population for the treatment of various diseases (1). The plant is also used in the Nyabatso agroforestry system for quality crop cultivation between the trees. So far, no studies have been carried out on the leaves of this species which could provide a clue for both traditional medication and the mentioned agroecosystem. Here we report the chemical leaf wax composition.

The surface lipids were extracted from leaves by immersing tissues in chloroform for 30 s and the extractives analysed by GC-MS and GC-FID. Derivatization of sample aliquots was performed using BSTFA as silylating reagent. The average total wax load for the leaves of *N. laevis* was found to be 11.5 µg/cm^2. The triterpenoids oleanolic and ursolic acid constitute the most abundant wax chemical components (9.7 µg/cm^2) with the latter representative as dominating compound (72%), followed by a series of long-chain homologous alkanes (C_{26} – C_{33}) (1.7 µg/cm^2) and traces of fatty acids (C_{22} – C_{33}) (0.15 µg/cm^2), which represent typical constituents of epicuticular waxes. With regard to the seen profile, the presence of ursolic acid in considerable amounts is significant in that this compound is well known to possess antimicrobial (2) and antifeeding properties (3) as well as antitumoural activity (4).

References: 1. Burkill, H.M. (1985) The Useful Plants of West Tropical Africa, Vol. 1, Royal Botanic Gardens, Kew, London. **2.** Collins, M.A. and Charles H.P. (1987) Food Microbiol. 4: 311. **3.** Valencia, E. et al. (1997) Fitoterapia 68: 556. **4.** Liu, J. (1995) J. Ethnopharmacol. 49: 57.

B043 Quantitative analysis of metabolite profiling of root extracts from *Lithospermum erythrorhizon*

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The root extracts of *Lithospermum erythrorhizon* has been used as by itself or in combination with other herbal extracts for wound healing, anti-inflammation, anti-infection and as a dye for staining textiles and pigment for food coloring. Shikonin, the red naphthoquinone pigment, and acetylshikonin have been identified as the bioactive principles in *L. erythrorhizon*. However, a quantitative metabolite profiling analysis of *L. erythrorhizon* root extracts has not yet been reported. In nutraceutic and pharmaceutic point of views, it is important to develop an optimal and reproducible extraction procedure as well as quantitative methods for analyzing index and/or active compounds in order to best quality control in herbal manufacturing. In this paper, we demonstrated that using *n*-hexane as extraction solvent and under optimized extraction conditions the yield of root extract reached approximately 4% (weight/dry weight of root tissues). Thin-layered chromatography, high performance liquid chromatography, IR, and 1D- and 2D-NMR were performed in this study to analyze and identify the compound profile of root extract of *L. erythrorhizon*. In addition to shikonin, we have obtained seven shikonin derivatives, namely acetylshikonin, β-acetoxyisovaleryshikonin, isobutyrylshikonin, β,β-dimethylacrylshikonin, isovalerylshikonin, β-hydroxy-isovalerylshikonin, and deoxyshikonin. The contents of shikonin, acetylshikonin, β-acetoxyisovaleryshikonin, isobutyrylshikonin, β,β-dimethylacrylshikonin, and isovalerylshikonin in the total root extract were 1%, 6%, 5%, 19%, 10%, and 12% (w/w), respectively, as determined using HPLC analysis. These compounds are potentially good candidates as the referencing/index compounds of root extract of *L. erythrorhizon* and four out of the six compounds are first time to be quantitatively characterized. Antioxidant activity of total extracts, shikonin and its derivatives were evaluated and compared using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay.

B044 On-line identification of unstable iridoids from *Jamesbrittenia fodina* by LC-MS and LC-NMR

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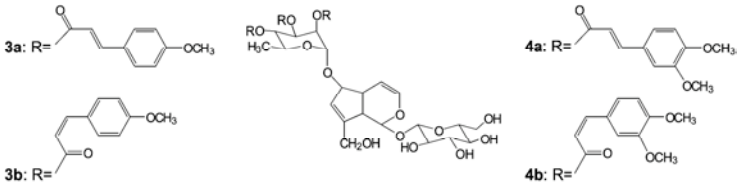
LC-UV-MS analysis of the methanol extract of *Jamesbrittenia fodina* Wild (Scrophulariaceae) revealed the presence of different iridoid cinnamic acid esters. Isolation of these constituents was prevented by instability problems. LC/UV/MS and LC/NMR analysis of the mixtures obtained after a tentative isolation revealed that, in a first instance, instability was due to a light induced *cis/trans* isomerisation of the cinnamoyl moiety (1). Further investigation of related compounds showed an additional instability linked to other chemical transformations. A detailed LC/NMR/MS study of these fractions demonstrated that the modifications occurred on the rhamnose moiety of these iridoids. It could be concluded that the second type of instability was attributable to trans-esterification of the cinnamoyl moiety on the rhamnose unit. The recording of stop-flow LC/NMR spectra on specific LC-peaks permitted the direct monitoring of these transformations. Based on these on-line data, four new unstable aucubin derivatives were efficiently characterised.



Reference: 1. Cogne A.-L. et al. (2002) Phytochem. Anal., in press.

B045 New diterpenes from freshwater macrophytes *Potamogeton lucens* and *P. pectinatus*

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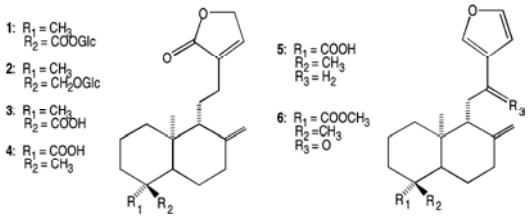
In order to evaluate the type of constituents produced by aquatic macrophytes, a widespread hydrophyte from Léman's Lake, *Potamogeton lucens* L. (Potamogetonaceae), was investigated and among various diterpenes two new glycosilated labdanes diterpenes were isolated (**1**, **2**). LC-UV-MS analysis of the methanolic extract showed that these new compounds and various widespread flavonoids were part of its main constituents. Their aglycones were obtained by enzymatic hydrolysis with β-glucosidase and used as standards for LC-UV-MS profiling of dichloromethane crude extracts of *Potamogeton lucens*, *P. pectinatus*, *P. crispus* and *P. perfoliatus*. The aglycone of **1**, previously described as nivenolide (**3**), was detected in *P. lucens*. A new aglycone, the epimer of demethylpinusolide (**4**), was also characterized in *P. lucens* and *P. pectinatus*, and isolated from *P. pectinatus*. The LC-UV-MS analyses did not reveal any of the new lactone diterpene glucosides in the extracts of *P. pectinatus*, *P. crispus* or *P. perfoliatus*, but they allowed the detection of related compounds in all macrophytes. Furanoids labdane diterpenes, daniellic acid (**5**) and its 12-oxo methyl ester derivative (**6**), were also isolated from *P. pectinatus* and detected in *P. lucens* lipophilic extract by LC-UV-MS. Labdane diterpenes seem thus to be characteristic of the *Potamogeton* genus and a few previous studies have also mentioned their occurrence in other members of the Potamogetonaceae. The antialgal properties of these constituents are currently under investigation for a better understanding of their ecological significance.



B046 Phytochemical investigation of *Vismia guineensis* by LC/UV-DAD, LC/MS-MS and LC/^{1}H-NMR

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Vismia guineensis (L.) Choisy (Hypericaceae) is a typical shrub of tropical West Africa locally called "Karidjakouma"; its bark and roots are employed in decoctions for internal and external usages in many skin diseases, such as dermatitis, leprosy, syphilis, herpes, scabies and eczemas (1, 2). In order to avoid disappearance of this widely used species, a comparative investigation of its root and leaf composition was initiated in order to evaluate the possible use of the leaves in traditional medicine. A secondary metabolite profiling of the lipophilic extracts of both leaves and roots (hexane and chloroform) was performed by means of LC/UV-DAD and LC/MS-MS. HPLC separation was carried out on a C-18 column with an acetonitrile/water gradient. The ionisation in LC/MS was achieved by APCI (Atmospheric Pressure Chemical Ionisation) in both positive and negative ion modes. The LC/UV-DAD analysis showed the presence of four classes of metabolites having specific chromophores: flavonoids, anthraquinones, vismiones and bianthrones. The molecular weights and characteristic fragments obtained by LC/MS analyses allowed the identification of different compounds by comparison with literature data. MS^n experiments were carried out for the determination of characteristic fragments of the unknown structures present in the extracts. Three additional isomeric bianthrones were detected in *V. guineensis*. LC/^{1}H-NMR was evaluated for the on-line identification of these latter isomers. Comparison between the leaf and root extracts showed that the same classes of constituents are present but only a minority of their derivatives are shared between these organs. In order to establish a definitive phytoequivalence of both organs, further pharmacological investigations are needed.

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B047 A global metabolomic approach involving LC/MS and GC/MS for a detailed study of the changes occurring in *Arabidopis thaliana* under stress conditions

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Metabolite profiling provides a deeper insight into the ultimate functions of gene expression and is the key to understanding how changes at the level of the genome and proteome affect cellular function. Unlike genomics and proteomics, a single analytical technique does not exist that is capable of profiling all metabolites of a given organism. In order to study in depth the metabolic changes that occur upon stress induction in plants, *Arabidopis thaliana* (ecotype Columbia) has been chosen as a model. Recent advances in molecular biology and total genome sequencing make this plant a key target for such type of analysis. The plant was submitted to various general stresses including wounding by forceps or superoxide exposure by paraquat spraying in order to obtain a general view of all changes that might occur at the level of secondary metabolite production. Frozen fresh plant material was extracted at low temperature (-40°C) by solvents of increasing polarities by miniaturised pressurised extraction in order to minimize interferences by enzymatic reactions. This novel microextraction procedure was applicable already to few mg of plant material with a satisfactory reproducibility. The lipophilic extracts (CH_2Cl_2 and 2-propanol) were analysed by LC/MS on a long phenyl column (50 cm), while the more polar extracts (MeOH) were analysed on a C-18 column (50 cm). Various MeCN-water gradients were applied in order to obtained a high LC resolution of most of the metabolites present. Complementary GC/MS analyses were also performed in order to assess changes that occur in the more volatile constituents and especially the oxylipins. Comparison of all the MS chromatograms obtained were performed using a comprehensive chemometric method which enabled to reconstruct automatically single ion traces characteristic for changes in metabolite production. The results demonstrated that noticeable changes occurred upon elicitation of *A. thaliana* in all extracts qualitatively and quantitatively. A characterisation of the most interesting induced constituents is underway. Based on the metabolite profiling methods presented, studies related to the production of defence compounds, molecules involved in signalling and relation between metabolite induction and defence gene expression are foreseen.

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B048 Capillary GC-MS analysis of tropane alkaloids from the roots and stem-bark of *Schizanthus grahamii*

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The genus *Schizanthus* contains a wide range of tropane alkaloids. The formation of ester derivatives from mainly angelic, tiglic, senecioic, itaconic or mesaconic acids, as well as the formation of dimers, constitutes the peculiar characteristics of this genus (1). The potential of direct identification of tropane alkaloids in crude extracts from roots and stem-bark of endemic Chilean *Schizanthus grahamii* (Solanaceae) by GC-MS is presented and discussed.

Notwithstanding the success of some combination of chromatography with spectrometric techniques, GC-EIMS remains the prominent technique for performing qualitative analysis. However, some drawbacks may arise in the differentiation of structural isomers showing superimposable mass spectra. In order to detect eventual artefacts or thermodegradation in the hot and surface-active injection port, leading to erroneous peak identification, different injection techniques are evaluated.

In this study, crude alkaloid extracts and some purified fractions were analysed by GC-EIMS using split, splitless and on-column injection. The latter was used as the reference method because it avoids artefact formation in the injection port. Thus, the different series of isomeric compounds in the extracts were confirmed. More than twenty alkaloids including four series of isomers were detected in the stem-bark, while only ten minor alkaloids were identified in the roots. Hygrine derivatives, tropine, pseudotropine, tropinone, 3α,7β-dihydroxytropane together with hydroxytropanes esterified with isomeric C_5 acids are present in both plant parts. However monomeric and dimeric tropanol diesters of mesaconic and itaconic acids were solely detected in the stem-bark. The difficulty to assign these isomeric compounds is pointed out.

Reference: 1. Muñoz, O. (1992) Química de la flora de Chile, Departamento Tecnico de Investigation Universidad de Chile, Santiago.

B049 Quantitative analysis of hyoscyamine in *Hyoscyamus reticulatus* L. by GC-MS

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Hyoscyamus reticulatus is used as a allucinogenic drug in East part of Turkey. Hyoscyamine content of leaves and root samples of *H. reticulatus* L. from Bulanık-Mus were investigated by capillary GC-MS. Gas chromatography-mass spectrometry was carried out on a Varian-Chrompack 3800 gas chromatograph coupled to a Saturn 2000 mass detector. Mass spectrometer with ion trap detector in full scan (80-325 amu) under electron impact ionization (70 eV) was used. The chromatographic column for the analysis was Chrompack WCOT-Fused Silica CP-Sil 5CB capillary column (30 m x 0.25 mm i.d, film thickness 0.25 µm). The carrier gas used was helium at a flow rate of 1 ml/min. Dried and powdered roots and leaves of *H. reticulatus* samples were extracted with methanol in a Soxhlet apparatus for 2 hours. The methanol was evaporated in vacuo at 50 ºC and the crude alkaloid fractions were obtained using the alkaloid extraction procedure (1). 1 µl crude alkaloid fractions were injected and analysed with the column held initially at 125 ºC for 1 min and then increased to 250 ºC with a 10 ºC/min heating ramp and then kept at 250 ºC for 5 min. The injection was performed in splitless mode at 280 ºC. Hyoscyamine, the predominant compound, reached 0.036 ± 0.004 % in the leaves and 0.056 ± 0.011 % in the root. These findings are in accordance with the reports on hyoscyamine content in other *Hyoscyamus* species (2,3). The limit of detection was calculated to be 3.125 µg/mL and the limit of quantification was calculated to be 6.25 µg/mL for hyoscyamine. The method has been shown to be linear and sensitive.

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B050 *Physalis angulata* L. – MPLC as an improved technique to obtain physalins

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The ergostane derivatives, physalins, with twenty eight carbon atoms are produce by several species of *Physalis* genus. These compounds possess a 13,14–seco–16,24-cycloergostane skeleton with a carbonyl group at C–15. Physalins have shown to be active against neoplasic tumors, inflammatory and tropical endemic diseases, as well as, in some immunological disorders (1). The main aim of this work is to develop a new methodology for optimizing the yield in the obtention of physalins. From the stems ethanolic extract a "pool" of those derivatives were obtained according to Mabry's modified technique (2). The "pool" was separated by a single medium performance liquid chromatography MPLC, (Büchi apparatus) using Lichroprep Si 60 column (50 cm height x 2.5 cm diameter), as stationary phase and cyclohexane–chloroform gradient system, as eluting phase (flux 20 mL/min). The pure substances percentages are pointed the out in Table 1, side by side, with the results of open column (CC) having Si gel 60 (30.0 cm height x 2.0 cm internal diameter), as stationary phase. Physalins were detected in hexane–chloroform (3:7) solvent system. That methodology allows obtention of four pure compounds in an overall yield ranging from 16% to 20%, a much better result than those described, so far, in the literature (3).

Physalins	Yield % CC	Yield % MPLC
B	5,0	5,5
F	-	8,2
G	3,5	4,6
D	4,3	8,0

Table 1: Yields of physalins.

Acknowledgement: Far-Manguinhos/Fiocruz.

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B051 Low molecular plant constituents as novel suppliers for characters in cladistic phylogenetic analyses: alkaloids in the Convolvulaceae

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The Convolvulaceae comprise 54 genera and about 1850 species showing cosmopolitan distribution with its centre in the tropics. Systematic uncertainties mainly exist regarding intrafamilial (tribal) relationships. A cladistic analysis of the family based on morphology included 128 characters such as habit, vegetative morphology and anatomy, reproductive structures, embryo features and chromosome numbers (1). To date, this family has not yet been the subject of broad molecular phylogenetic work. However, preliminary attempts in this direction were presented recently (2). Since convolvulaceous species produce a wide variety of alkaloids such as pyrrolidines, tropanes, pyrrolizidines, ergolines, 140 alkaloid characters of 8 different structural types (including the 4 above-mentioned ones) were chosen for a cladistic assay performed by PAUP/MacClade. 120 species taken from 29 genera were analysed by GC-MS.

The broad and differential occurrence of tropane alkaloids (simple tropanes, aliphatic and aromatic tropanol esters) in the different genera and tribes turned out to be of distinct significance. Moreover, pyrrolizidine alkaloids as well as ergoline alkaloids are valuable markers in certain clades of the family. Combined cladistic phylogenetic analysis of both, phytochemical and morphological characters should be promising.

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B052 Secondary metabolites of willow *Salix alba* L. and *Salix fragilis* L. vary in different regions of central Balkan peninsula

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Salix alba L. (White willow) and *Salix fragilis* L. (Crack willow) are closely related species with a widely sympatric distribution throughout Europe (1). The lack of clear-cut diagnostic characters, together with the fact that intermediate morphological forms largely dominate in the field and that interspecific controlled crosses are possible lend support to the hypothesis that *S. alba* and *S. fragilis* may hybridise frequently in nature (2). Phytochemical determination of tannins and total flavonoids are interesting markers to document metabolic diversity within and between populations from pure species and hybrids as defined by morphology and genotyping.

The samples (leaves, catkins and branches) from different population and their hybrids from different regions of central Balkan peninsula were collected during different stages of vegetation. Total phytochemical were extracted and the amounts of flavonoids and tannins were analysed. Flavonoid and tannin contents varied between 1-5 and 5-21%, respectively. The main flavonoids are isoquercitrin and naringin and its glucosides.

Our results provide detailed information about the metabolic diversity in natural populations of *S. alba* L. and *S. fragilis* L and their hybrids. The data derived from our phytochemical and morphological analysis may be helpful to study genetic diversity and DNA polymorphism in both willow species.

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B053 Phytochemical profile of *Leontopodium alpinum* Cass. in comparison to other Asian *Leontopodium* species

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The genus *Leontopodium* comprises more than 50 species that grow mainly in high mountainous regions of Central Europe and East Asia. In this presentation we report the isolation and structure elucidation of the sesquiterpenes β-isocomene, silphinene and modhephene from *L. alpinum* Cass. and 5-hydroxyobliquine from *L. leontopodioides* Beauverd. Isolation was performed by means of silica gel CC, silica gel $AgNO_3$ CC and Sephadex® LH-20 CC; structure elucidation by means of GC-MS, HR mass spectrometry and 1D- and 2D-NMR spectroscopy. For the fingerprint analysis of the secondary metabolite pattern of the *Leontopoidum* species *L. calocephalum* Beauverd, *L. campestre* Hand.-Mazz., *L. dedekensii* Beauverd, *L. franchetii* Beauverd, *L. sinense* Hemsl. ex Forb. & Hemsl., *L. subulatum* Beauverd, *L. leontopodioides* Beauverd. appropriate chromatographic methods were developed. Root extracts were analysed by TLC, RP-HPLC-UV/MS and GC/MS allowing the assignment of the characteristic constituents: bisabolane derivatives (1), benzofurane glycosides (2), lignanes (2), coumarins (2) and most of the described sesquiterpenoids (2-4). For the analysis of flavonoids and phenolic acids (5,6) of the aerial parts RP-HPLC-UV/MS methods were used. Comparison of the investigated species showed significant differences of the bisabolane and coumarin pattern in the root extracts. *L. franchetii* and *L. sinense* e.g. contained none of the known bisabolane derivatives. The known ligan derivative occurred in all investigated species. Root extracts of *L. alpinum*, *L. campestre* and *L. leontopodioides*, three closely related species, showed a similar secondary metabolite pattern. Analysis of the aerial parts revealed no significant differences between the investigated species.

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B054 On-line identification of metabolites in crude extracts of various *Justicia* species (Acanthaceae) by LC/UV/APCI-MS^n

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LC/MS/MS and LC/MS^n represent very important tools for the on-line identification of natural products in crude extracts (1, 2). This study aimed at improving the knowledge on the chemical composition of the dichloromethane extract of *Justicia secunda* (stems) and to monitor variations in profiles of the isolated compounds from *J. secunda* and other non identified compounds in *J. refractifolia* and *J. graciliflora* by liquid chromatography with ultraviolet and mass spectrometric detection. MS^n fragmentation experiments were very useful to identify the known compounds present in the extracts. The compound classes peptide alkaloids, phenylalanine derivatives, indoloquinoline alkaloids, triterpenes, phenolic and olefinic amides and 2,5-diaryl-3,4-dimethyltetrahydrofuranoid lignans, were determined via on-line identification by LC/UV/APCI-MS^n analysis. The most frequently encountered metabolite among *Justicia* species was auranamide (phenylalanine derivative) while distribution of quindoline (indoloquinoline alkaloid) was limited to *Justicia secunda*.

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B055 Analysis of different tissues of six *Eleutherococcus* (*Acanthopanax*) species (Araliaceae) by LC/UV-APCI-MS and LC-NMR

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Eleutherococcus senticosus is an indigenous shrub from the northern regions of Asia. This plant is endangered by over-harvesting, especially as only roots are used in health-food. However, there are other species in the genus such as *E. koreanum* Nakai, *E. sessiliflorum* Seemann, *E. sieboldiana* Makino, *E. chiisanense* Nakai, *E. divaricatus* Seemann in South Korea (1). It may be possible to substitute the extract of the roots of *E. senticosus* with other tissues (stems, leaves and fruits) and/or with other *Eleutherococcus* species, as far as the similarity in their chemical constituents and the clinical activities are concerned. The chromatographic profiles of each tissue of the 6 species were compared by atmospheric pressure chemical ionization liquid chromatography-mass spectrometry (LC/UV-APCI-MS) and liquid chromatography coupled with a nuclear magnetic resonance (LC-NMR). The content of eleutherosides B & E, coniferin, chlorogenic acid and hyperin was also determined using hyphenated techniques (2). In general, the content of eleutherosides B and E in stems was higher than that in roots and the chromatographic profile of *E. sessiliflorum* and *E. chiisanense* was almost the same in all tissues. In the fruits, the content of eleutheroside E was higher in *E. sessiliflorum* and *E. chiisanense* than in *E. senticosus*. The results of this work clarified the chemical composition of each tissue of various *Eleutherococcus* species as basic data for future clinical trials.

Acknowledgements: This work was supported by the Post-doctoral Program of Korea Science & Engineering Foundation (KOSEF).

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B056 Chemotaxonomic investigations of the *Achillea millefolium* group with IR spectroscopy

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The *Achillea millefolium* group contains several taxa of different ploidy, morphology and chemistry (1). As the taxa are partly very similar concerning the morphology, chemical screening methods are needed that provide not only a fingerprint of the plant but also some information about the structures of the main compounds. The IR spectroscopy is a useful tool for structure elucidation of sesquiterpenes, a group that is important for the pharmacological effects of the plant as well as for chemotaxonomic questions (2). Therefore a method for screening single plants by IR spectroscopy was developed.

100 mg of dried flowerheads of single plants were extracted with 1ml dichloromethane. This extract was divided by VLC on silicagel 60 in two fractions by elution with dichloromethane (fraction 1) and dichloromethane-acetone (7: 3, fraction 2). The sesquiterpenes were enriched in fraction 2 which was evaporated. The residue was redissolved in a few drops of methanol and put on a silicium plate for IR measurement.

Experiments with the taxa *A. aspleniifolia* Vent., *A. ceretanica* Sennen, *A. roseo-alba* Ehrend., *A. setacea* W. et K. (2n), *A. ceretanica* Sennen, *A. collina* Becker, *A. pratensis* Saukel & Länger (4n), *A. millefolium* L. S. l., *A. millefolium* subsp. *sudetica* Opiz, *A. styriaca* Saukel ined. (6n) and *A. pannonica* Scheele (8n) from different locations showed characteristic patterns. The recorded spectra correlated well with the IR spectra of the sesquiterpenes isolated from the respective taxa. With this method it was possible to determine whether sesquiterpenes with an α-methylen-γ-lactone or with a saturated lactone dominate in the plant. This can be important because sesquiterpenes with an α-methylen-γ-lactone moiety are known to be responsible for allergic reactions (3-5). Accordingly IR spectroscopy seems to be a useful method for the rapid screening of *Achillea* species in combination with TLC.

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B057 Analysis of flavonoids and caffeoylquinic acids in the *Achillea millefolium* group

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Flavonoids, due to their antioxidative, antiphlogistic and spasmolytic activities (1), probably contribute to the pharmacological activity of the drug Herba millefolii, which is widely used in folk medicine (2). Furthermore these compounds may be of chemotaxonomical relevance. Thus, the composition of the flavonoid complexes of eleven different taxa of the *Achillea millefolium*-group were investigated by capillary electrophoresis. Aerial parts of the flowering plants of *A. setacea* W. et K., *A. aspleniifolia* Vent., *A. roseo-alba* Ehrend., *A. ceretanica* Sennen (2n), *A. collina* Becker, *A. pratensis* Saukel & Länger, *A. ceretanica* Sennen (4n), *A. millefolium* L.S.I., *A. millefolium* subsp. *sudetica* Opiz, *A. styriaca* Saukel ined. (6n) and *A. pannonica* Scheele (8n), which occur in Austria and the surrounding regions were analysed. 17 flavonoids (luteolin-7,4'-O-diglucosid, apigenin-7-O-glucoside, luteolin-7-O-glucoside, isorhamnetin-3-O-rutinoside, vicenin-2, schaftoside, isoschaftoside, 6-hydroxy-luteolin-7-O-glucoside, rutin, luteolin-4'-O-glucoside, luteolin-7-O-glucuronide, isoorientin, vitexin, apigenin and 3 yet not identified flavonoids), being the main compounds in the different species, were selected for the comparison of the flavonoid patterns. The similarities in one species and remarkable differences between the taxa proved the chemotaxonomic relevance of the flavonoids. The determined amounts of 0.3 to 2.1 % in the drug pointed to their contribution to the pharmacological effects.

Investigations of the phenolcarbonic acids in these *Achillea* taxa showed chlorogenic acid in all samples, besides four dicaffeoylquinic acids and related substances. The quantitative variability between the samples was observed, but the patterns of the caffeoylquinic acid-derivatives showed no remarkable differences. Because of the minor differences between the taxa these phenolics are of low chemotaxonomic value, but with amounts up to 3.1 % they may also contribute to the pharmacological effects of Herba millefolii.

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B058 APCI-MS – a helpful tool to identify sesquiterpenes in species of the *Achillea millefolium* group

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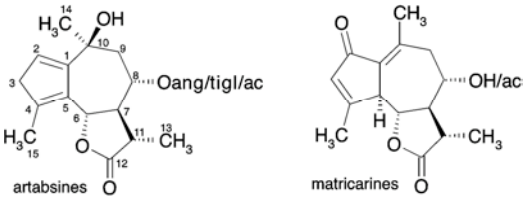
Several species of the polyploid *Achillea millefolium* group are characterized by labile proazulenes (8α-tigloxy- / 8α-angeloxy- / 8α-acetoxy-artabsin) and stable matricarine-derivatives (1). Their analysis is performed by HPLC on RP 8 material using a methanol-water gradient and diode array detection (220 nm and 255 nm) (2). Coupling of mass spectrometry as additional method for detection and identification is presented. Due to the low polarity of the compounds APCI was employed for ionization in the positive mode whereas ESI did not yield useful mass spectra. The matricarines show high intensity of their quasimolecular ions in contrast to the labile proazulenes which only yield fragments at the respective conditions. Addition of ammonium acetate to the water in a concentration of 10 mM and adaption of the respective lens voltages cause higher stability of the quasimolecular ions of the proazulenes. They are detectable with low intensity beside a prominent mass peak corresponding to the adduct $[M+NH_4]^+$. In contrast, the matricarines do not show any adduct with ammonium acetate. The influence of the temperature and respective lens voltages is discussed. This method represents a useful technique to identify and characterize labile sesquiterpenoids during analyses of different extracts and during isolation procedures.



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B059 Analysis of flavones in species of *Teucrium* from Macedonian flora

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Assay of flavones is performed in samples of *Teucrium chamaedrys* L., *T. montanum* L. and *T. polium* L., Lamiaceae, collected in several locations in Macedonia. Liquid-liquid extraction in 70 % ethanol during 24 hours with continuous stirring at room temperature was employed for extraction of flavonoid compounds from the plant material. The bulk extract was then concentrated under low pressure and fractionated by subsequent extractions with diethylether, ethylacetate and *n*-buthanol. Flavone aglycones were then analyzed in the diethylether extracts by reversed phase HPLC using C18 column (250x4.6 mm, 5 µm) and gradient elution with a mobile phase composed of water, acetonitrile and methanol. UV diode-array detector was used for identification of flavones based on comparison of retention times and UV-spectra to the ones obtained for authentic samples. For positive identification of the flavones, isolation and purification was performed using column chromatography on silica and TLC and then HPLC and UV-spectroscopic analysis (1). *T. polium* was found to be rich in luteolin, apigenin, cirsimaritine and a flavone (**F**) with the same UV-spectrum that cirsilineol, but with a significantly shorter retention time. Minor quantities of luteolin, diosmetin, cirsimaritine and **F**, and only traces of apigenin were identified in extracts of *T. montanum*. Significant quantities of the flavone **F**, lesser amounts of luteolin and cirsimaritine, and only traces of apigenin and diosmetin were found in *T. chamaedrys* extracts. A nonparameter approach for estimating the effect of -OH and -OCH_3 groups in various positions in the flavone ring on the retention time was employed for prediction of the most probable structure of the unknown flavone **F** (2, 3). This method implied that the flavone **F** is most probably cirsiliol, a 3'-OH derivative of cirsilineol. This is the reason for the identical UV-spectrum but shorter retention time of cirsiliol (**F**) compared to cirsilineol.

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B060 Essential oil constituents from Iranian *Phlomis herba-venti* L. leaves

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Phlomis herba-venti L. (Labiatae) is a wild plant growing in Mazandaran province (1). Several *Phlomis* species are used in herbal medicine, e.g. for diseases of the respiratory tract or externally for treatment of wounds (2). The leaves of *Phlomis herba-venti* L. were collected in June 2001 from the suburb of Sari, Mazandaran province, north of Iran. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Mazandaran University of Medical Sciences. The leaves were subjected to hydrodistillation using a Clevenger-type apparatus for 5 h to yield 1.1% of yellowish oil. The oil after preparation was submitted to GC (Perkin-Elmer 8500 gas chromatograph with FID and a DB-5 capillary column 30 m × 0.25 mm; film thickness 0.25 µm) and GC/MS (Hewlett Packard 6890 series, with a similar DB-5 capillary column) analysis. The 23 components of the oil (about 97.2%) were identified by their retention time, retention indices relative to C_9-C_{28} n-alkanes, and by comparison of their mass spectra with those of authentic samples or with data already available in the literature. The relative percentage of compounds was calculated from the total chromatogram by the computer. Germacrene-D (33.9%), hexadecanoic acid (12.9%) and α-pinene (9.4%) were identified as major constituents.

Acknowledgements: We thank Dr. Gh. Amin (Department of Pharmacognosy, Tehran University of Medical Sciences) for identification of the plant.

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B061 The essential oil of *Salvia aethiopis*

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Salvia aethiopis L. belonging to the family Labiatae, is an aromatic shrub, which grows wild in Iran (Mazandaran, Azerbaidjan, Khorasan) (1). *S. aethiopis* has been used in Iranian herbal medicine as a carminative and tonic agent (2); there is not any report on the volatile constituents of this plant. The medicinal properties attributed to the essential oils of the genus *Salvia* prompted us to investigate the chemical constituents of the oil of *S. aethiopis*. The aerial parts of *S. aethiopis* were collected in March 2001 from the suburb of Sari, Mazandaran province, north of Iran. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Mazandaran University of Medical Sciences. The aerial parts were subjected to hydrodistillation using a Clevenger-type apparatus for 5 h to yield 1.6% of yellowish oil. The oil after preparation was submitted to GC and GC/MS analysis. The 28 components of the oil (about 96.9%) were identified by their retention time, retention indices relative to C_9-C_{28} n-alkanes, and by comparison of their mass spectra with those of authentic samples or with data already available in the literature. The relative percentage of compounds was calculated from the total chromatogram by the computer. β-Caryophyllene (17.0%), α-copaene (16.3%), germacrene-D (13.8%), β-cubebene (9.7%), spathulenol (8.3%), δ-cadinene (7.7%) and α-humulene (6.9%) were identified as major constituents.

Acknowledgements: We thank Dr. Gh. Amin (Department of Pharmacognosy, Tehran University of Medical Sciences) for identification of the plant.

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B062 Constituents of the essential oil of *Commiphora myrrha* (Nees) Engl. var. *molmol*

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Myrrh oil has been used since Ancient Greek times to heal wounds. It makes a good expectorant, used in chest rubs for bronchitis and catarrhal colds (1,2). The oleo-gum resin of *Commiphora myrrha* (Nees) Engl. var. *mol - mol* was prepared from Shiraz, Fars province in Iran, in 2001 and identified by Department of Pharmacognosy, Tehran University of Medical Sciences. A voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Crushed air-dried oleo-gum resin of this plant (100 g) was subjected to hydrodistillation for 6h using a Clevenger-type apparatus to give oil in 3.1% yield (3.1 g). The chemical composition of the essential oil obtained from the oleo-gum resin was examined by using GC and GC/MS. The 32 components of the oil (about 94.6%) were identified by their retention time, retention indices relative to C_9-C_{28} n-alkanes, and by comparison of their mass spectra with those of authentic samples or with data already available in the literature. Among the 32 components identified in this oil, curzerene (40.1%), furanoeudesma-1,3-diene (15.0%), β-elemene (8.4%) and 2-O-acetyl-8,12-epoxygermacra-1(10),4,7,11-tetraene, isomer I (6.5%) were found to be the major constituents.

Acknowledgements: We thank Dr. Gh. Amin (Department of Pharmacognosy, Tehran University of Medical Sciences) for identification of the plant.

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B063 Application of solid-phase microextraction (SPME) with GC-MS for investigation of the composition of the essential oil from herb and fruits of *Peucedanum tauricum* Bieb.

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Solid-phase microextraction (SPME), a new sample preparation technique was developed by Arthur and Pawliszyn (1). The main advantages of SPME are: small sample volume, solventless, simplicity, rapidity, high sensitivity if used with GC, and high selectivity if connected to GC-MS (2,3).

Investigations of the composition of essential oil from non crushed and crushed (*in-situ)* dried herb and mature dried fruits of *Peucedanum tauricum* Bieb. (Umbelliferae) collected by SPME technique in comparison with the composition of the essential oil obtained from dried herb and dried mature fruits of this plant with hydrodistillation with *m*-xylene, was the aim of this work. Composition of obtained essential oils was monitored by GC-MS (FID). The same compounds (above 90% sesquiterpenes) were detected (GC-MS) by both techniques. SPME technique can be used for a quick determination of qualitative composition of essential oils with small amounts of plant material, without tedious extraction procedures or hydrodistillation techniques and chemical solvents.

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B064 Investigations on the quality of volatile oils from the chosen coriander lines

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Coriandrum sativum L. (Apiaceae) is an aromatic, annual, herbaceous plant known as coriander. Coriander is native to Southern Europe and Asia. It is widely cultivated in many countries favored by a suitable climate. Coriander is well-known as a culinary herb and its fruits and volatile oil, which is obtained from the fruits, are used in food industry, in perfumes, in flavoring alcoholic beverages and in medical preparations such as tonics and stomachics. Coriander has also several pharmacological activities (antifertility, antihyperglycemic, antihyperlipidemic, antioxidant, hypotensive, anticonvulsant, etc.). The volatile oil of coriander is characterized by high amounts of linalool, depending on geographical locations and other factors.

In the Turkish flora the genus *Coriandrum* is represented by two species; *C. sativum* and *C. tordylium*. *C. sativum* is also cultivated in different regions of Turkey. The fruits of the plant are used in food industry and as a folk remedy.

In this study, we investigated the quality of the oils of the winter cultures from chosen ten coriander lines, belonging to different origins. The plant materials were collected from the Botanical Garden of Faculty of Agriculture in Ankara, in September 2000 and 2001.

The dried and crushed fruits of ten samples were water-distilled in a Clevenger type apparatus for 3 hours. And then the oils were analyzed by GC and GC/MS using a fused-silica capillary column coated with OV-1. The constituents were identified by comparing their retention times and Kovats indices with those of authentic reference compounds and by comparison with published MS data and from Computer library searches.

Yields of the volatile oils of the fruits from the chosen coriander lines were 0.28-0.77%. The major component of the different samples was linalool (71.1-79.6%). The other components of the volatile oils of the samples differed from each other according to the origin.

B065 Chemical composition of the leaf essential oil of *Senecio myricaefolius* Bojer

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Senecio myricaefolius Bojer (Compositae) is a toxic wild-growing tree, an aromatic plant indigenous to Madagascar. A review of the literature reveals that the genus *Senecio* is characterized by the occurrence of 13-membered macrocyclic pyrrolizidine alkaloids (1). These are toxic to both humans and livestock. Other than the previous LPN staff studies (2,3), there have been no investigations reported concerning either the alkaloid contents or the volatile constituents of *S. myricaefolius*. This paper presents the chemical composition of the essential oil hydrodistilled from the fresh leaves of *S. myricaefolius*, obtained at very low yields of 0.03 to 0.04 %. This oil is a clear mobile liquid, pale yellow in color, and having a sweet odor. The components of *S. myricaefolius* oil were identified using the combination of TLC, GC/MS, GC/FTIR and NMR data. The main components are oxygenated monoterpenes (53.9%), with 1,8-cineole (44.1%) as the major compound and oxygenated sesquiterpenes (17.1%); (–)-spathulenol occurs in significant amounts (13.1%). The hydrocarbons are represented mainly by 4 monoterpenes (17.1%). The 10 identified sesquiterpene hydrocarbons account for only 9.3%, among which α-humulene (4.2%) predominates. The 26 identified and assigned constituents represent about 97.30% of the total oil. Further fractionation carried out on the total oil yielded hydrocarbons and oxygenated compounds. Olfactory examination of the former fraction have been performed by a local specialist. From the point of view of the perfumer, the characteristic grassy, hay-like odor with a minty undertone is an interesting olfactory "touch". In conclusion, the complex mixture of hydrocarbons of *S. myricaefolius* essential oil is responsible of its sweet, commercially interesting odor. This is a noteworhy result described for the first time by the present investigation.

Acknowledgments : Dr M.-F. Grenier-Loustalot staff at CNRS/SCA Vernaison (France) is gratefully acknowledged for providing the GC/MS, GC/FTIR data and for conducting the NMR experiments.

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B066 Composition of the essential oil of *Ononis viscosa* L. subsp. *breviflora* (DC.) Nyman

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Ononis viscosa L. is a small annual plant of the Leguminosae family that grows in West Mediterrenaen area in Turkey. In a previous work, *O. viscosa* was shown to have antibacterial activity against Gram positive bacteria (1). Resorcinol derivatives were found in the chloroform extract of the aerial parts of this plant in Spain (2). The plant subjected to the present study was collected in July 2001, in Fethiye, Turkey. Dried aerial parts were water distilled using a Clevenger-type apparatus to produce essential oil in 0.24 % yield. The oil was analyzed by GC/MS. Hexahydrofarnesylacetone (12.5 %), carvacrol (10.0 %), lauric acid (8.3 %), nonanal (5.5 %), (*E*)-geranylacetone (4.8 %) and dodecanal (4.8 %) were identified as major constituents.

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B067 Thalictricoside, a new phenolic compound from *Thalictrum orientale*

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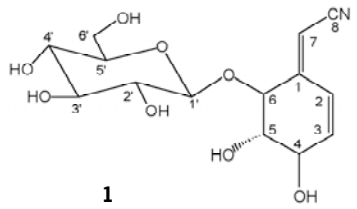
Nine species and three varieties of *Thalictrum* (meadow rue) are known to grow in Turkey. *Thalictrum* species have been used as aperient, diuretic, tonic and antiseptic in folk medicine. Their hypotensive, antimicrobial and antitumor effects have been observed in pharmacological tests. *Thalictrum* species contain alkaloids, glycosides, etc. (1,2,3). In this study, the underground parts of *T. orientale* collected from Niğde-Ulukışla in Turkey were investigated. The BuOH-soluble part of the methanolic extract was fractionated by vacuum liquid chromatography (VLC). One of the fractions yielded lithospermoside (**1**), a cyanoglycoside and thalictricoside (**2**), a new phenolic glycoside which was isolated by medium-pressure liquid chromatography (MPLC). Their structures were established by spectroscopic techniques. Lithospermoside has previously been obtained from *T. rugosum* (4). This is the second isolation of this compound. However, thalictricoside is a new natural compound.



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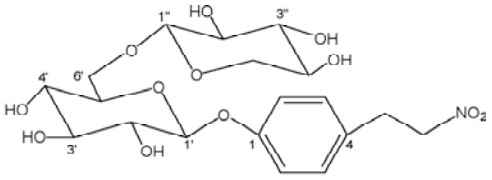

2

References: 1. Schiff, P.L. et al. (1970) Lloydia 33: 403-452. **2.** Başer, K.H.C. (1986) New Trends Nat. Prod. Chem. 26: 45-58. **3.** Wagner et al. (1971) Phytochemistry 10: 2553-2554. **4.** Wu et al. (1979) J. Nat. Prod. 42: 500-511.

B068 Chemical composition and variability of *Inula graveolens* (L.) from Corsica

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Inula graveolens (L.) Desf. [syn. *Dittrichia graveolens* (Desf.) Greuter] is a herbaceous plant of the Compositae family widespread in the Mediterranean area. The chemical composition of one essential oil from Iran has recently been reported (1).

The aim of the present work was to get a better knowledge of the essential oil of *Inula graveolens* from Corsica. First, we studied a commercial sample by combination of chromatographic (CC, GC) and spectroscopic techniques (MS, ^{13}C-NMR). The second part concerned the analysis of essential oils produced from plants collected in different regions of Corsica or at different stages of development.

The identification of the individual components of samples was based: (i) on comparison of their GC retention indices (RI) on apolar and polar columns, with those of authentic compounds, (ii) on computer matching with laboratory-made and commercial mass spectra libraries and comparison with spectra of authentic samples or literature data, (iii) on comparison of the resonance in the ^{13}C-NMR spectrum of the mixture with of those of authentic samples or literature data compiled in our spectra libraries with the help of laboratory-made software.

In total, 86 compounds were identified which represented 94% of the total amount. The main components are bornyl acetate (56.8%), borneol (7.6%) and τ-cadinol (7.8%). The composition of *Inula graveolens* oil is stable along the vegetative life of the plant. All the laboratory-produced samples belong to the bornyl acetate/borneol chemotype, although the contents of these two compounds differ from sample to sample.

References: 1. Mirza M and Ahmadi L (2000) J Essent Oil Res, 12: 507.

B069 The essential oil of wild carrot aerial parts from Corsica

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Daucus carota L., which belongs to the Umbelliferae family, is a tall robust annual spiny-fruited herb growing wild in dried-out fields or meadows. It is native to Europe and is the precursor of the cultivated carrot.

A review of the literature reveals that the volatiles of the carrot umbels have been the subject of only one study in which the authors identified one hundred components by GC and GC/MS (1). The essential oil is characterised by high contents of monoterpene hydrocarbons, α-pinene, sabinene, myrcene and limonene being the major components.

In our present study, we report the chemical composition of a collective oil of wild carrot aerial parts from Corsica. The identification of compounds has been carried out by ^{13}C NMR spectroscopy according to an original method created in our laboratory (2). It consists of identifying the individual components of a complex mixture using ^{13}C NMR spectroscopy without previous separation. This method allows the direct identification of the main components of essential oils and extracts. The quantification of these components is carried out by GC/RI (Retention Indices).

The corsican essential oil exhibited a quite different composition with polish oil: the major constituents are two phenylpropanoids: (*E*)-iso methyl eugenol (33%) and elemicin (11.4%), and a monoterpene hydrocarbon, α-pinene (24.9%).

References: 1. Staniszewska M and Kula J (2001) J Essent Oil Res 13: 439. **2.** Tomi F et al (1995) J Magn Reson Anal, 1: 25.

B070 Composition of essential oil of wild and cultivated *Satureja khuzestanica*

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Satureja kuzestanica (Persian name: Marzeh Khozestani) is an endemic plant of Iran that is widely distributed in southern part of Iran. It is renowned as analgesic and antiseptic in folk medicine (1-3). In this research, the composition of the essential oil of the aerial parts, obtained by hydrodistillation using a Clevenger type apparatus, was determined using GC-MS. Varian 3400 GC with a CP Sil DB1 column, 60m x 0.25 mm, combined with a Varian MAT (70 eV) temperature programmed, 4°C/min from 50 to 250°C, with He as the carrier gas was used. Identification of components was based on comparison of their retention times with those of analytical standards of available terpenoids, and matching mass spectral data of oil constituents according to the literature (4,5). The major constituents of the oil were carvacrol (90%), eugenol (3.6%), 2-methoxy-4-isopropylphenol (1%) and *p*-cymene (0.8%). The remaining compounds (thirteen components) were less than 0.7%.

The major constituents of the cultivated plant were carvacrol (81%), *p*-cymene (5%), myrcene (1.5%), γ-terpinene (2%), and 4-terpineol (2%). The remaining 19 components were less than 1%.

In both, the wild and cultivated plant, carvacrol was the major component. However, either the percentage or the structure of other major components were not the same.

References: 1. Jamzad, Z. (1994) Iran J. Botany 6(2), 215-218. **2.** Mozaffarian, V. (1998) a dictionary of Iranian plant names, 2nd ed. Farhang Moaser. **3.** Al-Biruni, Kitabe al-Saydaneh-fi-al Tibb, Edited and English translation by Hakim Mohammad Said, (1973), Al-Biruni's Book on Pharmacy and Materia Medica Hamdard National Foundation, Karachi, Pakistan. **4.** Ryhange R. et al. (1963) Acta Chem. Scand., 17, 2025-2035. **5.** Sydow, E. V. et al. (1963) Acta Chem.Scand, 17, 2504-2512.

B071 New pyrrolizidine alkaloids from *Lithospermum canescens* Lehm

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Lithospermum canescens (Indian paint or hoary puccoon) is a common prairie plant (1). It belongs to the Boraginaceae family so the presence of pyrrolizidine alkaloids (PA) should be presumed. Based on structural aspects, double-bond in position 1,2 and estrification at both necic OH-functions, PA can show toxic side effects. Therefore aerial parts of plant *L. canescens* were investigated using methods described earlier (2).

Seven PA were isolated and their structures determined by GC-mass spectroscopy and homo- as well as heteronuclear 2D-NMR correlated spectroscopy.

Four of them have not been described previously. The known alkaloids belong to the retronecine-type and are O^9-(-)-viridifloryl-retronecine (lycopsamine), its O^7-acetylderivative (acetyllycopsamine) and O^7-acetyl-O^9-(+)-trachelanthoyl-retronecine (acetylintermedine).

The new PA show the structures of O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine (O^7-3-hydroxy-3-methyl-butanoyl)-rinderine), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(-)-viridifloryl-heliotridine (O^7-3-hydroxy-3-methyl-butanoyl)-echinatine), O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine, O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)- O^9-(-)-viridifloryl-heliotridine.

Acknowledgements: Saskatchewan Herb Research Centre, Department of Horticulture Science, University of Saskatchewan, Canada, Dr. Branka Barl.

References: 1. Wiedenfeld, H. et al. (1998) Abstracts of Plenary Lectures Short Lectures and Posters 46th Annual Congress Society for Medicinal Plant Research, Vienna, Austria. **2.** Roeder, E., Wiedenfeld, H. (1977) Phytochemistry 16, 1462-1463.

B072 A new dinitrogenous alkaloid from *Cyrtanthus obliquus*

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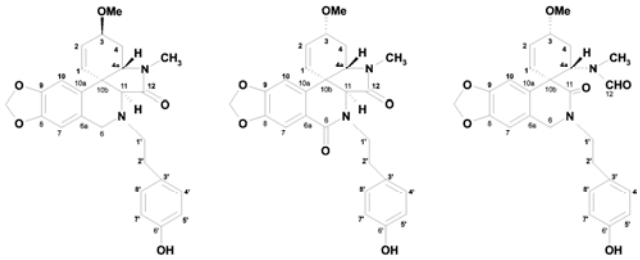
As part of our ongoing phytochemical and cytotoxicity studies on the isoquinoline alkaloids from South African Amaryllidaceae used in traditional medicine, we investigated *Cyrtanthus obliquus* (L.f) Ait, a species indigenous to the Western Cape, Eastern Cape and KwaZulu Natal Provinces of South Africa. We describe the isolation and characterization of the novel dinitrogenous alkaloid (–)-obliquine (**1**), together with the known structures, 11α-hydroxygalanthamine, 3-epimacronine, narcissidine, tazettine and trisphaeridine. Obliquine represents the third member of a new subgroup of the Amaryllidaceae alkaloids, where a nitrogen atom replaces the oxygen atom in position 5 of a tazettine type molecule, and that nitrogen atom is substituted by a 6-hydroxyphenethyl moiety, and follows the isolation of (+)-plicamine (**2**) and (–)-secoplicamine (**3**) (**1**). The structure and stereochemistry of **1** were determined by detailed 1D and 2D NMR techniques and HREIMS. In contrast to **2** and **3** and based on the magnitude of the coupling constants between H-3α and H-4a and H-4α and H-4a, a β-orientation was assigned to the 3-OMe group in obliquine. All the alkaloids were screened for cytotoxicity using the MTT assay against two mammalian cell lines, namely CHO and HepG2 cells, and were not cytotoxic at concentrations up to 100 µg/ml.



1 **2** **3**

Reference: 1. Ünver, N. et al. (1999). *Phytochemistry* 50: 1255-1261.

B073 Structural investigation of the carbohydrate moiety of an arabinogalactan protein from the roots of *Baptisia tinctoria*

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Baptisia tinctoria (L.) R. Brown (wild indigo), is a native plant from Northern America. Root extracts are used as an unspecific stimulant of the immune system. Polysaccharides and glycoproteins are thought to be involved in immunostimulation (1,2).

The aim of this work was to isolate an arabinogalactan protein (AGP) from an aqueous extract of dried roots of the plant and to study the chemical structure of the carbohydrate moiety of the macromolecule.

Roots were extracted with water and the extract was divided into a high molecular weight fraction (HMF) and a low molecular weight fraction (LMF) by tangential flow filtration with a MWCO of 30.000 Da. After dialysis of the HMF an AGP was isolated by precipitation with β-glucosyl-Yariv reagent. Methylation analysis of the AGP and the products of acid hydrolysis and reduction of uronic acids was performed to have an idea of the chemical structure of the AGP. The results show a molar ratio between branching sugar components, backbone sugar components and terminal sugar components of 1:1:1 which leads to the assumption that the macromolecule is highly branched. The molecular weight of the AGP, determined by SEC shows a hydrodynamic volume of about 60.000 Da using pullulans (linear polysaccharides) as standard whereas the absolute molecular weight was estimated to be about 300.000 Da using a light scattering detector. These values support the results of methylation analysis by indicating a non-linear compact highly branched molecule.

References: 1. Beuscher, N., Kopanski, L. (1989) Planta Med. 55: 358-363. **2.** Wagner, H. et al. (1985) Arzneim. Forsch. 35, 1069-1075.

B074 A new coumarin from *Sanguisorba minor*

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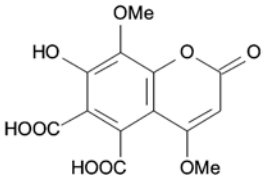
Faculty of Pharmacy, Ain -Shams University, Cairo, Egypt.

Wild Rosaceous plants of Egypt are known in folk medicine to produce extracts of hypoglycemic activity. The present study describes the isolation and structure elucidation of nine phenolics from the aqueous/ethanolic whole plant extract of *Sanguisorba minor*. Only one of these compounds was new and was found to possess a quite unique carboxy coumarin structure, namely 4,8-dimethoxy-7-hydroxy-2-oxo-2H-1-benzopyran-5,6-dicarboxylic acid (**1**). This followed from the brown $FeCl_3$ test, the electrophoretic mobility, the UV maxima in MeOH at 252, 310, 325 nm, the IR bands at υ 1685, 1700 and 1720 cm^{-1} (consistent with 6-, 5- and 2-CO groups), recovery of the compound unchanged after normal acid hydrolysis and also, from the molecular ion exhibited in negative ESI-MS at m/z: 309 and from the fragment ions at m/z: 264.9 and 221.0 (consistent with [M – COO] and [M – 2 COO]), as well as from the resonance singlets, in 1D-^{1}H NMR spectrum of the compound (DMSO-d_6) at δ ppm 3.82, 3.99 (4- and 8-OMe), 6.55 (H-3) and 12.35 (hydrogen bonded H-7). Both decoupled and gated decoupled ^{13}C NMR have confirmed the achieved structure and showed resonances at δ ppm 172.8(s), 169.1(s), 163.3(d, *J*=1.5 Hz), 56.5(q, *J*=130 Hz), 59.7(q, *J*=130 Hz) and 95.0 (d, *J*=169.3 Hz), assignable to (C6-COOH), (C5-COOH), (C-2), (OMe-4), (OMe-8) and (C-3), respectively. The known compounds, 1-*O*-β-galloyl-glucose; 2,3-hexahydroxydiphenoyl-(α/β)-glucose; gallic acid; 1-galloyl-2,3-hexahydro x ydiphenoyl-α-glucose; its β-isomer; quercetin-3-*O*-β-(6"-galloylgalactoside); kaempherol; quercetin and ellagic acid were also isolated and characterized.



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B075 Iridoid glycosides from *Globularia dumulosa*

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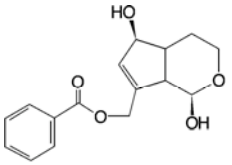
In the flora of Turkey, the genus *Globularia* (Globulariaceae) is represented by nine species (1,2). Our previous studies have resulted in the isolation of phenylethanoid glycosides and iridoid glycosides from *G. trichosantha* and *G. davisiana* (3-5) and sugar esters along with iridoid and phenylethanoid glycosides from *G. orientalis* (6). In the course of investigation of *Globularia* species growing in Turkey, we here report the isolation and structure elucidation of iridoids from an endemic species, *G. dumulosa* O. Schwarz. The powdered aerial parts of *G. dumulosa* were extracted with methanol. Chromatographic studies (VLC, MPLC and CC) on the water soluble parts of the methanolic extract resulted in the isolation of two new iridoids (**1**, **2**) in addition to seven known iridoid glycosides, davisioside, aucubin, melampyroside, catalpol, 10-*O*-benzoylcatalpol, alpinoside and deacetylalpinoside. The structures of all compounds were established by means of spectral (UV, IR, 1D, 2D NMR and MS) evidence.



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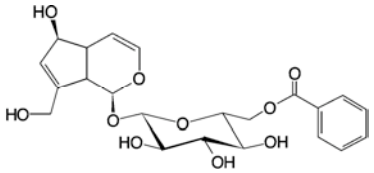

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References: 1. Davis, P. H. (1982) Flora of Turkey and East Aegean Islands. Vol. 7, University Press. Edinburgh. **2.** Duman, H. (2001) Bot. J. Linn. Soc. 137: 425-428. **3.** Çalış, I. et al. (1999) J. Nat. Prod. 62: 1165-1168. **4.** Çalış, I. et al. (2001) J. Nat. Prod. 64: 60-64. **5.** Çalış, I. et al. (2002) Chem. Pharm. Bull. 50 (in press). **6.** Çalış, I. et al. (2002) Z. Naturforsch. C (in press).

B076 Phenolic compounds from *Globularia cordifolia*

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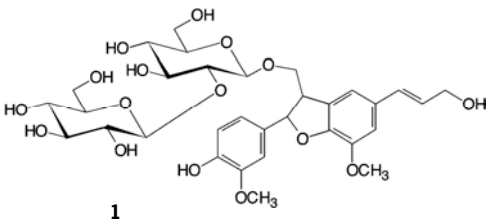
Globularia cordifolia L. (Globulariaceae) is a mat-forming shrublet growing in limestone cliffs in Central and South Europe (1). Several phytochemical studies exhibited that the main constituents of *G. cordifolia* were iridoid glycosides and flavonoids (2,3). As part of our work on isolation and identification of constituents from the genus *Globularia*, we report here the isolation of a new neolignan glycoside, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**1**) and a known neolignan glycoside, dehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside along with known flavone glycosides (chrysoeriol 7-*O*-β-allopyranosyl-(1→2)-β-D-glucopyranoside and stachyspinoside) and phenylethanoid glycosides (verbascoside, isoverbascoside, leucosceptoside A, martynoside and rossicaside A) from the underground parts of *G. cordifolia*. The structures of all compounds were elucidated by spectroscopic means, mainly by 1D and 2D NMR and MS.



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References: 1. Davis, P.H. (1982) Flora of Turkey and East Aegean Islands. Vol. 7, University Press. Edinburgh. **2.** Chaudhuri, R.K., Sticher, O. (1980) Helv. Chim. Acta 63: 117-120. **3.** Harborne, J.B., Williams, C.A. (1971) Phytochemistry 10: 367-368.

B077 Bonabilins, unique tropane alkaloids from *Bonamia spectabilis* (Convolvulaceae)

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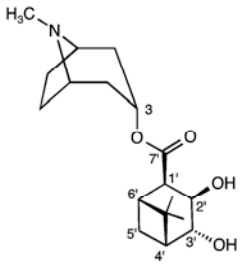
The Convolvulaceae family comprises about 1750 species mostly distributed in tropical and subtropical parts of the world. As a clear characteristic, it synthesizes a wide variety of tropane alkaloids, especially esters of 3α– and 3β-tropanol with simple aliphatic acids or substituted benzoic acids such as veratric, vanillic or kurameric acid (1, 2).

During our continuous studies on secondary metabolites of the Convolvulaceae, *Bonamia spectabilis* (Choisy) Hall. F., a twining shrub endemic to Madagascar and the tropical parts of East Africa, was investigated. The GC-MS analysis of the crude alkaloid fraction of the roots gave hints to the occurrence of so far unknown tropane alkaloids. Giving a positive reaction with Dragendorff´s reagent two major alkaloids were isolated by means of preparative TLC and their structures elucidated using ^{1}H-NMR, H,H-COSY, ^{13}C-NMR, C,H-COSY, HMBC, EI-MS, and HR-MS measurements.

They turned out to be tropan-3αol esters which we named Bonabilin A (**1**) and Bonabilin B (**2**). Their acyl moieties are rather unusual monoterpenoic acids, unique as acyl residues of tropanol derivatives.



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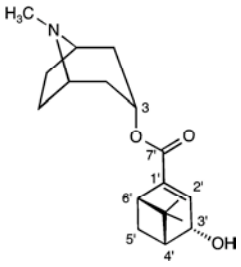

2 (rel. config.)

References: 1. Orechoff, A., Konowalowa, R., (1934) Ber. Dtsch. Chem. Ges., 67, 1153-1156. **2.** Weigl, R. et al., (1992) Planta Med., 58, Supplement A 750.

B078 The diterpenoids of *Teucrium polium* subsp. *polium*

M. Bruno [a], A. Maggio [a], F. Piozzi [a], S. Puech [b] and S. Rosselli [a]

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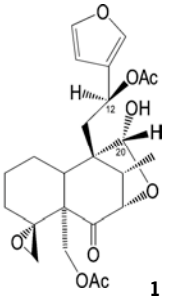
The genus *Teucrium* (Labiatae) is known as a rich source of neoclerodane diterpenoids (1). The species *T. polium* was object of several studies, but the samples harvested for these investigations in different countries were not sufficiently described, provided that no indication of the possible subspecies was given. As the section *Polium* is very complex, we decided to investigate a sure specimen of *T. polium* subsp. *polium*, collected in Southern France at Traviargues (Anduze-Gard).

Extraction of the dried aerial parts (acetone) and extensive column chromatography led to the isolation of three neoclerodane diterpenoids: the already known capitatin and auropolin, and the new 20-epi-auropolin **1**. The known products were identified by conventional ^{1}H-NMR and ^{13}C-NMR spectra. The structure of the new natural product was elucidated by the use of MS, ^{1}H-NMR, ^{13}C-NMR, NOE and ROESY NMR spectra.



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References: 1. F.Piozzi et al. (1998) Heterocycles 48, 2185 and bibliography therein.

B079 Composition of the essential oil of *Nepeta curviflora* Boiss.

F. Senatore [a], *F. Piozzi* [b] *and N. Arnold* [c]

[a] Dip. Chimica Sostanze Naturali, Univ. Napoli, Via Montesano 49, I-80131 Napoli, Italy. [b] Dip. Chimica Organica, Univ. Palermo, Viale Scienze, I-90128 Palermo, Italy. [c] Faculté de Sciences Agronomiques, Univ. Saint Esprit, Kaslik, Beyrouth, Lebanon.

The essential oil of *Nepeta curviflora* Boiss. (Lamiaceae), collected in Lebanon, was obtained by hydrodistillation of the dried aerial parts (yield 0.3 %) and analyzed by GC and GC/MS. No previous data were reported for the plant and the oil.

GC analyses were performed on a Perkin-Elmer Sigma-115 instrument with a DB-1 fused-silica column (30 m x 0.25 mm, film thickness 0.25 µm). Operating conditions: injector and detector temperature 250° and 285° respect., carrier gas He; oven temperature program 5 min isothermal at 40°, then at 2°/min up to 260° and then isothermal at 260° for 20 min. GC/MS analysis was performed using a Hewlett-Packard 5890 A apparatus, equipped with a HP-1 fused-silica column (30 m x 0.25 mm; film thickness 0.33 µm, linked on line with a HP Mass Selective Detector (MSD 5970 HP); ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature 295°. The identification of oil components was established from their GC retention times, by comparing their MS spectra with those reported in literature, and by computer matching with the NIST 98 and Wiley 5 libraries, as well, whenever possible, co-injections with authentic compounds.

Thirtyfive compounds were identified constituting 93.8 % of the oil, the major components being caryophyllene (50.2 %), caryophyllene oxide (6.4 %) and (*E*)-β-farnesene (5.3 %). The high percentage of caryophyllene in this *Nepeta* is quite unusual; in fact this sesquiterpene hydrocarbon occurs in many species of *Nepeta* but none has caryophyllene as the main component; for this reason we can consider this plant as the first example of a *Nepeta* species rich in caryophyllene.

B080 Alkaloidal constituents from *Aconitum jaluense* Komar

S.S. Kang [a], *S.H. Shim* [a], *J.S. Kim* [a], *K.H. Son* [b] *and K.H. Bae* [c]

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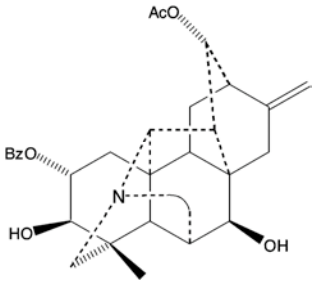
The root part of *Aconitum jaluense* Komar. (Ranunculaceae) has been used in folk medicine for the treatment of rheumatism and neuralgia. A new C_{20} diterpenoid alkaloid with a hetisine type skeleton, name jaluenine, was isolated from this plant. The dried roots were extracted with MeOH followed by fractionation with $CHCl_3$ and 3% aqueous NH_4OH to give alkaloidal fraction. The alkaloidal fraction was subjected to silica gel column chromatography to yield a compound (**1**), mp 114 - 116°C, $C_{29}H_{33}NO_6$. The structure of the new compound (**1**) was determined to be hetisane 2α,3β,7β,13α-tetrol 2-benzoate 13-acetate by spectroscopic methods including 2D NMR (^{1}H-^{1}H COSY, HMQC, HMBC, NOESY). In addition, the known compounds, mesaconitine, hypaconitine, lipomesaconitine, lipohypaconitine, neoline, 15-hydroxyneoline, and napelline were also isolated and identified. All the known compounds have been isolated for the first time from this plant.



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Acknowledgement: This research was supported by a grant (PF002104-04) from Plant Diversity Research Center of the 21st Centrury Frontier Reseach Program funded by Ministry of Science and Technology of Korean government.

B081 Two new neolignan derivatives of *Phyllanthus ussuriensis*

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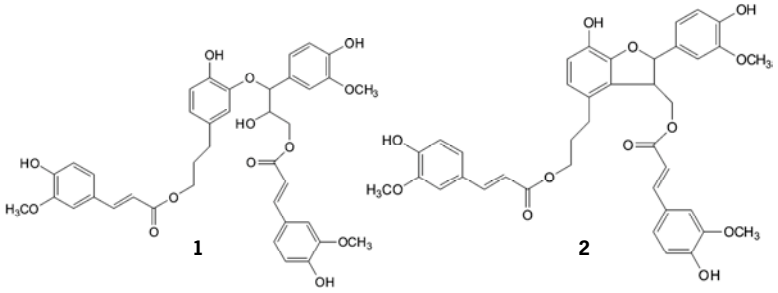
Phyllanthus ussuriensis Rupr. et Maxim. (Euphorbiaceae) is widely distributed in Korea, and has long been used in folk medicine to treat kidney and urinary bladder disturbances, intestinal infections, diabetes, and hepatitis. Reported chemical constituents of this species are only one flavonoid (quercetin-3-O-rutinoside), two gallotannins (gallic acid, methyl gallate), and two ellagitannins (corilagin, geraniin) (1).
An investigation of the $CHCl_3$ fraction of *P. ussuriensis* led to the isolation of two new neolignan derivatives, 3-(4-hydroxy-3-methoxy-phenyl)-acrylic acid 3-(4-hydroxy-3-{2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)-3-[3-(4-hydroxy-3-methoxy-phenyl)-acryloyloxy]-propoxy}-phenyl)-propyl ester (**1**) and 3-(4-hydroxy-3-methoxy-phenyl)-acrylic acid 3-{7-hydroxy-2-(4-hydroxy-3-methoxy-phenyl)-3-[3-(4-hydroxy-3-methoxy-phenyl)-acryloyloxymethyl]-2,3-dihydro-benzofuran-4-yl}-propyl ester (**2**). The structural elucidations of these compounds were based on the analysis of spectroscopic data.



References: 1. I. Ham et al. (2001) Yakhak Hoeji 45: 237-244.

B082 Inhibition of HIV-1 reverse transcriptase by phlorotannins from *Ecklonia cava*

Mi-Jeong Ahn [a], Kee-Dong Yoon [a], So-Young Min [a], Tae Gyun Kim [b], Seung Hee Kim [b], Jeong Ha Kim [c], Hoon Huh [a] and Jinwoong Kim [a]
[a] College of Pharmacy, Seoul National University, Seoul 151-742, Korea. [b] National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul 122-704, Korea. [c] Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, Korea.

The bioassay-directed isolation of a Phaeophyton, *Ecklonia cava* afforded four phlorotannin derivatives, eckol (1), dieckol (1), bieckol (2), and phlorofucofuroeckol (3). Among these compounds, bieckol and dieckol exhibited the inhibitory activity on human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) with IC_{50} values of 0.51±0.34, 5.3± 2.8 μM, respectively. The inhibitory activity of bieckol was comparable to that (IC_{50} value of 0.28±0.15 μM) of nevirapine, a reference compound. Enzyme kinetic assay showed that bieckol inhibited the RNA dependent DNA synthesis (RDDS) activity of HIV-1 RT competitively against dUTP/dTTP with a Ki value of 0.84 μM. This result suggest that bieckol may act as a selective HIV-1 RT inhibitor through binding to the dNTP binding site.

Acknowledgements: This research was supported by a grant from the Ministry of Maritime Affairs & Fisheries of Korea (19980024).

References: 1. Fukuyama, Y. et al. (1985) Chem. Lett. 739-742. **2.** Fukuyama, Y. et al. (1989) Chem. Pharm. Bull. 37: 2438-2440. **3.** Fukuyama, Y. et al. (1990) Chem. Pharm. Bull. 38: 133-135.

B083 Polyoxygenated leaf flavonoids of *Eugenia edulis*

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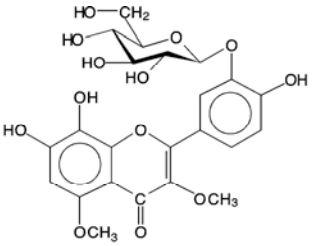
Extracts of *Eugenia edulis* (Myrtaceae) are used in Egyptian folk medicine to treat infectious diseases. In the present study, the aqueous alcoholic leaf extract which showed anti-bacterial activity, was fractionated over Sephadex LH-20 columns to afford 14 phenolics, among which three were new. They were identified as gossypetin-3,5-dimethyl ether-3'-*O*-β-glucopyranoside (**1**), gossypetin-3,5-dimethyl ether (**2**) and myricetin-3,5,3'-trimethyl ether (**3**). Structures were established by conventional methods and confirmed by ESI-mass and NMR spectral analysis. The three compounds exhibited diagnostic ^{13}C-NMR spectral patterns which reflected the presence of a 3,5-dimethyl etherification of the flavonoid moieties. This followed from the characteristic upfield shifts of the resonances (172.3, 173.1 and 172.7 ppm, respectively) of the carbonyl carbons C – 4 and from the down field shifts of the resonances of the C – 3 carbons (δ values 139.4; 140.0 and 140.4 ppm, respectively) of the aglycones in comparison with the corresponding resonances in the spectra of gossypetin and myricetin.



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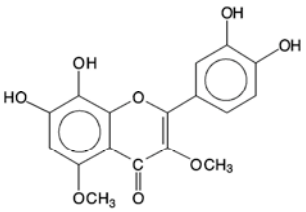

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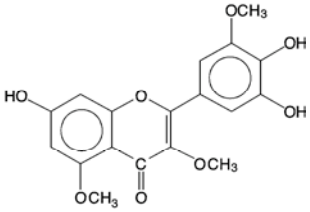

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B084 Pelargoniin E, a new ellagitannin, and accompanying phenols from *Pelargonium reniforme* Curt.

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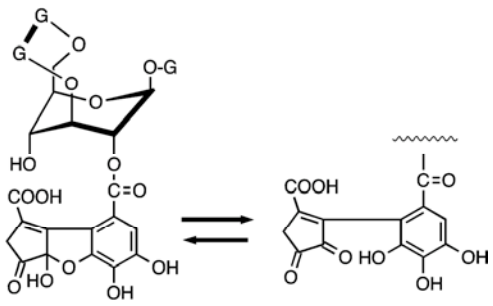
Pelargonium reniforme Curt. (Geraniaceae) is highly estimated by traditional practitioners and the native population in areas of southern Africa for its curative properties. The therapeutic significance of this plant is also documented by its present utilisation in modern phytotherapy for the treatment of respiratory tract infections (1).

Further investigation of the aerial parts of *P. reniforme* led to the isolation of the new ellagitannin pelargoniin E (**1**). Identification of **1** not only extends the series of ellagitannins based on a glucose core which itself adopts the less favourably $^1C_4$ conformation, but also introduces another example of rarely found dehydroellagitannins having an oxidatively modified DHHDP moiety attached to just C-2 of the glucose core. Besides the presence of common phenolics, the extract also yielded the new natural product gallic acid *n*butyl ester (**2**), and a series of sporadically found metabolites. These include 4,6-dihydroxy-2β-glucopyranosyloxyacetophenone, 1-O-galloylglycerol, 6″-O-galloyl-salidroside and (α,β)-3,4-di-O-galloylglucose. The structures of these compounds were established from spectroscopic studies.



1 (G = galloyl)

References: 1. Kolodziej, H. (2000) Curr. Topics Phytochem. 3: 77.

B085 New lactoyl glycoside quercetin from *Melia azedarach* leaves

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Melia is a fast growing deciduous tree, native to southwestern Asia, and is cultivated and naturalized in many warm and temperate countries of the world. Different parts of the tree, such as the bark and the leaves are used in folk medicine (1). The aqueous ethanolic extract of the aerial parts of this plant afforded four known flavonoids namely: kaempferol-3-O-rutinoside, 3-O-rhamnoside, quercetin-3-O-rutinoside and 3-O-rhamnoside (two of which were previously isolated from this plant (2); along with two new compounds; quercetin -3-O-[rhamnosyl 1→6 (4"-lactoyl glucoside)]-4'-O-glucoside and cinnamoyl-1-α-L-rhamnoside. The structure elucidation was based on ^{1}H- and ^{13}C-NMR together with the different physical and chemical investigations.

Among all flavonoids so far tested, quercetin and its derivatives showed pharmaceutical activities e.g. cytotoxic activity *in vitro* or *vivo* (3), strong spasmolytic activity (4) and influence on the metabolism of blood vessel walls, while the cinnamic acid derivatives play a multiplicity of roles in environment (3). For the above reasons the pharmacological activity of the isolated compounds are under investigation.

References: 1. El-Hadidi, M.N. and Boulos, L. (1988) The Street Trees of Egypt, The American University in Cairo Press. **2.** Marco, J.A. et. al. (1986), J. Nat. Prod., 49(1), 170. **3.** Wagner, H. (1977) in Biochemistry of Plant Phenolics (Eds.T. Swain, J.B. Harborne and C.F. Van Sumere), Plenum Press, New York and London, P.589. **4.** Bohm, K. (1967) Die Flavonoide, Ed. Cantor KG, Aulendorf/Wurtt.

B086 Constituents isolated from *Patrinia saniculaefolia* Hemsley

Ren-Bo An [a], *Kun Ho Son* [b], *Hyun Pyo Kim* [c], *Sam Sik Kang* [d], *Hyeun Wook Chang* [e], *Young Ho Kim* [a], *KiHwan Bae* [a]

[a] College of Pharmacy, Chungnam National University, Taejon 305-764, Korea. [b] Department of Food and Nutrition, Andong National University, Andong, 760-749, Korea. [c] College of Pharmacy, Kangwon National University, Chunchon, 200-701, Korea. [d] Natural Products Research Institute, Seoul National University, Seoul, 110-460, Korea. [e] College of Pharmacy, Yeungnam University, Kyongsan, 712-749, Korea.

Patrinia saniculaefolia Hemsley (Valerianaceae) is an endemic species of the genus *Patrinia* in Korea (1), which has not been clarified as to its constituents. Several plants of the genus *Patrinia* have been used as traditional folk medicine in Korea and China for the treatment of initial stages of appendicitis, perityphlitis, neuralgia, insomnia in neurasthenia, psychoses, acute bacterial inflammation, and as emmenagogue (2). The whole plant was extracted with methanol; the extract was suspended in H_2O and successively partitioned with hexane, CH_2Cl_2 and BuOH. Repeated silica gel column chromatography and reversed phase HPLC from the hexane soluble fraction afforded two new iridoids (**1** and **2**), together with the known compounds β-farnesene, squalene, nardostachin, oleanolic acid, oleanonic acid, 3β,23-dihydroxy-urs-12-ene-28-oic acid, 3-O-α-L-arabinopyranosyl-oleanolic acid, β-sitosteryl-3-O-β-D-glucopyranoside, 3-O-β-D-glucopyranosyl-oleanolic acid, and 3-O-[β-D-xylopyranosyl-(1-3)-β-D-glucuronopyranoside-6-O-butyl ester].

The molecular formula of compounds **1** and **2** were $C_{22}H_{34}O_8$ by high resolution FABMS, and from an analysis of its ^{13}C- NMR and DEPT data. On the basis of ^{1}H-, ^{13}C-NMR, HMQC, HMBC and ^{1}H,^{1}H-ROESY spectral data, their structures were established as (1*S*, 3*R*, 5*R*,7a*R*)-3,5-dimethoxy-7-hydroxymethyl-1-(3-methylbutanoyloxy)-4-(3-methylbutanoyloxymethyl)-1,3,5,7a-tetrahydrocyclopent-4,6-diene[e]pyran and (1*S*,3*S*,5*R*,7a*R*)-3,5-dimethoxy-7-hydroxymethyl-1-(3-methylbutanoyloxy)-4-(3-methylbutanoyloxymethyl)-1,3,5,7a-tetrahydrocyclopent-4,6-diene[e]pyran, which were named patridoid 1 and patridoid 2, respectively.

References: 1. Lee, Y.N. (1996) Flora of Korea, Kyo-Hak Publishing Co., Ltd. **2.** Inada, A. et al. (1993) Shoyakugaku Zasshi 47: 301-304.

B087 Chemical constituents of *Chamaelirium luteum*

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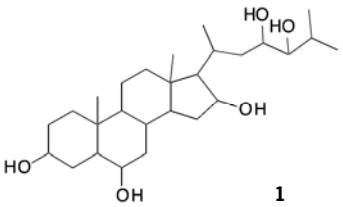
[a] Department of Chemistry, The University of Queensland 4072, Australia. [b] Centre for Magnetic Resonance, The University of Queensland 4072, Australia, [c] Mediherb, P. O. 713, Warwick 4370, Australia.

Results of an investigation of the rhizome and roots of *Chamaelirium luteum* (L.) A. Gray (syn. *Helonias dioica*, Liliaceae) are presented. Traditionally and in homeopathy this herb is used as a uterine tonic, emmenagogue and also to treat a broad range of female symptoms (1,2). Little is known about the chemical constituents of *C. luteum*, with most present day secondary literature based upon scientific data from the late 19th century (3,4). *C. luteum* is reported to contain chamaelirin (diosgenin glycoside) (1-4), helonin (a glycoside) (2,3), and diosgenin (1,2,5). However, no chemical structure has ever been attributed to either chamaelirin or helonin. HPLC (Evaporative Light Scattering Detector) analysis of the alcoholic extracts of the dried underground parts of *C. luteum* reveals four major constituents. The principal constituent was characterized as a steroidal saponin upon chromatographic purification. It has a molecular weight of 922 g/mol, to which we attributed the molecular formula of $C_{45}H_{78}O_{19}$. The sugar components of the steroidal saponin were identified by GC/MS analysis as glucose and fucose, via a procedure involving degradation and derivatisation. After preliminary 1D and 2D NMR studies (750 MHz ^{1}H, ^{13}C, COSY, TOCSY, HSQC and HMBC), we propose that the structure of the aglycone ($C_{27}H_{48}O_5$) of the major steroidal saponin is **1**.



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References: 1. (1992) Hagers Handbuch der pharmazeutischen Praxis, 5th Ed., Springer Verlag, Berlin. **2.** (1972) Hagers Handbuch der pharmazeutischen Praxis, 3rd Ed., Springer Verlag, Berlin. **3.** Greene, F.V. (1878) Am. J. Pharm., 250-253. **4.** Kruskal, N.C.I. (1892) Jahresber. Pharm. 27, 570. **5.** Marker, R. et al. (1942) J. Am. Chem. Soc. 64, 1283 – 1285.

B088 Degradation of andrographolide under heat accelerating condition

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[a] Department of Pharmaceutical Chemistry, and [b] Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand.

Andrographolide is the major active diterpene lactone from *Andrographis paniculata* (Burm. f.) Wall. ex Nees, a medicinal herb widely used in many Asian countries for the treatment of common cold, fever, and non-infectious diarrhea. Despite its keen potential, the herb itself, as herbal drug, has very short shelf life according to Chemical Specification of Thai Herbal Drugs, with higher than 26% loss in total lactone content upon 1-year storage at ambient condition (1). This leads to the limitation of the further development of *A. paniculata* for wider clinical uses. Here, we wish to report the preliminary result on degradation of andrographolide in dry, solid form. Upon an elevated temperature (70ºC, 75% relative humidity), crystalline andrographolide appeared highly stable with neither chemical nor physical observable change after 3 months. However, its amorphous form, prepared by solid-dispersion of andrographolide in PVP K-30 (1:2), decomposed under the same condition with higher than 50% degradation after 2 months. The main decomposition route was found to be the dehydration of 14-OH group, possibly via a concerted mechanism.

Acknowledgements: Research supporting grant; Faculty of Pharmaceutical Sciences, Prince of Songkla University.

References: 1. Dechatiwongse Na Ayudhya et al. (1993) Chemical specification of Thai Herbal Drugs, vol. I. Division of Medicinal Plant Research and Development, Ministry of Public Health. Bangkok.

B089 Novel isoprenylated chalcones and flavanones from two Madagascan *Cedrelopsis* species.

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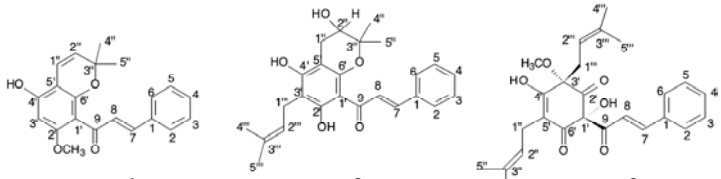
[b] Laboratory of Pharmacology, EES Sciences, University of Antananarivo, BP 906, Antananarivo, 101, Madagascar.

Cedrelopsis grevei, commonly called Katrafay, is amongst the many medicinal plants of Madagascar, being used to relieve muscle fatigue when the bark is soaked in hot water (1). Previous investigations have found this plant to contain chromones and coumarins (2,3,4,5). Two limonoids of unusual structure, cedmilinol and cedmiline have also been isolated from *C. grevei* (1). The dichloromethane extract of *C. grevei* yielded a dihydrochalcone, uvangoletin, a flavanone, 5,7-dimethylpinocembrin, two hydroxylated chalcones, cardamonin and flavokawin B and three isoprenylated chalcones, 2'-methoxyhelikrausichalcone, and the novel compounds, cedreprenone (**1**) and cedrediprenone (**2**).

The leaves of *Cedrelopsis microfoliata* have ethnopharmacological importance as they are used to prepare a decoction for woman to drink after childbirth. This is the first phytochemical investigation of *Cedrelopsis micro - foliata*.

The hexane extract yielded three compounds, a novel chalcone, microfolian (**3**) and two flavanones (microfolione, a novel flavanone and agrandol). The dichloromethane extract yielded four compounds, three coumarins (cedrecoumarin A, obliquin, and a novel coumarin, microfolicoumarin, and a sesquiterpenoid (sesquichamaenol).



1 **2** **3**

References: 1. Mulholland, D. et al. (1999) Tetrahedron, 55, 11547. **2.** Eshiett, I.T. et al. (1968) J. Chem. Soc (C), 481. **3.** Dean, F.M et al. (1971) Phytochem, 10, 3221. **4.** McCabe, P.M. et al. (1967) J. Chem. Soc (C), 145. **5.** Kotsos, M. et al. in press.

B090 Novel mexicanolide and phragmalin limonoids from two Madagascan Meliaceae species

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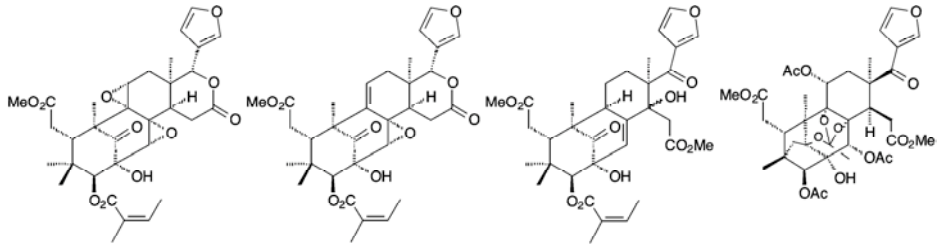
[b] Laboratory of Pharmacology, EES Sciences, University of Antananarivo, BP 906, Antananarivo, 101, Madagascar.

Five novel mexicanolide limonoids have been isolated from the Madagascan species *Quivisia papinae* Baillon ex Grandidier (Meliaceae). These include quivisianolide A (**1**), possessing a hitherto unreported 9α,11α-epoxide ring, the corresponding $\Delta^{9(11)}$ double bond analogue quivisianolide B (**2**), and the 17-keto ring D *seco* quivisianone (**3**).

The Madagascan Meliaceae *Neobeguea leandreana* Leroy has yielded three novel phragmalin limonoids, including the rare 17-keto ring D *seco* leandreanin A (**4**).

The structural elucidation of these compounds, principally by 1-D and 2-D NMR spectroscopy, will be presented, and the chemotaxonomic implications of these findings will be discussed.



quivisianolide A quivisianolide B quivisianone leandreanin A

1 **2** **3** **4**

B091 Some chemical constituents of *Scilla natalensis* and *Urginea altissima* (Hyacinthaceae)

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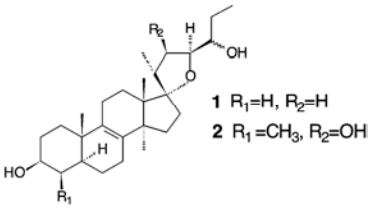
[a] Natural Products Research Group, School of Pure and Applied Chemistry, University of Natal, Durban, 4041, South Africa. [b] Ethnobotany Unit, National Botanical Institute, PO Box 52099, Berea Road 4007, South Africa.

The Hyacinthaceae family is one of the two most widely used plant families by the Zulu of KwaZulu-Natal (1). Recent investigations in our laboratory of several members of the three Southern African sub-families of the Hyacinthaceae have yielded a range of novel homoisoflavanones, nor-triterpenoids, bufadienolide glycosides, cholestane glycosides, chalcones, and benzopyranones. These include a novel bufadienolide glycoside, urginin, (3β-*O*-{α-L-rhamnopyranosyl-[(1→4)-β-D-glucopyranosyl] 1→3)-α-L-rhamnopyranoside}) which precipitated out from the methanol extract of the bulbs of *Urginea altissima*, and the novel trisnor-triterpenoid, (23*S*)-17α,23-epoxy-3β,24ξ-dihydroxy-27,28,29-trisnor-lanost-8-ene, **1**, and bisnortriterpenoid, (22*R*,23*S*)-17α,23-epoxy-3β,22,24ξ-trihydroxy-27,28-bisnor-lanost-8-ene, **2**, isolated from the dichloromethane extract of the bulbs of *Scilla natalensis*. Compounds **1** and **2** were isolated using column chromatography over silica gel and structures were determined using 2D-NMR techniques and LC-MS/MS methods for urginin and GC-MS for compounds **1** and **2**.



Acknowledgements: We are grateful to Mr D. Jagjivan, Mr M. Watson and Mr Bret Parel for technical assistance, and gratefully acknowledge funding by the NRF, the University of Natal Research Fund, and the Wellcome Trust (Equipment Grant number 052451).

References: 1. Pohl,T.S. et al. (2000) Curr. Org. Chem. 4: 1287-1324.

B092 Chemical constituents of the Zulu medicinal plant *Galtonia princeps* (Hyacinthaceae)

K. du Toit [a], *N.R. Crouch* [a,b], *S.E. Drewes* [c], *D.A.Mulholland* [a] *and E. Ndlovu* [a]

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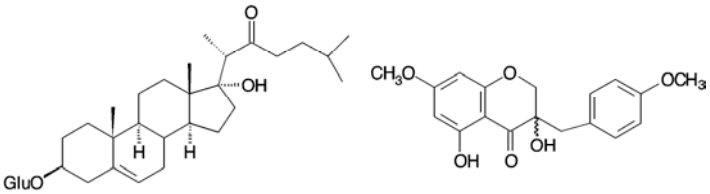
Galtonia princeps is a member of the Ornithogaloideae subfamily of the Hyacinthaceae family. Bulbs of this plant are used for magical purposes by the Zulu people of KwaZulu-Natal. An investigation of the chemistry of the bulbs of this species has yielded a novel cholestane glucoside from the methanol extract, and a homoisoflavanone from the dichloromethane extract, as shown below. This is the first report of the isolation of a homoisoflavanone from outside the Hyacinthoideae sub-family of the Hyacinthaceae (1). Compounds were purified by means of column chromatography over silica gel and structures were determined using 2D-NMR and MS techniques. The identity of the sugar was determined using an acid hydrolysis and identification of the sugar obtained.



Acknowledgements: We are grateful to Mr D. Jagjivan, Mr M. Watson and Mr Bret Parel for technical assistance, and gratefully acknowledge funding by the NRF, the University of Natal Research Fund, and the Wellcome Trust (Equipment Grant number 052451).

References: 1. Pohl, T.S. et al. (2000) Curr. Org. Chem. 4: 1287-1324.

B093 4'- Deoxy iridoid glycosides from the roots of *Centranthus longiflorus* ssp. *longiflorus*

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The genus *Centranthus* (Valerianaceae) is represented by three species in the flora of Turkey (1). *Centranthus longiflorus* ssp. *longiflorus* is traditionally used as sedative (2). In a previous paper we described the isolation and characterization of a new iridolactone (longifloron), a valepotriate (valtrat hydrine B8), two known iridoid glycosides (patrinoside and kanokoside A), and in addition two steroids (oleanolic acid and sitosterol) and a flavonol glycoside (quercetin 3-O-rutinoside) from the methanolic extract of the aerial parts of this plant (3).
In this study two new iridoid glycosides, 4´-deoxykanokoside A, 4´-deoxykanokoside C, have been isolated from the roots of *C. longiflorus* ssp. *longiflorus* together with three known iridoid glycosides, kanokoside A, kanokoside C, valerosidatum. The structure elucidation of the isolated compounds was performed by spectroscopic (UV, IR, 1D and 2D NMR, ESI-MS) methods.

References: 1. Davis, P. H. (1972) Flora of Turkey and East Aegean Islands University press, 4, pp. 558-559 **2.** Baytop, T. (1984) Türkiye'de Bitkilerle Tedavi, Press, Istanbul, pp. 282 **3.** Demirezer, L.Ö. et al. (1999) Phytochemistry, 51, 909.

B094 Iridoid and phenolic glycosides from *Wulfenia carinthiaca*

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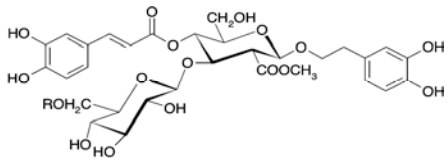
Wulfenia carinthiaca Jacq. (Scrophulariaceae) is a Tertiary relic, whose distribution is limited to two small areas in Carinthia (Austria) and in the Prokletjia Mountains in the Balkans.
We report on the isolation and structure elucidation of four compounds (two new phenolic glycosides and two iridoids) from the methanolic extract of the underground parts. The new compounds are [**1**] 2'-O-acetylplantamajoside and [**2**] 2'-O, 6"-O-diacetylplantamajoside. These compounds are closely related to plantamajoside (1) which was first isolated from *Plantago major* (Plantaginaceae). Additionally, the iridoids globularin (2) and isoscrophularioside (3) were isolated. These compounds are known to occur in other members of the Scrophulariaceae. Compounds were isolated by silica gel column chromatography and semi preparative reversed phase HPLC. Structure elucidation was performed by HR-mass spectrometry and 1- and 2-D NMR spectroscopy. Evaluation of the compounds for anti-inflammatory activity is in progress.



1 R = H
2 R = OCH_3

References: 1. Ravn, H., Brimer, L. (1988) Phytochemistry 27: 3433-3437. **2.** Calis, I. et al. (1991) Helv. Chim. Acta 74: 1273-1277. **3.** Junior, P. (1981) Planta Med. 43: 34-38.

B095 Iridoid glucosides from *Veronica* Species

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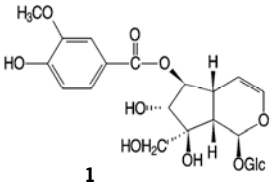
In the flora of Turkey, the genus *Veronica* L. (Scrophulariaceae) is represented by 79 species, 26 of which are endemic (1). Some of these *Veronica* species are used as diuretic and for wound healing in traditional Turkish medicine (2). *Veronica* species contain mainly iridoid glucosides, some phenylethanoid and flavonoid glycosides (3-5). Our previous research has demonstrated that the water soluble portion of MeOH extracts of some *Veronica* species show suppressive effect on nitric oxide production in lipopolysaccharide-stimulated mouse peritoneal macrophages (6). In a continuation of this study, we present here the isolation and the structure elucidation of two highly oxygenated, new iridoid glucosides, urphoside A (**1**) and urphoside B (**2**) together with nine known iridoid glucosides, pikuroside, aucubin, catalpol, veronicoside, catalposide, verproside, amphicoside, 6-*O*-veratroyl catalpol, and verminoside from the active fractions of *Veronica pectinata* var. *glandulosa*, *V. persica* and *V. hede - rifolia*. The planar as well as the stereo structures of the isolated compounds were determined by means of extensive 1D- and 2D-NMR spectral analysis. Molecular formula of urphoside B (**2**) which was the chlorinated derivative of urphoside A (**1**) was established by HR-ESI-MS.



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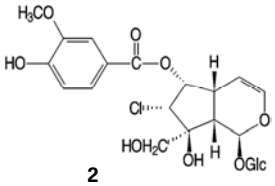

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References: 1. Davis, P.H. (1978) Flora of Turkey and The East Aegean Islands. Vol. 6. University Press. Edinburgh. **2.** Baytop, T. (1999) Therapy with Medicinal Plants in Turkey (Past and Present). Publications of Istanbul University. Istanbul. **3.** Taskova, R. et al. (1998) Phytochemistry 49: 1323-1327. **4.** Ozipek, M. et al. (1999) Chem. Pharm. Bull. 47: 561-562. **5.** Chari, V.M. et al. (1981) Phytochemistry 20: 1977-1979. **6.** Harput, U.S. et al. (2002) Biol. Pharm. Bull. 25: 483-486.

B096 Flavonoid glycosides from *Veronica pectinata* var. *glandulosa* and *V. persica*

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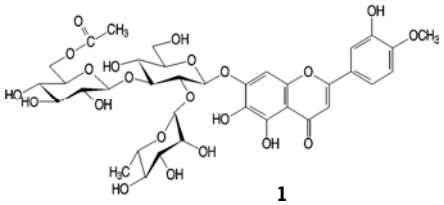
In the flora of Turkey, the genus *Veronica* L. (Scrophulariaceae) is represented by 79 species, 26 of which are endemic (1). Some of these *Veronica* species are used as diuretic and for healing of wound in Turkey (2). Previously, a large variety of flavone aglycones such as luteolin, apigenin, chrysoeriol, scutellarein, isoscutellarein and their acylated glycosides were reported from *Veronica* species (3). Here we report the isolation of a new (**1**) and three known (**2**-**4**) flavon glycosides as well as a known flavon aglycone (**5**) from *Veronica pectinata* var. *glandulosa* and *V. persica*, respectively. Their structures were determined as 3'- hydroxy, 4'-*O*-methylscutellarein-7-*O*-[2"-*O*-αL-rhamnopyranosyl-3"-O-(6""-*O*acetyl-βD-glucopyranosyl)]-βD-glucopyranoside, named sarachoside (**1**), isoscutellarein-7-*O*-2"-*O*-(6"'-*O*-acetyl-β-D-allopyranosyl)-β-D-glucopyranoside (**2**), 4'-*O*-methylisoscutellarein-7-*O*-2"-*O*-(6"'-*O*-acetyl-β-D-allopyranosyl) -β-D-glucopyranoside (**3**), 3'-hydroxy, 4'-*O*-methylisoscutellarein-7-*O*-2"-*O*-(6"'-*O*-acetyl-β-D-allopyranosyl)- β-D-glucopyranoside (**4**) and circilineol (**5**) by spectral analysis.



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References: 1. Davis, P.H. (1978) Flora of Turkey and The East Aegean Islands. Vol. 6. University Press. Edinburgh. **2.** Baytop, T. (1999) Therapy with Medicinal Plants in Turkey (Past and Present). Publications of Istanbul University. Istanbul. **3.** Chari, V.M. et al. (1981) Phytochemistry 20: 1977-1979.

B097 Steroidal saponin E from *Convallaria majalis* L.

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Convallaria majalis L. (lily of the valley) is a plant of the family Liliaceae, widely distributed in Europe. The overground parts of *C. majalis* contain cardenolidic glycosides and its roots and rhizomes were found to contain several steroidal saponins. Tschesche *et* al. (1) have isolated furostanol saponin, *i.e.*, convallamaroside, the aglycone of which is convallamarogenin, that has been identified as Δ^{25}-5β,20β,22α-spirosten-1β,3β-diol. In addition to convallamaroside, other steroidal spirostanol saponins (13 compounds) have been isolated (2). In this report we describe the isolation and structural characterization of saponin E. Powdered roots and rhizomes of *C. majalis*, 1900 g, were macerated for 48 h with aqueous 50% methanol. The extract was partitioned between $CHCl_3$ and *n*-BuOH with H_2O. Compound E (m.p., 198-204°C) was isolated, 370 mg, by column chromatography from a fraction of less polar compounds. The structure of saponin E was elucidated in terms of ^1H and ^{13}C NMR spectra involving the 2D techniques (HETCOR, HMBC, ROESY). The compound E was identified as Δ^{25}-5β-spirostene-1α,2β,3β,5β-tetraol 5-O-β-D-arabinoside.
Conformational analysis including the orientation of -OH groups and of the sugar moiety was performed by the semiempirical MO method. For the optimized low energy structure, the NMR shielding constants were calculated by using the *ab initio* GIAO CHF method.

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B098 New triterpene saponins from *Mimosa pudica* seeds

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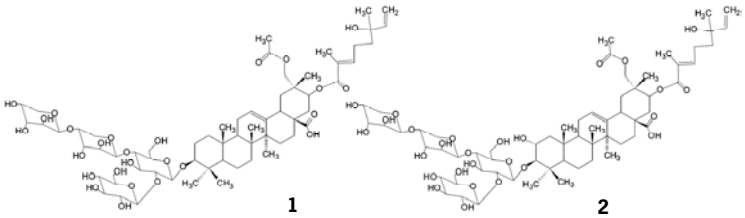
In the framework of our research programme on bioactive products from the Mimosaceae family (1-2) we investigated the presence of saponins in *Mimosa pudica* L. seeds. In a previous phytochemical work on the "sensitive plant", we reported the occurence of flavonoid glycosides (3-4) in aerial parts. A refluxing ethanolic seed extract was partitioned with *n*-butanol / water. The concentrated butanolic fraction was submitted to extensive preparative RP-HPLC to give two new monodesmosidic oleanane-type saponins bearing unusual 29-acetyl and 21-geranyl chains (**1**-**2**).
Their structural elucidation was performed mainly by 2D NMR techniques and HR-FAB-MS.



1 **2**

References: 1. Jiang, Y. (1991) Saponins from *Mimosa tenuiflora* (Wild) Poiret, Mimosaceae, PhD Thesis, ULP Strasbourg. **2.** Englert J. (1995) Saponins from *Mimosa pigra* L., Mimosaceae, PhD Thesis, ULP Strasbourg. **3.** Englert J. et al. (1993) Planta Med., 60: 194. **4.** Lobstein A. et al. (2002) Biochem. Syst. Ecol., 30: 375.

B099 Mentzeliol - a new compound from *Mentzelia chilensis* Gay "Cordillera Negra"

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Decoctions of the aerial parts of *Mentzelia* sp. (Loasaceae), well known in Peruvian traditional medicine as *angua - raté*, are used as cicatrizant of gastric ulcers and for dyspeptic disorders (1,2). The wild growing and cultivated plant material obtained from Pamparomas, Departamento Ancash, and traded as *anguaraté* is here provisionally called *Mentzelia chilensis* Gay "Cordillera Negra", since the taxonomy of *Mentzelia* sp. in Peru has not yet been completely resolved (3). Previously we identified mentzeloside **1** as an antiinflammatory compound in *M. chilen - sis* (4). Continuing our phytochemical investigations we now isolated from the MeOH extract of the stems the C9-iridoids 5-OH-mentzeloside (scabroside) **2** and 11-β-D-Glucosyl-epoxydecaloside **3**, as well as a new natural compound, mentzeliol **4**. The latter was isolated by VLC on a cyclohexyl-RP-phase column and semipreparative HPLC on a polar endcapped reversed phase column. Structure elucidation using 1- and 2-dimensional NMR spectroscopy as well as GC-MS analysis revealed that **4** was (*1R*, 2S*, 3S**)-4-(hydroxymethyl)-3-(1-hydroxyprop-2-en-2-yl)-cyclopent-4-en-1,2-diol, a new natural compound which we designated as mentzeliol. **2** was previously identified only in *Deutzia* sp. (5), **3** in *Mentzelia* sp. (6). By TLC **4** could be detected in both, roots and stems of *M. chilensis*. Further investigations are in progress to clarify the role of **4** in the chemical taxonomy of *Mentzelia* sp. of Peru as well as its biological properties regarding the traditional use of *anguaraté*.

Acknowledgements: Alsitan GmbH, Greifenberg, Germany, is acknowledged for providing plant material and for financial support.

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B100 Five new medicagenic acid saponins from the roots of *Muraltia ononidifolia*

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In continuing our studies on the genus *Muraltia* (Polygalaceae) (1), we isolated five new triterpene saponins **1-5** from the ethanolic extract of the roots of *Muraltia ononidifolia* E. Mey which is an herbaceous plant indigenous to Southern Africa. The crude saponin mixture was fractionated by column chromatography over Sephadex LH-20 and repeated medium-pressure liquid chromatography (MPLC) over normal Silica gel, followed by semi-preparative HPLC on a reversed phase (C18) column yielding five pure compounds. Their structures were elucidated mainly by 600 MHz NMR analysis including 1D and 2D-NMR spectroscopy (COSY, TOCSY, NOESY, HSQC, HMBC) and FAB-MS as 3-*O*-β-D-glucopyranosyl-medicagenic acid-28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**1**), 3-*O*-β-D-glucopyranosyl-medicagenic acid-28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**2**), 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-medicagenic acid-28-*O*-βD-xylopyranosyl-(1→4)-[βD-apiofuranosyl-(1→3)]-αL-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**3**), 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-medicagenic acid-28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**4**) and 3-*O*β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-medicagenic acid (**5**), respectively.

Reference: 1. Elbandy, M. et al. (2002) J. Nat. Prod. 65: 193-197.

B101 Four new triterpene saponins from *Acanthophyllum glandulosum*

G. Gaidi [a], *T. Miyamoto* [b], *M. Ramezani* [c] *and* M.A. Lacaille-Dubois [a]

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Acanthophyllum glandulosum Bunge ex Boiss. (Caryophyllaceae) is one of the 6 species of the section *Pleiosperma* endemic to Iran (1). The plant has been used as an expectorant, emetic and detergent. No previous phytochemical investigation has been reported. Here, we describe the isolation and structure elucidation of four new triterpene saponins, glandulosides A-D (**1**-**4**). The methanolic extract of the roots was purified by column chromatography over Sephadex LH-20 and by successive medium pressure liquid chromatography (MPLC) on normal and reversed phase (C18) Silica gel column yielding four pure saponins. Their structures were elucidated mainly by 600 MHz NMR analysis including 1D and 2D NMR spectroscopy (COSY, TOCSY, NOESY, HSQC and HMBC) (2) as 23-*O*-β-D-galactopyranosyl-gypsogenic acid-28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→6)]-β-D-galactopyranoside (**1**), 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-*O*βD-xylopyranosyl-(1→3)-βD-xylopyranosyl-(1→4)-αL-rhamnopyranosyl-(1→2)-4-*O*acetyl-β-D-fucopyranoside (**2**), 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-*O*βD-xylopyranosyl-(1→3)-βD-xylopyranosyl-(1→4)-αL-rhamnopyranosyl-(1→2)-[4-*O*acetyl-βD-quinovopyranosyl-(1→4)]-β-D-fucopyranoside (**3**), 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosyl-gypsogenin-28-*O*βD-xylopyranosyl-(1→3)-βD-xylopyranosyl-(1→4)-αL-rhamnopyranosyl-(1→2)-3,4-di *O*-acetyl-β-D-fucopyranoside (**4**).

References: 1. Rechinger, K.H. (1988) Flora Iranica, Flora des iranischen hochlandes und der umrahmenden Gebirge, Caryophyllaceae II; Akademische Druck und Verlagsanstalt: Graz, Austria. **2.** Gaidi, G. et al. (2001) J. Nat. Prod. 64: 920-924.

B102 Acylated flavonoid glycosides from *Marrubium velutinum*

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The genus *Marrubium* L. comprises around 30 species, indigenous in Europe, the Mediterranean area and Asia (1). *Marrubium velutinum* Sibth & Sm. (Lamiaceae) is an endemic herb of central and southern Greece. The air-dried powdered aerial parts of the plant were extracted with petroleum ether, ether, ethyl acetate and methanol. The dried methanol extract was subjected to VLC over silica gel. Further fractionation with repeated CC on silica gel and Sephadex LH-20 led to the isolation of one new acylated flavonoid glucoside, chrysoeriol 7-*O*-(3'',6''-*O*-*E*-di-*p*-coumaroyl)-β-D-glucopyranoside, together with ten known flavonoids: apigenin 7-*O*-(3''-*O*-*E*-di-*p*-coumaroyl)-β-D-glucopyranoside, apigenin 7-*O*-(3''-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside, isorhamnetin 3-*O*-(6''-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside, isorhamnetin 7-*O*-(6''-*O*-*E*-*p*-coumaroyl)-β-D-glucopyranoside, isorhamnetin 3-*O*-β-D-glucopyranoside, isorhamnetin 3-*O*-β-D-rutinoside, quercetin 3-*O*-(6''-acetyl)-β-D-glucopyranoside, isoquercitrin, kaempferol-3-*O*-β-D-rutinoside and chrysoeriol. The structures of the isolated compounds were established by means of UV, 1D and 2D NMR spectroscopy (^{1}H, ^{13}C/DEPT, COSY, HMQC, HMBC, ROESY), as well as mass spectrometry (HR-MALDI). Earlier flavonoid surveys of *Marrubium* sp. (2-7), revealed a nearly complete dominance of flavones and the presence of only one flavonol (kaempferol) isolated from *M. peregrinum* (7). Therefore the presence of flavonol glycosides at *M. velutinum* appears as a characteristic feature of this plant.

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B103 Two new sesquiterpens from the leaves of *Laurus nobilis* L.

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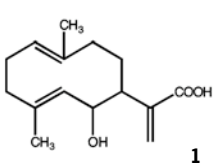
Laurus nobilis L. (bay laurel, laurel) is a small aromatic tree native to the Mediterranean regions. The leaves are much used in cookery for flavouring as spice in marinating and pickling foods. As medicinal plants, bay leaves and berries have been employed against rheumatism and as stomachic, antiseptic, carminative, diaphoretic and insect repellent. The essential oil is used by the cosmetic industry. In our chemical and biological investigation on bioactive compounds from medicinal plants, we examined the methanolic extract from the leaves of *Laurus nobilis* L. collected in Campania's hills during summertime. The methanolic extract was subjected to a modified Kupchan's partitioning methodology to obtain four extracts: *n*-hexane, CCl_4, $CHCl_3$ and *n*-butanol. Twelve known components: blumenol C, dendranthemoside A, dihydrodendranthemoside A, lyoniside, ampelopsisioniside, citroside A, icariside B1, alangionoside A and its aglycone, dihydroalangionoside A, kaempferol-3-O-α-L-(3",4"-di-*E*-*p*-coumaroyl)-rhamnoside and kaempferol-3-O-α-L-(2"-*E*-*p*-coumaroyl)-rhamnoside were isolated and characterized from *n*-butanol and chloroform extracts. Two new compounds from chloroform soluble fraction were identified as the germacrane-type **1** and the sesquiterpene **2**. The structures of the new compounds were elucidated by a combination of NMR techniques including $^1H,^1H$ (COSY, TOCSY, ROESY) and $^1H,^{13}C$ (HMQC and HMBC) spectroscopy and FAB-MS spectrometry.



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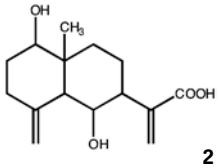

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B104 Neoclerodane diterpenoids from *Croton eluteria*

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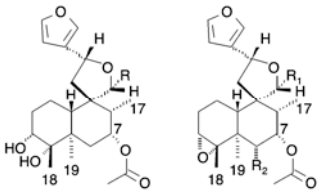
Croton eluteria Bennett (Euphorbiaceae), is a tropical shrub or small tree, native from the West Indies and northern South America.

In previous papers, its dried bark, called "cascarilla" was shown to contain terpenes derivatives (**1-4**).

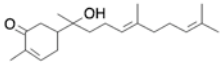
This investigation of non-polar constituents were continued. From the chloroformic fractions, partitioned by, CC, MPCC, and preparative HPLC, five new neoclerodanes have been isolated, called Cascarillin E-I, and a diterpene, 7-hydroxy-1,6-cyclo-2,10,14-phytatrien-4-one, already identified in *C. linearus* L. (**5**).

In this communication, we describe the structural elucidation of these compounds, determinated by MS, NMR 1-D and 2-D data.



R: OCH_3 Cascarillin E
R: OH Cascarillin F

R_1: OH ; R_2: $OCOCH_3$ Cascarillin G
R_1: OH ; R_2: H Cascarillin H
R_1: $OCOCH_3$; R_2: OH Cascarillin I



7-hydroxy-1,6-cyclo-2,10,14-phytatrien-4-one

References: 1. Hagedorn, M.L. et al. (1991) Flavour Frag. J. 6, 193-204. **2.** Halsall, T.G. et al. (1965) Chem. Commun. 11, 218-219. **3.** Claude-Lafontaine, A. et al. (1976) Bull. Soc. Chim. Fr. 88-90. **4.** Vigor, C. et al. (2001) Phytochemistry 57, 1209-1212. **5.** Alexander I.C. (1991) Phytochemistry 30, 1801-1803.

B105 Iridoids from *Putoria calabrica* (Rubiaceae)

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The genus *Putoria* Pers. (Rubiaceae) only includes two species. *Putoria calabrica* (L. f.) Pers. is a undershrub widely distributed on the mountain slopes of the Mediterranean area.
Previous investigations of the aerials parts resulted in the isolation of phytol and β-sitosterol, of several anthraquinones and of naphtalene-derived pigments belonging to the lapachenol and tectol series (1). No previous chemical work dealing with terpenoid glycosides has been recorded on the genus *Putoria*.
In the course of searching for cytotoxic, hypotensive agents from medicinal herbs, fresh aerial parts of *P. calabrica* were extracted with MeOH (3x5 L) at room temperature. The methanol extract, after separation by filtration of a white solid precipitated and crystallized in EtOAc (ursolic acid), was subjected to repeated flash column chromatography over silica gel to afford five known iridoid glycosides: asperuloside, paederoside, asperulosidic acid, 3-methoxy-3,4-dihydroasperuloside (V3 iridoid) and geniposide.
The structures of the compounds were determined on the basis of the spectral data (UV, IR, MS, ^{1}H NMR and ^{13}C NMR) of both natural and peracetylated glycosides, identical with those previously described (2-5).
Iridoid glycosides, particulary asperuloside, asperulosidic acid and geniposide, are good chemotaxonomic markers of the Rubiaceae family (6).
Paederoside is a much more interesting asperuloside derivative, due to its sulfur containing structure. Previously, it had been only isolated from species of *Paederia* (*P. scandens* and *P. foetida*).

References: 1. Gonzales, A. G. et al. (1974), An Quim. 70: 858. **2.** Bailleul, F. et al. (1977) Phytochemistry 16: 723. **3.** Sainty, D. et al. (1981) Planta Med. 42: 260. **4.** Suzuki, S. et al. (1993) Heterocycles 35: 895. **5.** Bojthe-Horvàth, K. et al. (1982) Phytochemistry 21: 2917. **6.** Jensen, S. R. et al. (1975) Bot. Notiser 128: 148.

B106 New guaianolides from *Tanacetum fruticulosum* Ledeb.

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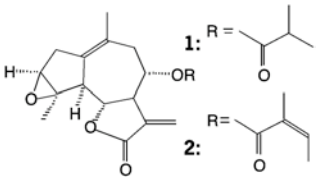
Sesquiterpene lactones have been reported to have multiple biological effects including cytotoxic, antibacterial, anti-inflammatory, hypotensive and many others. The guaianolides represent one of the largest groups of sesquiterpene lactones covering over 600 known naturally occurring compounds. Much attention has been paid to the antitumor properties associated with their cytotoxicity. The genus *Tanacetum*, with ca. 200 species, is distributed over Europe and West Asia.
As a part of our continuing studies on Iranian plants, we examined *T. fruticulosum*, collected in the Hamedan area, N. West of Iran in 1998, and isolated two new guaianolide sesquiterpene lactones (**1**,**2**) by extraction and chromatographic procedures. The air-dried aerial parts (500 g) were extracted with $CHCl_3$. The extract obtained was defatted with MeOH and first separated by CC (silica gel). The fractions obtained with Et_2O-Petrol (1:3) were separated by prep. TLC (silica gel) affording a mixture which was further separated by HPLC (RP-8, MeOH-H_20; 7.5:2.5) to give 8 mg **1** and 12 mg **2**. The molecular formula of **1** $C_{19}H_{24}O_5$ and **2** $C_{20}H_{24}O_5$ were deduced from high resolution EIMS. With ^{1}H-NMR signals ($CDCl_3$, 500MHz), ^{13}C-NMR ($CDCl_3$) and using 2D-NMR spectroscopy, (^{1}H,^{1}H-COSY, ^{1}H,^{13}C-COSY HMQC), we were able to assign all the ^{1}H and ^{13}C chemical shifts. The observed NOEs support the proposed stereochemistry.



Reference: 1. Weyerstahl, P. et al. (1999) Flavour Fragr. J., 14,112-120.

B107 Iridoids isolated from *Pterocephalus sanctus* Decne

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Continuing our studies on some endemic medicinal plants growing in South Sinai, Egypt (1), we investigated *Pterocephalus sanctus*, belonging to Dipsacaceae family (2), which is a very rare wild plant growing in Saint Catherine mountain. As far as we know, this is the first report of phytochemical studies of the plant.

We investigated the 80 % aqueous methanol extract of *P. sanctus* herb (600 g). The obtained extract (25 g) was defatted and subjected to column chromatography over Silicagel eluted with hexane and a gradient by addition of ethyl acetate. The fraction Fr-V, (6 g) eluted with 75% ethyl acetate was further separated by Silicagel flash column chromatography eluting with a gradient increase of methanol in ethyl acetate. Four fractions were collected (Fr-V-a; Fr-V-b Fr-V-c Fr-V-d). Fraction (Fr.-V-c) was further purified over Sephadex LH-20 eluted with 20% aqueous ethanol. The obtained fraction P-1 (2.15 g) was rechromatographied over flash Silicagel chromatography eluted with a gradient of methanol in chloroform (5-20%). Two major fractions were collected, P-1a (0.5 g) and P-1b (50 mg). Fraction P-1a showed two main spots in TLC and was subjected to Silicagel column chromatography using 10% methanol in chloroform. Pure substance A (34 mg) was obtained and was identified as swerosid by analysis of the 2D-NMR spectra (H,H-COSY, HMQC, HMBC) and comparison with NMR data of sweroside-6'-O-glucoside (3). The other pure substance B (41 mg) was identified as loganin by means of the 2D-NMR spectra (H,H-COSY, HMQC, HMBC) in comparison with reference data (4).

This is the first report of isolation and identification of two iridoids, swerosid and loganin, from *P. sanctus*.

References: 1. Adel Kamal Zaki et al. (2000) Book of Abstracts of Natural Products Research, the Netherlands, 779. **2.** Tackholm (1974), Cited in "Student Flora of Egypt" Beirut. **3.** R.X. Tan, et al. (1996) Phytochemistry 42: 1305. **4.** T.T. Thuy et al. (1999) Journal of Chemistry, 37: 64-69.

B108 New abietane and seco-abietane diterpenoids from the herb of *Salvia candelabrum*

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Salvia candelabrum Boiss. (Lamiaceae) is a herbaceous species, native to South Spain and is used in the traditional medicine as febrifuge. Previous phytochemical studies on the aerial parts have yielded the abietane candelabrone (1), and 3,4-*seco* rearranged abietane diterpenes, candesalvones A and B (2) besides triterpenes, β-sitosterol and essential oil. Our recent study on 11 European *Salvia* species revealed pronounced antioxidant activity of the extract of *S. candelabrum* in both enzyme-dependent and enzyme-independent systems of lipid peroxidation. Phytochemical analysis of the active extract showed the presence of potent antioxidant agents e.g. rosmarinic acid, caffeic acid and flavonoids. However, the total amount of phenolic compounds was found to be definitely higher than the sum of these constituents. In order to characterise further compounds of *S. candelabrum*, the methanolic extract of the aerial parts was investigated in detail.

The plant material was collected from the experimental field of the Institute of Ecology and Botany of the H.A.S., Vácrátót, Hungary. The methanolic extract of the herb was subjected to open column chromatography on polyamide and on silica gel, and then fractionated repeatedly by preparative TLC to afford five compounds. The structures were established by ESI-mass spectroscopy and advanced two-dimensional NMR methods, including ^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC experiments. The present paper reports on the isolation and structure elucidation of three new 3,4-*seco*-abietane and one new abietane diterpenes along with the known candelabrone. The chemical names of the new compounds are as follows: 12-hydroxy-7,11,14-trioxo-3,4-*seco*-4(18),8,12-abietatrien-3-oate; 11,14-dihydroxy-12-methoxy-7-oxo-3,4-*seco*-4(18),8,11,13-abietatetraen-3-oic acid; 11,12,14-trihydroxy-7-oxo-3,4-*seco*-4(18),8,11,13-abietatetraen-3-oate; 3,7,11,14-tetraoxo-8,12-abietadiene-12-ol.

Acknowledgements: This research was supported by grants OTKA F 029 249, OTKA T 035 200 and FKFP 0024/2001.

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B109 Isolation of mangiferin and structure revision of shamimin from *Bombax malabaricum*

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Bombax malabaricum DC. (Bombacaceae) (Syn. *B. ceiba* L. and *Salmalia malabaricum* DC) is also known as silk-cotton tree. It is commonly found in the Indo-Pakistan subcontinent and other parts of Asia and Australia (1). The plant is well reputed for the treatment of diarrhoea, tumors, fever, dysentery, kidney and bladder ulceration, and chronic inflammation (2,3). Phytochemical investigation of different parts of this plant resulted in the isolation of naphthol, naphtoquinones, polysaccharides, anthocyanins and lupeol (4).
The 70 % alcoholic extract of the leaves of *B. malabaricum* was concentrated, kept in the refrigerator overnight, and centrifugated. The supernatant was successively extracted with $CHCl_3$, EtOAc and BuOH. Repeated column chromatography of the BuOH fraction yielded compound **1** which was identified as 2*C*-β-D-glucosyl-1,3,6,7-tetrahydroxyxanthone (mangiferin) (4,5). The ^1H and ^{13}C-NMR data were in complete agreement with those reported for shamimin or 6*C*-β-D-glucosyl-3,5,7,2',4',5'-hexahydroxyflavone reported before from *Bombax ceiba* (3). Therefore we conclude that the structure of shamimin has to be revised, and that it is identical to mangiferin.

References: 1. Chanda, Y.R. (1962) The Wealth of India, Raw Material, vol. IX, pp. 175-183, CSIR Hillside Rood, New Delhi, India. **2.** Hocking, M.G. (1997) A Dictionary of Natural Products p. 119, Plexus Publishing, Inc. **3.** Shahen Faizi, Muhammed Ali (1999) Planta Med. 65, 383-385. **4.** Wada, H. et al. (1995) Chem. Pharm. Bull. 43 (3) 461-165. **5.** Hano, Y. et al. (1991) Planta Med. 57, 172-175.

B110 Structure elucidation of three new acetylated flavonoid glycosides from *Centaurium spicatum*

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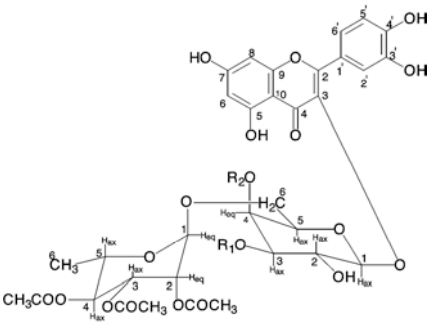
Centaurium spicatum (L.) Fritsch (Gentianaceae) is an annual herb occurring in Southern Europe and Northern Africa, where it is used together with other *Centaurium* species like *C. pulchellum* in traditional medicine. Alkaloids and secoiridoids have been reported before from *Centaurium spicatum*, but this is the first report on flavonoids from this plant. Three new acetylated flavonol glycosides, quercetin 3*O*(2,3,4-triacetyl-α-rhamnopyranosyl)-(1→6)-β-galacto-pyranoside (**1**), quercetin 3-*O*-[(2,3,4-triacetyl-α-rhamno-pyranosyl)-(1→6)]-3-acetyl-β-galactopyranoside (**2**), and quercetin 3-*O*-[(2,3,4-triacetyl-α-rhamnopyranosyl)-(1→6)]-4-acetyl-β-galactopyranoside (**3**) have been isolated and identified. Structure elucidation, especially the localisation of the acetyl groups, and complete ^1H and ^{13}C NMR assignments were carried out using one- and two-dimensional NMR methods, including ^1H and ^{13}C NMR, DEPT-135 and DEPT-90, and gradient-assisted experiments such as DQF-COSY, TOCSY, HSQC and HMBC (1).



1 $R_1 = R_2 = H$
2 R_1 = acetyl, $R_2 = H$
3 $R_1 = H$, R_2 = acetyl

References: 1. Shahat A.A. et al. (2001) *Magn. Reson. Chem.* 39, 625-629.

B111 Isolation, characterisation and chemical modification of related analogues of abietane and totarane diterpenes with antiplasmodial activity from *Harpagophytum procumbens*

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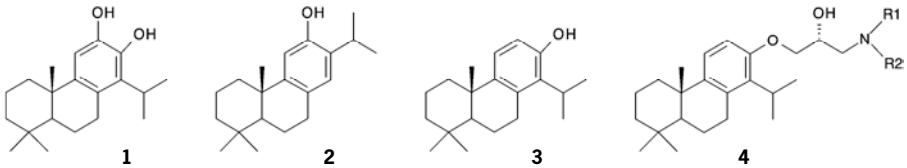
Reliance on traditional medicines (largely plant based) in the developing world is considerable and represents a wealth of information not only as chemotherapeutic agents, but also as a potential source of novel antimalarial drugs. In the course of our research on the antimalarial activity of traditional medicines, we investigated the *in vitro* antiplasmodial activity of one of South Africa's medicinal plants, *Harpagophytum procumbens*, also commonly known as Devil's Claw. Bioassay guided fractionation led to the isolation, identification and full characterisation of the totarane diterpene (**1**) and the abietane diterpene (**2**), which showed significant i*n vitro* antiplasmodial activity (IC_{50} <1µg/ml) against both drug sensitive and resistant strains of *Plasmodium falciparum*. Although the compounds are known, they are not known to possess antiplasmodial activity and have different structural features to current antimalarial drugs. *In vitro* cytotoxicity screenings showed that the compounds were not toxic to mammalian cells (CHO and HEPG2) at the concentrations required to kill the parasites. Chemical modification of a commercially available analogue totarol (**3**), led to 5 new synthetic β-amino alcohol derivatives of the general structure (**4**), which were tested for *in vitro* cytotoxicity and antiplasmodial activity. Although the compounds showed an improved activity and were equally active against drug sensitive (D10) and resistant (K1) strains of *P. falciparum*, no definite structure-activity conclusions could be made at this stage. Considering their antiplasmodial activity and lack of toxicity, the isolated compounds are promising templates for the development of a novel group of antimalarial drugs.



1 **2** **3** **4**

B112 A new branched acylated glycoside luteolin from *Mentha x piperita* leaves

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Mentha x piperita (Labiatae) has many medicinal and daily uses; infusion of its leaves is stomachic, aphrodisiac, carminative, appetizer especially when mixed with tea (1). In the past ten years, there have been several studies on the flavonoid constituents of family Labiatae (2,3). The 5,6-dihydroxy flavone and free flavone aglycones were isolated from *Mentha x piperita* (4,5). The aqueous acetone extract of *M. piperita* leaves afforded one new flavone glycoside luteolin-7-O-3^G ($3^{""}$ acetyl rhamnosyl) rutinoside besides the known flavonoids: luteolin-7-O-glucoside, 7-O-rhamnoside and 7-O-rutinoside with the three aglycones; luteolin, chrysoeriol and diosmetin.
All the above isolated compounds were isolated and purified using different physical and chemical methods and their structures were elucidated using ^1H and ^{13}C-NMR spectroscopy.

References: 1. Boulos, L. (1983) Medicinal Plants of North Africa Reference publications, Inc. Michigan 48001, U.S.A. **2.** Tomas-Barberan,F.A. and Wollenweber, E. (1990) Plant Syst. Evol., 173: 109. **3.** Tomas-Barberan,F.A. and Gil, M.I. (1990) Rev. Latinoam. Quim., 21: 134. **4.** Voirin, B. and Bayet, C. (1992) Phytochemistry, 31: 2299. **5.** Passannanti, S. et.al. (1990) Fitoterapia 61: 54.

B113 A new lupane triterpene from *Euphorbia portlandica*

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Euphorbia portlandica L., an Euphorbiaceae , is commonly found in the coast of Portugal, especially in sand and rocks near the beaches. The *Euphorbia* genus, with more than 1600 species, has been a source of biological active compounds.
The whole dried plant was extracted with acetone. The acetone extract was suspended in MeOH/H_2O and extracted with *n*-hexane. Fractionation of the hexane extract yielded a new pentacyclic triterpene alcohol with a lupane skeleton which was established as 3α-hydroxy-19αH-lup-20(29)-ene (**1**). The known pentacyclic triterpene glutinol, identified by comparison of its spectroscopic data with those described in the literature (1), was also isolated. The characterisation of the new compound and its acetylated derivative was based on NMR data, including 2D NMR, and mass spectrometry. Its structure and configuration was confirmed by X-ray diffraction analysis, using Cu radiation. The molecule crystallises in the monoclinic non centrosymmetric space group P2. The compound is an isomer of lupeol with a 3α-hydroxyl group at C-3 and a β isopropenyl chain at C-19.
Lupeol and its esters, structurally related with compound **1**, are known for their biological activity, namely their anti-inflammatory and antitumour activities (2, 3, 4).

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B114 Methoxylated flavones from *Artemisia rupestris*

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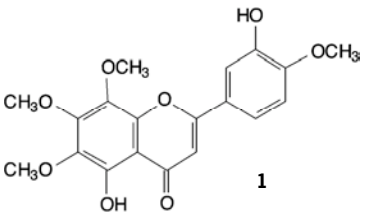
Artemisia rupestris L. (Compositae) is used by the Uyghur people of Xinjiang Uyghur Autonomous Region of China, for a variety of anti-inflammatory conditions including influenza, dermatitis, measles, burns, hepatitis and snakebite (1). Previous research on this plant have resulted in isolation of seven known compounds, including flavonoids and sesquiterpenes such as rupestric acid, rupestonic acid and iso rupestonic acid (2-4). Methanolic extracts of the aerial parts of air-dried, powdered plant material followed by preparative chromatography using a polyamide column yielded three compounds. The most abundant compound was identified as 5,3'-dihydroxy-6,7,8,4'-tetramethoxyflavone **1**, also known as gardenin D, by UV, MS and NMR spectroscopic methods. These compounds may possibly contribute to the anti-inflammatory effect, since several lipophilic flavonoids have been shown to inhibit eicosanoid synthesis (5). The anti-inflammatory and anti-oxidant screening of extracts and other constituents is in progress.



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B115 A rapid and sensitive LC/NMR method for the absolute configuration determination of two 6-alkylated α-pyrones at the microgram level

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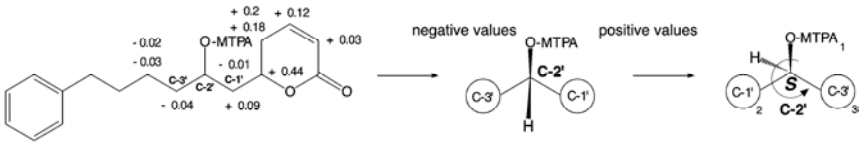
Determination of the absolute configuration at the asymmetric centers of two α-pyrones isolated from *Ravensara crassifolia* (Lauraceae) was performed using Mosher's method (1). Conventional analysis of the ester derivatives by ^{1}H NMR was replaced by LC/NMR (2) analysis of the crude reaction mixture. Completion of the reaction was checked by APCI LC/MS on 5% of the total mixture and LC-^{1}H NMR spectra were recorded on the 95% remaining in the stop-flow mode. The main advantages of this new method are its rapidity and sensitivity. Typically only a few micrograms have to be injected on-column and no clean-up procedures are necessary. These aspects are very important in natural product chemistry since often the sample amounts are very limited.



References: 1. Ohtani, I. et al. (1991) J. Am. Chem. Soc. 113: 4092-4096**. 2.** Wolfender, J.-L. et al. (2001) Phytochem. Anal. 11: 1-22.

B116 Chemical constituents of *Inula verbascifolia* subsp. *methanea* and *I. pseudolimonella* (Asteraceae) growing in Greece. Biological activities

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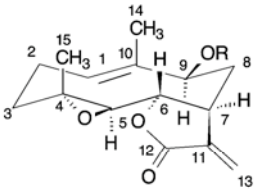
The aerial parts of *Inula verbascifolia* subsp. *methanea* yielded three new epoxygermacranolides, compounds **1**-**3**, in addition to the previously known 9β-hydroxyparthenolide **4** and the flavonoid apigenin **5**. From *Inula pseudolimonella* were also isolated: 9β-hydroxyparthenolide **4**, inusiniolide **6**, dammaradienyl acetate **7** and dammaradienol **8**. All isolated compounds were identified by means of spectral data (IR, ^{1}H NMR, ^{13}C NMR, HRFABMS and CIDMS).



1: R = $COCH_2C(CH_3)(OH)CH_2CH_3$

2: R = $COCH_2C(CH_3)(OH)CH_3$

3: R = $COCH(CH_3)CH(OH)CH_3$

The *in vitro* cytotoxic activities of compounds **1-3** were evaluated against six human solid tumor cell lines. Compounds **1-3** showed the most potent activity against the three colon cancer and PC-3 androgen insensitive cell-lines, but moderate one against the MCF-7 and LNCaP cells. Compound **3** was the most active against HCT-116 colon cell line (IC_{50}, 0.39 μg/mL). Compounds **1-4**, **6-8** were also assayed for their antimicrobial activities against six Gram (±) bacteria as well as against three pathogenic fungi. All the tested compounds showed an interesting profile against Gram (±) bacteria with **4** to exhibit the strongest antibacterial activity and **8** the strongest antifungal one.

B117 Synthesis of diterpene glucosides from kaurenoic acid

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The diterpene *ent*-kaur-16-en-19-oic acid (kaurenoic acid) occurs abundantly in some Brazilian Asteraceae and Annonaceae species. Several biological activities, such as antiviral, antimicrobial, trypanosomicidal, antiinflamatory, anti-hypertensive, miracidicidal and growth hormonal, have been reported for this acid and related compounds (1). Moreover, natural kaurane glycosides such as wedeloside, atractyloside and carboxyatractyloside have been shown to be as toxic as strychnine causing specific inhibition of ADP-ATP transport through mitochondrial membrane (1,2). Aiming to synthetise new potentially bioactive kaurane glycosides, we firstly prepared the methyl *ent*-kaur-16-en-19-oate (methyl kaurenoate ester) by methylation of kaurenoic acid. The alcohols *ent*-kaur-16-en-19-ol and methyl *ent*-17-hidroxi-16α-kauran-19-oate were obtained by reduction and hydroboration-oxidation of the methyl kaurenoate ester, respectively. The glucosidation of the alcohols, followed by de-*O*-acetylation, afforded novel *O*-glucosyl derivatives. All the obtained compounds were characterized by spectroscopic methods (^{1}HNMR, ^{13}CNMR, MS).

Acknowledgements: UESB, UFMG.

References: 1. Ghisalberti, E.L. (1997) Fitoterapia 68: 303-24**. 2.** Eichholzer, J.V. et al. (1981) Tetrahedron 37: 1881-91.

B118 Lignans from *Linum meletonis*

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Lignans constitute a widely distributed class of natural products with a wide range of physiological functions and of great medicinal importance. Plants belonging to the genus *Linum* are known to contain lignans (1-5). In the present work, the lignans of *Linum meletonis* Hand.-Mazz. collected in Turkey were analyzed using a GC-MS method developed by us (6). Dried and powdered aerial parts of *L. meletonis* (100 mg) were sonicated with 80 % methanol for 1 h. Then 4 ml of dichloromethane and 4 ml of water were added. The tube was closed, mixed and centrifuged at 1000 g for 6 min. One and a half ml of the organic layer was evaporated, the residue was re-dissolved in 1.5 ml of methanol and subjected to GC-MS analysis, using a WCOT fused-silica CP-Sil 5CB column. Compounds were identified by comparison of MS and retention times with those of authenticated standards and also by comparison with published MS data. Seven lignans were identified in the aerial parts of *L. meletonis*: isojusticidin, podophyllotoxin, 6-methoxypodophyllotoxin, polygamain, hinokinin, morelensin and bursehernin. This is the first report of the lignans from this species, and the four last lignans were not previously described from any *Linum* species.

References: 1. Broomhead AJ and Dewick PM (1990) Phytochemistry 29: 3839. **2.** Konuklugil B (1997) Bio. Chem Eco. 25: 75. **3.** Konuklugil B (1998) Bio. Chem Eco. 26: 795. **4.** Van Uden W et al. (1992) J. Nat. Prod. 55: 102. **5.** Meagner LP et al. (1999) J. Biol. Chem. 47: 3173. **6.** Koulman A et al. (2001) Planta Med. 67: 858.

B119 New iridoid glycosides from *Rhinanthus glacialis*

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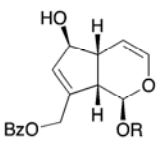
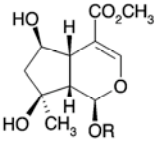
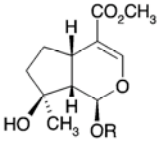
Rhinanthus glacialis Personn., a widespread herb of alpine meadows, belongs to the family of Scrophulariacae, which is known for its variety of iridoid glycosides (1). This class of compounds proofed not only to be of chemotaxonomic interest within the Scrophulariaceae (2) but has also shown a broad variety of bioactivities, among these antimicrobial, antitumoral, hemodynamic, choleretic, hepatoprotective and anti-inflammatory activities (3). *Rhinanthus glacialis* has to our best knowledge never been the aim of any phytochemical research attempt. In this presentation we report on the isolation and structure elucidation of the known iridoid-glycosides melampyroside (**1**), 6'-O-benzoylshanzhiside methylester (**2**) and the aglycon (5) shanzhigenin methylester (**3**) and two new iridoid glycosides namely 6'-O-benzoylmussaenoside (**4**) and 6'-O-benzoylmelampyroside (**5**). The structures were elucidated by utilizing different 1D- and 2D-NMR techniques as well as MS and HPLC-MS/MS (ESI).



1 R = Glc
5 R = 6'-O-benzoyl-Glc



2 R = 6'-O-benzoyl-Glc
3 R = H



4 R = 6'-O-benzoyl-Glc

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B120 Xanthones from *Polygala vulgaris*

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With the aim to search for new antitumor agents from plants we considered *Polygala vulgaris* (Polygalaceae).

Members of Polygalaceae are well known for containing a variety of different chemical constituents, many of which exhibit significant biological activity. In fact, previous phytochemical investigations on different *Polygala* species yielded a large number of different compounds: lignans, xantones and styrylpyrones (1, 2).

In the course of our studies, the chloroform extracts from both roots and aerial parts showed activity against LoVo cell line. Fractionation of the active extracts led to the isolation of several phenolic compounds.

This paper reports the characterization of a new chloroxantone, 7-chloro-1,2,3-trihydroxy-6-methoxyxanthone, and two polyoxygenated xantones, 1,3-dihydroxy-2,4,7-trimethoxyxanthone and 4,7-dihydroxy-2,3-methylendioxyxanthone, from the chloroform extract of aerial parts of *P. vulgaris*. The presence of chloroxantones in higher plants were firstly reported by Hu *et al.* from *Hypericum ascyron* (3). Structures of isolated compounds were elucidated by 1D and 2D NMR techniques including 1D TOCSY, HMBC, HMQC, COSY, NOESY and by HR-MS. Compounds were tested for antiproliferative activity against human intestinal adenocarcinoma cell lines (LoVo) and its drug resistance sub clone (LoVo/Doxo). As reference compound doxorubicine hydrochloride was used. The chloroxantone showed the highest activity.

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B121 Polysaccharides from the lichen *Thamnolia vermicularis* var. *subuliformis*

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About 13500 species of lichens have been described, however less than 100 species have been investigated for polysaccharide constituents (1). Many polysaccharides from lichens have been found to have immunological activities (1). The lichen *Thamnolia vermicularis* var. *subuliformis* (Ehrh.) Schaer has been shown to contain a complex heteroglycan with an unusual rhamnopyranosylgalactofuranan structure, which was active in the phagocytosis assay and anti-complementary assay (2). The purpose of this study is the isolation and structural elucidation of other polysaccharides from this lichen.

The polysaccharides were extracted with hot water and 0.5 M NaOH, isolated by ethanol precipitation and dialysis, chromatographically purified with ion-exchange, gel filtration and preparative HP-GPC. The polysaccharides were structurally characterized by ^{1}H and ^{13}C NMR spectroscopy, methanolysis, methylation analysis using GC-MS, enzymatic and week acid hydrolysis followed by analysis of oligosaccharides. The molecular weights were determined by HP-GPC.

In addition to the complex heteroglycan previously described, the *T. vermicularis* var. *subuliformis* was shown to contain a gel forming β-glucan not found in lichens before. The glucan was isolated in about 9% yield from the alkali extract and was shown to have a (1→3)-β-D-glucopyranosyl backbone with (1→6)-βD-glucopyranose sidegroup for every third unit in the backbone, and a molecular weight of about 67 kD. Two rhamnose containing galactomannans were isolated from the water extract and shown to have molecular weights about 18kD and 200kD respectively.

In conclusion we find that this lichen produces polysaccharides with unusual structures and besides being of interest as possible immunostimulators, these new structure could also be of interest from a chemotaxonomical point of view.

Acknowledgements: Dr. S. Jonsdottir, Science Institute, University of Iceland, K. Ingolfsdottir, University of Iceland, Faculty of Pharmacy.

References: 1. Olafsdottir, E.S., Ingolfsdottir, K. (2001) Planta Med. 67: 199-208. **2.** Olafsdottir, E.S. et al. (1999) Phytomedicine 6: 273-279

B122 Approach to total synthesis of abietatriene diterpenes isolated from genus *Salvia*

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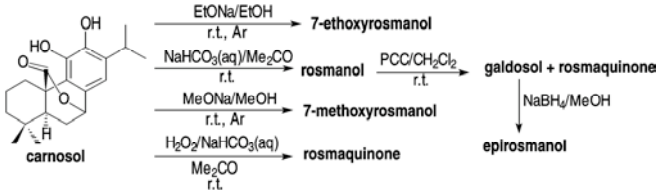
A member of the Labiatae family, the genus *Salvia* consists of some 500 species found worlwide. Since ancient times, many species of the genus have been credited with medicinal properties (1,2), and thus reward investigation. *Salvia* extracts have shown interesting biological activity as antibacterial (3) and antioxidant (4). Some abietane diterpenes isloated from the endemic Canary Island plant *Salvia canariensis* L. have shown interesting antimicrobial and cytotoxic activities (5,6). Many of these compunds are isolated from the extract of the plant in very low quantities. We have interested in the synthesis of these type of diterpenes and their related compounds. We describes the transformation of carnosol in the already known compounds rosmanol (7), rosmaquinone, 7-methoxyrosmanol, 7-ethoxyrosmanol (8), galdosol (9) and epirosmanol (10).



Acknowledgements: We thank to FEDER (Grant Nº 1FD97-0703-C02-01) and the CYTED Project IV.11 for financial support.

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B123 Phenolic and terpenic compounds from *Sideritis stricta* Boiss. & Heldr. apud Bentham

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The genus *Sideritis* L. (Lamiaceae) is represented by more than 150 species which are distributed especially in the Mediterranean region (1). In Turkish flora, 46 *Sideritis* species are known (2) and some of them are used in traditional medicine and as herbal tea (3). In this study, we have investigated the acetone extract of an endemic species, *Sideritis stricta* Boiss. & Heldr. apud Bentham, on which no previous phytochemical study has been reported. By employing a combination of chromatographic methods (VLC, MPLC, Si gel CC, Sephadex LH-20 and Polyamide CC) a phenylethanoid glycoside, verbascoside, two flavonoids with acetylated sugars, isoscutellarein 7-*O*-[6'''-*O*-acetyl-β-D-allopyranosyl-(1→2)]-β-D-glucopyranoside and isoscutellarein 7-*O*-[6'''-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6''-*O*-acetyl-β-D-glucopyranoside and five kaurene type diterpenes, sideridiol, isosidol, sidol, isolinearol and linearol were isolated. The structures of the compounds were elucidated by 1D- and 2D- NMR techniques (^{1}H, ^{13}C, DEPT-135, DQF-COSY, HMBC, HSQC, HSQC-TOCSY, HSQC-NOESY) and HRMS. Isoscutellarein 7-*O*-[6'''-*O*-acetyl-β-D-allopyranosyl-(1→2)]-6''-*O*-acetyl-β-D-glucopyranoside is being reported from the genus *Sideritis* for the first time.

References: 1. Obon de Castro, C., Rivera Nunez, D. (1994) A Taxonomic Revision of the Section Sideritis (Genus Sideritis) (Labiatae), Berlin-Stuttgart. **2.** Aytaç, Z., Aksoy, A. (2000), Flora Mediterranea, 10, p. 181-4. **3.** Baytop, T. (1999) Therapy with Medicinal Plants in Turkey (Past and Present), Nobel Tıp Kitabevleri, Istanbul p.193, p.375.

B124 Flavonoids and caffeetannins from *Mentha piperita* leaves and *Thymus serpyllum* herb

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Caffeetannins called also labiataetannins having caffeoyl group in their molecules, are represented by rosmarinic acid and other caffeoyl or dihydrocaffeoyl derivatives. Rosmarinic acid, consisting of two phenylpropanoid units, is widely distributed in the family Lamiaceae. Caffeic acid trimers and tetramers of related structures and glycosides of flavanones and flavones have also been found in some Lamiaceae plants (1-3). *Mentha piperita* L. and *Thymus serpyllum* L. are popular medicinal herbs in Europe which have been used as spasmolytic, carminative, and cholagogue drugs and as spices in a variety of food preparations since ancient times. The volatile oil yield of both taxa is 0.5-4% (2). Other constituents like polyphenols have been less characterised. Subsequent separation of the aqueous acetone extracts from the dried aerial parts of both analysed species on octadecyl, Sephadex LH20 and silica gel columns led to the isolation of 5 flavonoid glycosides and 2 caffeetannins: eriodictyol 7-*O*-rutinoside (eriocitrin), luteolin 7-*O*-rutinoside, hesperidin, diosmin, luteolin 7-*O*-glucuronide, rosmarinic acid and salvianolic acid K. Structures of identified compounds were elucidated by chemical methods (mainly co-chromatography, hydrolytic degradation, melting point) and spectroscopic techniques (UV, MS, 1D and 2D NMR)(3). Using RP-HPLC we revealed that the peppermint tea gives polyphenols in a high amount. Eriocitrin has been recognised as a main constituent in concentration 3.0-15.3%. The second compound is luteolin 7-*O*-rutinoside in 0.7-3.3%. Hesperidin and diosmin, which are 4'-*O*-methylated derivatives of previous flavanone and flavone glycosides, occur in a small amount about 0.1-0.6%. The concentration of rosmarinic acid ranged from 0.1-0.85%. In contrary, serpyllum herb supplies caffeetannins about 1.8-4.3% and luteolin 7-*O*-glucuronide as a predominant flavonoid in concentration 0.6-1.1%. Based on the qualitative and quantitative

B125 New sterol glycosides from *Ajuga salicifolia*

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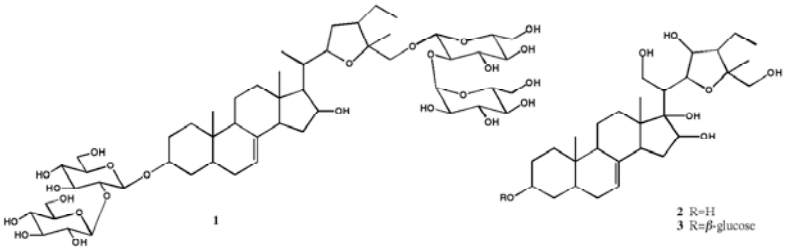
In the flora of Turkey the genus *Ajuga* L. is represented by 11 species (1) some of which are traditionally used in wound healing, as diuretic as well as against diarrhea and high fever (2). Previously we reported the isolation and structure elucidation of ionone and iridoid glycosides (3), and novel sterol glycosides (4) from the aerial parts of *Ajuga salicifolia*, collected from Ankara. Further investigations on the dichloromethane extract of the title plant by VLC (silica gel, RP-18), subsequent CC (silica gel), and HPLC (RP-18) resulted in the isolation of three new sterol glycosides (1-3). The structures of the compounds were elucidated by one and two dimensional NMR techniques (^{1}H, ^{13}C, ^{13}C/DEPT, DQF-COSY, HMBC, HSQC, HSQC-TOCSY, ROESY) and high resolution mass spectrometry.



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B126 Quinolizidine alkaloids from the curare plant *Clathrotropis glaucophylla*

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Clathrotropis is a small genus of the Fabaceae family, with 6 species endemic to the tropical South America. *C. glaucophylla* Cowan was collected in the rainforests of the upper Orinoco in Venezuela in 1999 during ethnobotanical fieldwork among the Yanomami Amerindians. The ethnobotanical investigation has revealed that *C. glaucophylla* is of great importance among the Yanomami, the seeds playing a significant role in alimentation, and the bark being used as ingredient of curare arrow poison.

A new quinolizidine alkaloid, (–)-13α-hydroxy-15α-(1-hydroxyethyl)-anagyrine ((–)-clathrotropine), was isolated from the alkaloid extract of *C. glaucophylla* bark, together with eleven known quinolizidine alkaloids: (–)-lupanine, (–)-6α-hydroxylupanine, (+)-5,6-dehydrolupanine, (–)-anagyrine, (–)-thermopsine, (–)-baptifoline, (–)-epibaptifoline, (–)-rhombifoline, (–)-tinctorine, (–)-cytisine and (–)-N-methylcytisine. The isolation and structure elucidation have been performed with the aid of chromatographic (TLC, HPLC and CC) and spectroscopic (UV and 1D/2D NMR) methods, and mass spectrometry.

It is known that quinolizidine alkaloids have toxicological and pharmacological activities. They interact with ACh receptors as agonists and some inhibit Na^{+} and K^{+} channels, which might lead to respiratory paralysis and ventricular arrest at high dosis (1,2). This suggests that *C. glaucophylla* is an active component in the curare. To our knowledge this is the first time quinolizidine alkaloids have been isolated from an arrow poison ingredient. It is also the first report on *Clathrotropis* species being used in arrow poison.

References: 1. Wink, M. (1998) Alkaloids: Biochemistry, Ecology, and Medical Applications. Plenum Press. New York and London. **2.** Kinghorn, A.D. and Balandrin, M.F. (1984) Alkaloids: Chemical and Biological Perspectives. John Wiley & Sons, New York.

B127 Isolation and structure elucidation of alkaloids from *Consolida orientalis*

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Diterpene alkaloids have attracted considerable interest because of their complex structure, interesting chemistry, and noteworthy physiological effects. This specific type of alkaloids occurs in certain species of the Ranunculaceae, Garryaceae, Compositae, Saxifragaceae, and Rosaceae. Structurally, two categories of compounds can be differentiated: the highly functionalized C_{19} norditerpenoid alkaloids, and the C_{20} diterpene alkaloids with two or three oxygen functions. Norditerpene alkaloids have been found to exert antiinflammatory, analgesic and various cardiovascular effects, and also inhibitory activity against acetylcholinesterase.

In the course of a search for biologically active compounds from Hungarian Ranunculaceae species, we have examined the alkaloidal constituents of *Consolida orientalis* (Gay) Schrödiger, a species widely distributed in the Iberian peninsula and in south-eastern parts of Europe, and occurring abundantly in south-eastern Hungary. Previous phytochemical studies demonstrated the occurrence of lycoctonine type alkaloids in *C. orientalis* collected in Spain and in Turkey.

The present paper reports the isolation and structure elucidation of a new norditerpene alkaloid, 18-demethylpubescenine, together with four known compounds, 14-demethyltuguaconitine, takaosamine, gigactonine and delcosine, obtained from a Hungarian population of *C. orientalis*. The compounds were isolated from the methanolic extract of the whole fresh plants using combined chromatographic methods. Extensive NMR studies, including ^{1}H,^{1}H COSY, HSQC, HMBC and NOESY experiments, resulted in complete and unambiguous ^{1}H assignments of all compounds and the reassignment of some ^{13}C NMR chemical shifts for previously known compounds.

Acknowledgemets: This work was supported by grants OTKA T035200, OTKA T038390, FKFP 0024/2001, and ETT 11503/2001.

B128 Biogenetically diverse secondary metabolites from the fungus *Aspergillus versicolor* isolated from the marine sponge *Xestospongia exigua*

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In the search for new bioactive compounds from the sea, increasing attention is being given to microorganisms such as bacteria and fungi as potential sources of new natural products (1,2). Recent examples for secondary metabolites from marine sponge-derived fungi discovered by our own group include microsphaerones A and B (3), novel γ-pyrones from *Microsphaeropsis* sp. (isolated from the sponge *Aplysina aerophoba*), while a previous examination of the same fungus yielded inhibitors of protein kinases, namely betaenone derivatives and anthraquinone congeners (4).

In the present study, we examined an isolate of the fungus *Aspergillus versicolor* which we obtained from the marine sponge *Xestospongia exigua* collected in Indonesia. Following cultivation in a sea water based medium, six derivatives of 4-methoxy-2,6,7-trimethyl-1,4-dihydro-2H-3,5-dioxa-phenanthren-8-one named aspergiones A to F as well as aspergillitine (2,6,7-trimethyl-5-oxa-3-aza-phenanthren-8-one), representing novel angular tricyclic chromones were obtained from the mycelia and culture filtrate. Aspergillitine is one of the rare examples in nature of fungal-derived polyketides in which a nitrogen atom instead of an oxygen is incorporated into a heterocyclic system. Further investigation of the same fungal strain yielded a series of nine structurally unusual C_{21} terpenoids derived from 3-hexanoyl-6-hydroxy-1,1-dimethyl-1,4a,9,9a-tetrahydro-2-oxa-fluorene-5-carbaldehyde (aspergillones A to D) and 4-hydroxy-1-[5-hydroxy-2-(3-methyl-but-2-enyl)-phenyl]-non-1-en-3-one (asperones A to E), respectively, which apparently share a common biogenetic origin.

The structures of the new natural products were established based on extensive one and two dimensional NMR spectroscopic studies (^{1}H, ^{13}C, COSY, HMQC, HMBC, NOE difference spectra) as well as on mass spectral analysis.

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B129 Semiquantitative detection of antialgal activities in a TLC plate based bioassay

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The aim of our efforts was to develop a test system for the detection of cyanobacterial growth inhibitors extracted from microalgal culture media.
The separation of the complex composed culture media extracts was done by TLC. To simplify the screening on substances with antialgal activity, different alive microalgae were sprayed directly onto the TLC plates. Within 1-2 days active compounds led to significant zones of inhibition clearly recognizable in the removement of the green colour of the alga.
We found several unicellular and filamentous cyanobacteria being suitable as test organisms, namely *Chroococcus minutus, Nostoc carneum, Nostoc insulare, Spirulina laxissima, Synechocystis aquatilis* and a *Synechococcus* species. Conditions for suitability were sensitivity against active compounds and usability of the microalgal suspension as spray reagent.
When different concentrations of one culture media extract were tested, differences in the zones of inhibition (clearness and diameter) resulted dependend on substance concentration. Therefore this assay is suitable for semiquantitative rating of the concentration or activity of separated substances in complex composed extracts.

B130 A simple method to increase the yield of phycobiliproteins in cultures of cyanobacteria

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Cyanobacteria are well known as a source of different natural products such as (polyansaturated) fatty acids, vitamins, proteins and phycobiliproteins (PBPs). PBPs (a special group of photosynthetic pigments) are of commercial interest, because they can be used as natural dyes in food, drug and cosmetic industries replacing synthetic pigments, as highly sensitive fluorescent reagents in diagnostic tests, bioassays and others (1).
The production of PBPs in cyanobacteria depends on the cultivation conditions of the microalgae, especially on the nitrogen content of the medium and the light intensity (2).
The aim of our studies was to create a simple cultivation method, which led to higher amounts of PBPs in cultures of cyanobacteria.
Cultivation of cyanobacteria in closed systems under conditions developed for optimal growth led to high amounts of biomass but low percentage of PBPs. To increase the yield of PBPs in these cultures, a special treatment was developed based on the knowledge of the dependence of PBP production on specific cultivation conditions: Addition of nitrogen (+ 0.025% KNO_3) and transfer of the culture into complete darkness, both some hours before harvesting the culture. This simple treatment resulted in an increase of PBP-content in the range of 15-50% which was achieved within 8-16 hours.

References: 1. Becker, E.W. (1994) Microalgae – Biotechnology and Microbiology. Cambridge Studies in Biotechnology 10. Cambridge University Press. **2.** Volk, R.-B. (1996) Dissertation. University of Kiel, Germany.

B131 Complementary chromatic adaptation in the cyanobacterium *Chroococcus minutus*

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Besides of the photosynthetic pigments chlorophyll-a and carotenoids, cyanobacteria contain phycobiliproteins (PBPs) as accessory pigments. PBPs almost close the light-energy gap left by chlorophyll-a and the carotenoids, allowing the algae to use solar radiation more efficiently. The prosthetic groups (phycoerythrobilins or phycocyanobilins) determine the colour and absorption spectra of the different PBPs (1). Only some of the cyanobacteria, containing blue phycocyanin (PC) and red phycoerythrin (PE), can specifically modulate their content of PBPs to a change of the spectral quality of the light. These species have been classified in two groups: Species of group I can only alter their PE content, whereas species of group II can adjust both their PC and PE content (green light promoting the synthesis of PE and red light that of PC) (2).
In our studies we found *Chroococcus minutus* (Kützing) Nägeli, an unicellular cyanobacterium, being able to modulate its PBP content in dependence of spectral light quality as follows:
When cultivated under red light, *C. minutus* produced more PC relative to PE. When *C. minutus* was cultivated under green light, no significant difference in the PC/PE ratio to a white light culture became obvious. Therefore we propose to classify *C. minutus* to the group of cyanobacteria, which can only alter their PE content.

References: 1. Rowan, S.K. (1989) Photosynthetic pigments of algae. Cambridge University Press. **2.** Tandeau de Marsac, N. (1991) Chromatic Adaptation by Cyanobacteria. Cell Culture and Somatic Cell Genetics of Plants, Molecular Biology of Plastids and Mitochondria, 7 b. Academic Press, Inc. San Diego. 417-446.

B132 An immersion bioautography assay for compounds possessing antimicrobial activity of marine lichen

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In the search for bioactive principles from Marine natural products, the biological activites of lichens found in mangroves is still unexplored aspect of pharmacognosy. We examined *Roccella montagnei* a mangrove lichen for antimicrobial substances by bioautographic assay using *Bacillus subtilis* as the indicator. The biomedical potentials of lichens have been known to man since immemorial time. Growth inhibition using hole plate and agar disc diffusion assays were determined against three gram positive and seven gram negative bacteria and two fungi. In successive extracts, the chloroform and ethylacetate of *R. montagnei* showed maximum activity against *Serratia* sp. Chloroform extracts showed high inhibition against *Proteus vulgaris*. Methanol, ethylacetate, butanol, chloroform and ethanol extracts of *R. montagnei* inhibit all the tested bacteria. However, butanol extracts showed high inhibition against *Mycobacterium smegmatis* followed by acetone extracts showed against *Vibrio cholera* in the disc method. But in the hole plate method butanol extracts showed high inhibition against *Pseudomonas aerugi - nosa* and the lipid extracts showed complete inhibition against *Aspergillus niger*. Among the ten bacteria and two fungi tested , the column chromatographic fractions 2^{nd}, 6^{th}, 8^{th} and 9^{th} showed high inhibition against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus subtilis*. Due to the antibacterial activity found in the marine lichen we have extended the study in bioautography for detection of compounds. The TLC bioautogram of *R. montagnei*, the 8^{th} and 9^{th} fraction showed antibacterial compound with high inhibition and also showed bactericidal activity. These compounds were analysed by gas chromatography.

Acknowledgments: We thank our chairman, Thiru K. Paramasivam and the Correspondent Mr. P. Sathyamoorthy of our institution for their continuous encouragement. Our Principal, Mrs.R.Indralekha, Prof. Dr. A. Subramanian, Singapore, Prof. Dr. K. Kathiresan, Dr. P. Puspangodan, Director, NBRI, Lucknow, Dr. J. L. Rios of Spain, Dr. Kobus Eloff of South Africa for their valuable advice and suggestions. Dr. E. Arunnan and Mr. Rajaakumar of Indian Institute of Science for GC analyses.

B133 Comparison of the anti–inflammatory properties of farmed and wild *Stichopus mollis* sea cucumber

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Digestion, extraction and analysis of farmed and wild *Stichopus mollis* was carried out to assess any differences between the two materials, and to identify any novel bioactives.

Farmed material was obtained from an aquiculture research centre and wild material was acquired from a commercial fishery source. Both samples were digested with papain; high molecular weight materials isolated by dialysis and freeze dried to produce a fibrous material that demonstrated anti-inflammatory properties. Pepsin and Autolyse (kiwifruit enzyme) were also assessed with this outlined procedure (1).

An extraction of the glycosaminoglycan-like constituent of farmed *Stichopus mollis* was carried out by acetone extraction followed by papain digestion and ethanol precipitation (2). The extract was assessed for anti-inflammatory activity and showed improved anti-inflammatory properties above the initial digest only samples. Anti-inflammatory assessed in either a rat paw odema or polyarthritis model, or in a neutrophil bioassay.

Finally, biomass extraction with methanol of both farmed and wild *Stichopus mollis* was carried out and the extract separated on reverse phase with varying polarity elution carried out. Fractions were then assessed by NMR and TLC.

Variations between the two samples from extraction, TLC, NMR and bioactivity assessment were observed which suggest that there are significant differences in the two *Stichopus mollis* sources.

Acknowledgements: Eastland Marine Ltd, Wellington Medical School, Mr A Morgan, and Mr J Cave.

References: 1. Harris, A., Slim, G. (2002) Sea cucumber extraction. Provisional Patent. New Zealand. **2.** Mourao, P. Vieira, R. (1988) *J. Biochem.* 263: 18176-18183.

B134 Total synthesis and antimicrobial activity of (5*Z*,9*Z*)-5,9-hexadecadienoic acid

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The Δ5,9-diunsaturation is not common in natural fatty acids. Most known examples are of marine origin, in particular arising from the phospholipids of sponges, where the (5*Z*,9*Z*)-5,9-hexacosadienoic acid predominates (1). Another interesting example of a naturally occurring Δ5,9 fatty acid is the shorter-chain analog (5*Z*,9*Z*)-5,9-hexadecadienoic acid, which was originally reported from the cellular slime mold *Dictyostelium discoideum*, but later identified in several marine sponges (2). Work from our laboratory with the *iso*-branched analog (5*Z*,9*Z*)-14-methylpentadeca-5,9-dienoic acid revealed that the compound is antimicrobial against pathogenic Gram-positive bacteria, such as *Staphylococcus aureus*, but inactive against Gram-negative bacteria (3). Therefore, the question arises as to the antimicrobial potential of the normal-chain (5*Z*,9*Z*)-5,9-hexadecadienoic. For this purpose we developed two synthetic routes for the (5*Z*,9*Z*)-5,9-hexadecadienoic. In one approach a four-step synthesis was developed starting from 2-(2-bromoethyl)-1,3-dioxolane which was based on acetylide coupling to generate the Δ9 double bond and Wittig coupling to generate the Δ5 double bond (4). However, this methodology afforded a 10:1 mixture of the 5*Z* and 5*E* isomers. The second approach, although longer (six steps), only afforded the (5*Z*,9*Z*)-5,9-hexadecadienoic and it was based on a double acetylide coupling starting with 1,5-hexadiyne. The title compound displayed antimicrobial activity, specifically against Gram-positive bacteria such as *Staphylococcus aureus* (MIC 0.2 µmol/ml) and *Streptococcus faecalis* (MIC 0.08 µmol/ml). It was not, however, active against such Gram-negative bacteria as *Escherichia coli*. Hexadecanoic acid (16:0) showed no activity (MIC > 100 µg/ml) against any of these four microorganisms.

Acknowledgements: This work was supported by a grant from the National Institutes of Health (grant no. S06GM08102).

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B135 New active compounds from the soft coral *Muricea* c.f. *austera* (Gorgonaceae, Plexauridae)

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The pacific gorgonian *Muricea* c.f. *austera* (Plexauridae) was selected for study, because of the antibacterial activity shown (1). The specimen (1.52 kg) was extracted with methylene chloride:methanol (7:3), 2.5 Lx3 at room temperature and then the extract concentrated. This extract (72 g) was suspended in the same solvent mixture (200 mLx3) and the supernatant after concentration (45.3 g) was chromatographed on a silica gel column with hexane, hexane:toluene (1:1), toluene 100%, toluene:ethyl acetate (1:1), ethyl acetate 100 %,and methanol: H_2O (1:1) to give 6 fractions. Fraction 4 (1.34 g) was crystalized with methanol to yield 83 mg of pregna-5-ene-3β,20α,21-triol previously reported (2) which was active against *Bacillus subtilis* and *Sthaphylococcus aureus* at 250 µg/disc. Fraction 5 (400 mg) from several chromatographic column and HPLC gave two new pregnane derivatives: 3β-O-(β-D-glucopyranosyl)-pregna-5,20-diene (7.0 mg) and 3-β-D-(6'-O-acetyl-β-D-glucopyranosyl)-pregna-5,20-diene (7.4 mg).

Acknowledgment: Authors thank CONACYT (Ref. No. 34984-E) for financial support and Dr. William Fenical for the facilities provided for the determination of the chemical structure.

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B136 Studies on the structures of the exopolysaccharides produced by the cyanobacteria *Nostoc insulare*, *Chroococcus minutus* and *Synechocystis aquatilis*

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Some cyanobacteria are known for producing exopolysaccharides at a high level. (1) These are of great interest for example as thickeners, antitussiva or immunstimulants. (2) One exopolysaccharide each from *Nostoc insula - re, Chroococcus minutus* and *Synechocystes aquatilis* was isolated and purified from 8L-Batch cultures. Studies on the composition and structure were carried out by derivatization, gas chromatography and mass spectrometry. Furthermore the molecular weight was determined by size exclusion chromatography. The results show a high variability of the different exopolysaccharides in sugar composition and type of linkage. Interestingly many uncommon sugars were found. Especially in the exopolysaccharide from *Chroococcus minutus* methylated monosaccharides were dominant. A short characterization of the exopolysaccharides is given in the table below.

Cyanobacteria	Molecularweight	Sugar composition
Chroococcus minutus	995 kD	Glucose, Galactose, 6-Desoxy-2-O-methyl-hexose 2-O-methyl-hexoses, 3-O-Methyl-hexose
Nostoc insulare	1081 kD	Glucose, Arabinose,3-O-Methyl-pentose
Synechocystis aquatilis	996 kD	Fucose, Arabinose

References: 1. Schwart, R. (1990) Ind. J. Microbiol. 5: 113-124. **2.** De Phillips, R. (1993) App. A. Envir. Microbiol. 64/3: 1130-1132.

B137 New cytotoxic cembranoid diterpenes from the soft corals *Nephthea* sp. and *Sarcophyton* sp.

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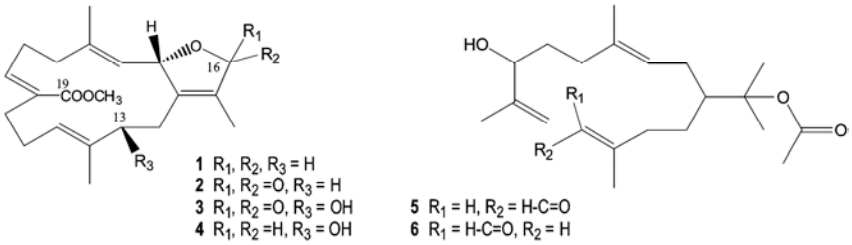
Two soft corals, *Nephthea* sp. and *Sarcophyton* sp., collected from the Fiji Islands and the Great Barrier Reef were investigated. After extraction with CH_2Cl_2 and MeOH, the organic extracts of the two soft corals were evaluated for biological activity. Simultaneous with these assays, investigation of the secondary metabolite chemistry of the samples was started. Chromatographic separation of the extracts using normal and C_{18} reversed phase VLC, SPE, and HPLC yielded three new cembranes from the *Sarcophyton* sp. (**1-3**), and two new seco-cembranoid acetates from the *Nephthea* sp. (**5** and **6**), together with the known compounds sarcoglaucol (**4**) and decaryiol. All structures were elucidated using IR, UV, EI-MS, ^{1}H-NMR and 2D-NMR techniques (HSQC, H,H-COSY and HMBC). Among the numerous cembranoids already isolated from coelenterates compounds **1**, **2**, and **3** represent rare examples of cembranoids functionalized at C-19. Compounds **1**, **3**, and decaryiol were found to be cytotoxic towards several tumor cell lines (GI_{50} values ranged from 0.15 to 8.6 µg/ml).



1 $R_1, R_2, R_3 = H$
2 $R_1, R_2 = O, R_3 = H$
3 $R_1, R_2 = O, R_3 = OH$
4 $R_1, R_2 = H, R_3 = OH$

5 $R_1 = H, R_2 = H\text{-}C{=}O$
6 $R_1 = H\text{-}C{=}O, R_2 = H$

B138 New and biologically active imidazole alkaloids from two sponges of the genus *Leucetta*

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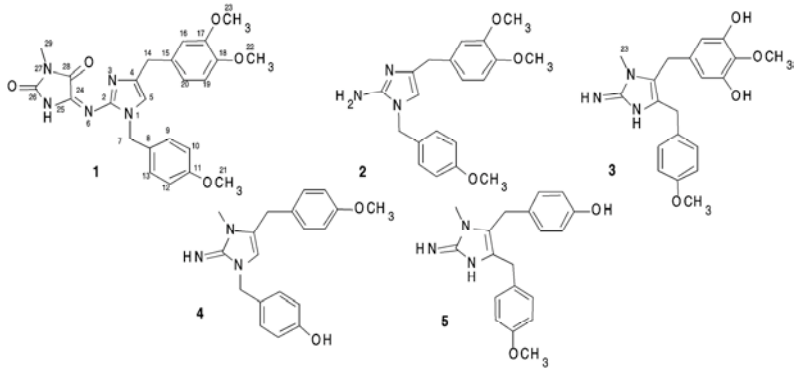
Chemical investigation of two sponges, *Leucetta chagosensis* and *Leucetta* cf *chagosensis*, collected from the Great Barrier Reef and the Fiji Islands, respectively, has led to the isolation of three new imidazole alkaloids (**1-3**), along with the known compounds isonaamine B (**4**) and naamine A (**5**). The structures of the new compounds (**1-3**) were elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). The structures of the known compounds **4** and **5** were determined by comparison of their ^{1}H and ^{13}C NMR spectroscopic data with published values. Compounds **1** and **2** were found to be cytotoxic towards several tumor cell lines (GI_{50} values ranged from 1.3 to 7.0 µg/mL).



B139 PCR based screening approach for the assessment of secondary metabolism in cyanobacteria

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Cyanobacteria are a rich source of secondary metabolites, in particular non-ribosomal peptides and polyketides (1). Cyanobacterial metabolites of this type not only exhibit a fascinating range of structural diversity, but also represent natural products with many interesting bioactivities; e.g. antibacterial, antifungal, antiviral and cytotoxic. Polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), which are responsible for the biosynthesis of these polyketides and peptides, form large multi-enzyme complexes with a modular structure.
Fifteen cyanobacterial strains from the genera *Synechocystis*, *Nostoc*, *Scytonema*, *Tolypothrix*, *Oscillatoria*, *Plectonema* and *Fischerella* were screened in an extended PCR study. Degenerate PCR primers were designed on the basis of conserved sequence motifs of PKS and NRPS (2). While the NRPS derived primers yielded gene ampilcons of the expected size from only one *Fischerella* strain, PKS fragments could, however, be amplified and sequenced from several cyanobacterial strains. Positive results from the PCR screening indicate the presence of the corresponding biosynthetic gene clusters in these cyanobacterial strains and provide valuable information about their metabolic potential.
In addition to this screening approach, the filamentous cyanobacterium *Plectonema* sp., which was found to be PKS-positive, was further investigated chemically. NMR spectroscopic measurements made with HPLC fractions of a methanol extract of this cyanobacterium indicated some fractions to have a high polypeptide content. From the NMR and MALDI-TOF data of these fractions it was possible to speculate that the peptides are composed of 15 to 20 amino acids.

References: 1. Dittmann, E. et al. (2001) Appl. Microbiol. Biotechnol. 57: 467-473. **2.** Beyer, S. et al. (1999) Biochim. Biophys. Acta 1445 : 185-195.

B140 Secondary metabolites of the North Sea bryozoan *Flustra foliacea* and their biological significance

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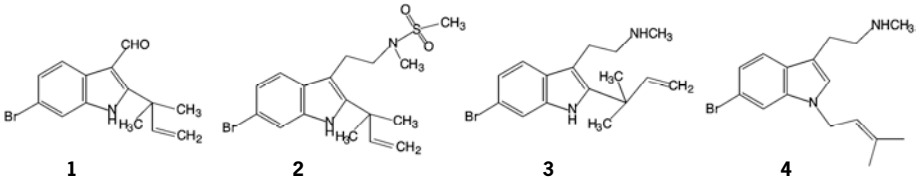
The marine bryozoan *Flustra foliacea* is abundant in various parts of the North Sea and contains biologically active brominated alkaloids and terpenoid metabolites. HPLC separation of the lipophilic extract of *F. foliacea* yielded twelve brominated indole alkaloids, four of which had unusual and new structures (**1 – 4**). GC-MS analysis of various *F. foliacea* extracts allowed to monitor the variation of the secondary metabolite content. It could be shown that samples of *F. foliacea* from different collection sites vary to a big extent, whereas the time of collection has no big influence. All compounds were tested for their antibacterial activity in agar diffusion assays towards bacteria isolated from *F. foliacea* itself and terrestrial bacteria. Significant activities were only found towards marine derived bacteria. Quorum sensing seems to be influcened by one of the metabolites. Pharmacological investigations show flustramin A to have an unspecific blocking activity on voltage activated Kv1.4 potassium channels.



1 **2** **3** **4**

B141 New antioxidant hydroquinone derivatives from the algicolous marine fungus *Acremonium* sp.

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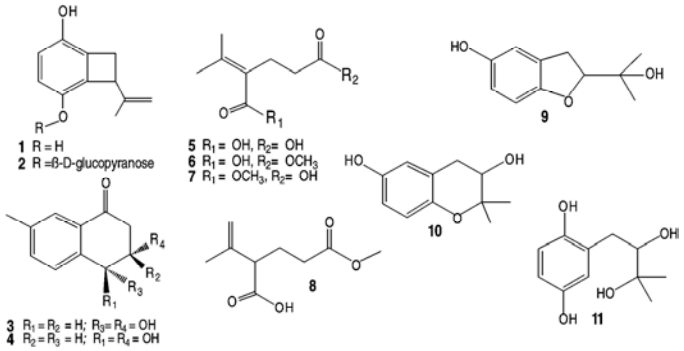
A marine fungal isolate, identified as *Acremonium* sp., was mass cultivated and found to produce two novel hydroquinone derivatives **1-2**. Compound **1** and its glucoside **2** possess a most unusual ring system. The new natural products **3-4**, were obtained as a 1:0.8 mixture. **5** was isolated for the first time as a natural product and its structure proven by x-ray analysis. In addition to these compounds an inseparable mixture of three new isomeric compounds (**6-8**) was also obtained. Isolated together with the new compounds were three known hydroquinone derivatives **9-11**. Compounds **1**, and **9-11** were found to have significant DPPH radical scavenging effects and are also able to inhibit peroxidation of linolenic acid (TBARS assay).



B142 The influence of antibacterial substances from marine fungi on the protein synthesis pattern of *Bacillus subtilis*

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As a part of an ongoing program designed to investigate marine fungi of the northern hemisphere for new antibacterial compounds we isolated ascochitine and the related new structure ascochital from a strain of the ascomycete *Kirschsteiniothelia maritima* (1). The compounds inhibit the growth of *B. subtilis* with a minimal inhibitory concentration of 0.1 µg/ml and 0.5 µg/ml resp. To identify their target in the bacterial cells we investigated their influence on the protein synthesis pattern in *Bacillus subtilis* using proteom analysis. The signature of many cytoplasmic proteins of *B. subtilis* could be analysed during the last years (2).

Changes in the protein synthesis rate were investigated by pulse-labeling experiments with L-[35S] methionine. Crude protein extracts of cells pulse-labeled at different time points after treatment with ascochitine or ascochital were separated on 2D gels. To identify newly synthesized or strongly induced proteins, dual-channel imaging (3,4) was used.

The incorporation of L-[35S] methionine added 60 min after test compound for 5 min was reduced by both compounds. The dual images showed that the synthesis pattern of cytoplasmic proteins in the pH range of 4 to 7 was significantly changed. The most dramatic effect was a strong induction of chaperones and stress-inducible proteases indicating that the test compounds cause protein stress in bacterial cells.

References: 1. Kusnick, C. et al. (2002) Pharmazie, in press. **2.** Büttner, K. et al. (2001) Electrophoresis 22: 2908. **3.** Bernhard, J. et al. (1999) Electrophoresis 20: 2225. **4.** Delta2D Software (DECODON GmbH Greifswald).

B143 Cytotoxic bromoindole derivatives and terpenes from a marine *Smenospongia* sp.

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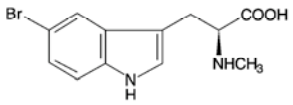
In the continuation of our investigations into the chemistry of marine organisms, we investigated a *Smenospongia* sp. collected from the Philippines. Detailed examination of this sponge resulted in the isolation of a variety of simple indole alkaloids, 5-bromo-*L*-tryptophan (**1**), 5-bromo-*L*-abrine (**2**), 5,6-dibromo-*L*-abrine (**3**) and 5-bromoindole-3-acetic acid (**4**). The pyrroloiminoquinone alkaloid makaluvamine O (**5**), 5,6-dibromotryptamine (**6**), aureol (**7**) and furospinulosin 1 (**8**) were also isolated and characterized. The structures of **1-8** were established by spectroscopic methods (UV, IR, 1D and 2D NMR, MS, $[\alpha]_D$). 5-bromo-*L*-abrine (**2**) and 5,6-dibromo-*L*-abrine (**3**) are new compounds. 5-Bromo-*L*-tryptophan (**1**) and 5-bromoindole-3-acetic acid (**4**) have been synthesized previously, but this is the first report on the isolation of these compounds from a natural source. All compounds were screened in HCT-116 colon carcinoma cell lines using an MTT assay. Compounds that showed at least moderate cytotoxicity were further examined in a set of isogenic HCT-116 cell lines consisting of p53 and p21 knockouts ($p53^{-/-}$ and $p21^{-/-}$) as well as the parental cell line of each ($p53^{+/+}$ and $p21^{+/+}$). Makaluvamine O (**5**), 5,6-dibromotryptamine (**6**), aureol (**7**), and furospinulosin 1 (**8**) all displayed significant activity in HCT-116 cell lines. With the exception of makaluvamine O (**5**), all compounds showed decreased activity against the $p53^{-/-}$ cell line indicative of a p53 dependant mechanism. Makaluvamine O (**5**) showed a promising activity profile showing very little differential between the p53 cell lines while showing an order of magnitude lower IC_{50} (2.3 µg/ml) against the $p21^{-/-}$cell line.



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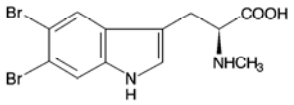

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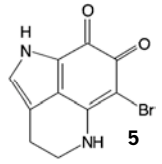

5

B144 Cytotoxic bisabolane type sesquiterpenes from a marine sponge, *Didiscus* sp.

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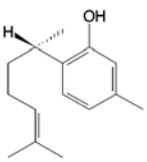
The p53 tumor suppressor gene is the most frequently mutated gene in human cancers. In response to DNA damage, p53 induces the expression of several genes, including p21 ($p21^{Waf1/Cip1}$), the key mediator of p53. In our continuing search for bioactive natural products from marine organisms, we screened marine invertebrate extracts in a set of isogenic colorectal cancer cells: wild type human colon tumor [HCT-116, $p53^{+/+}$ and $p21^{+/+}$], p53-deficient ($p53^{-/-}$) or p21-deficient ($p21^{-/-}$) HCT-116 cell lines in which the p53 and p21 genes were individually disrupted through homologous recombination. The crude MeOH extract of a Philippine marine sponge, *Didiscus* sp. showed some differential between the $p53^{+/+}$ and $p53^{-/-}$ HCT cell lines. Bioactivity-guided isolation carried out on the hexanes, $CHCl_3$ and aqueous MeOH extracts yielded two known bisabolane type sesquiterpenes, (+)-curcuphenol (**1**) and (+)-curcudiol (**2**), as well as β-sitosterol and phenethylamine. (+)-Curcuphenol (**1**) showed moderate activity against our panel of HCT-116 cells while (+)-curcudiol (**2**) was practically inactive at concentrations tested. Interestingly, **1** does not show a pattern indicative of a p53 dependant mechanism [IC50: 27 µg/ml ($p53^{+/+}$); 33 µg/ml ($p53^{-/-}$ and $p21^{+/+}$); 35 µg/ml ($p21^{-/-}$)], whereas the etoposide control clearly shows a dependence on p53 [IC_{50}: 2 µg/ml ($p53^{+/+}$); 10 µg/ml ($p53^{-/-}$), 2 µg/ml ($p21^{+/+}$) and 15 µg/ml ($p21^{-/-}$)].



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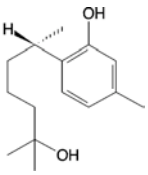

2

B145 *In vitro* cytotoxic activity of some Venezuelan marine organisms

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In the last 20 years, research has have been focused on the sea as source of substances with potential biological activity due to the discovery of novel compounds, which display biological properties such as antibacterial, antihelmintic and antitumoral. Venezuela possesses an appreciable biodiversity in its Continental Shelf, which remains mostly unexplored regarding biological properties. Consequently, the aim of the present research was to analyse the *in vitro* cytotoxic activity of some Venezuelan marine species. Various marine organisms were randomly collected at Playa Culí (Estado Sucre, Venezuela), using SCUBA and snorkelling. The marine organisms were immediately preserved in isopropyl alcohol. Samples were macerated, filtrated and concentrated under reduced pressure at 45 °C. Some extracts were fractionated by HPLC. A total of 8 extracts and 4 pure fractions (*Laurencia microladie*, *Purpura patula*, *Acmaea antillarum*, *Balanus* sp., *Diadema antillarum* and *Holothuria* sp. unidentified green-algae and a clam) were tested against the human cancer cell line, non-small lung cancer cell, applying the SRB *in vitro* assay for cell growth (3). Outstanding growth inhibition at three days exposure time was observed for the fraction Plocamium-1 and the extracts *Acmaea antillarum* and green algae (species unidentified), with IC_{50} values of 23.81, 25.54 and 5.28, respectively. The findings suggest that the marine extracts analysed could represent promising sources of novel active compounds with potential anticancer activity.

Acknowledgements: This research has been sponsored by Universidad de Oriente NS, Cumaná, Estado Sucre, Venezuela.

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B146 Brasilane-type sesquiterpenoids from the red alga *Laurencia obtusa*

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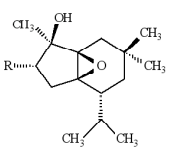
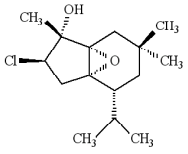
The red alga *Laurencia obtusa* (Huds.) Lamouroux (Rhodomelaceae) is found in most coastal ecosystems around the world and has received extensive attention from many research groups mainly because its structurally unusual secondary metabolites (1).

In the course of our continuing investigations towards the isolation of biologically active compounds from marine organisms of the Greek seas (2,3), we examined recently speciments of *L. obtusa* collected at Symi island in the Aegean Sea.

Three novel rearranged sesquiterpenes (**1-3**), along with the known metabolites brasilenol (**4**) and epibrasilenol (**5**), were isolated from the organic extract of the alga following chromatographic separations (VCC, TLC, HPLC). The new metabolites isolated in minute quantities, possess the unusual skeleton of brasilane and contain the unprecedented 1,6-epoxy moiety. The structures of these natural products, as well as their relative stereochemistry, were established by means of spectral data analysis, including 1D and 2D NMR experiments and MS.



1 R: -Cl
3 R: -Br



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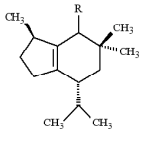

4 R: —OH
5 R: ····OH

References: 1. Faulkner, D.J. (2002) Nat. Prod. Rep. 19: 1-48. **2.** Iliopoulou, D. et al. (2002) Phytochemistry 59: 111-116. **3.** Mihopoulos, N. et. al. (2001) Tetrahedron Lett. 42: 3749-3752.

B147 Secondary metabolites from the marine sponge *Aplysina ocracea*

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During the searching for new metabolites with potential biomedical interest the study of the Caribbean sponges *Aplysina ocracea*, *A. archeri*, *A. lacunosa* and A. *fistularis* was performed. All the specimens were photographed *in situ* and voucher samples were registered and incorporated in the collections of the Zoological Museum of the University of Amsterdam.

The genus *Aplysina* belonging to the Verongida sponges (order Verongida, family Aplisinidae) has been characterized by the lack of terpenes, production of large amounts of sterols with the aplystane skeleton, and elaboration of bromotyrosine metabolites. These latter compounds have been considered chemical markers for Verongida sponges. Thus, they may be very helpful as additional distinctive characters in the identification of Verongida sponges because sometimes the anatomical characters are insufficient to attaining correct identifications and producing consistent taxonomic data (1).

From the organic extracts (CH_2Cl_2 or CH_2Cl_2-EtOH) of *Aplysina* species analysed the major bromotyrosine compound, 3,5-dibromo-1-hydroxy-4-dimethoxy-2,5-cyclohexadien-1-acetamide, was isolated by flash chromatography on a SiO_2 column with a solvent gradient (*n*-hexane/EtOAc/MeOH) and identified on the basis of IR, MS and NMR (^1H, ^{13}C, DEPT-135, COSY, HMQC, HMBC) and by comparison with spectroscopic data (IR, MS and ^1H NMR) previously reported (2). Formation of a mono-O-acetyl derivative indicated the presence of a single hydroxyl group on the molecule and confirms the assign structure. To the best of our knowledge this is the first time that this ketal was isolated from *A. ocracea* and *A. lacunosa*.

References: 1. Ciminiello, P. et al. (2000) J. Nat. Prod. 63: 263-266. **2.** Sharma, G.M. et al. (1970) J. Org. Chem. 35 (8): 2823-2824.

B148 Bolinaquinone, a marine sesquiterpenoid hydroquinone with acute and chronic anti-inflammatory properties

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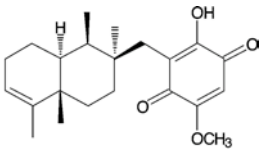
Marine organisms are a source of natural products with a high pharmacological potential (1). In this respect, we have studied the anti-inflammatory properties of bolinaquinone, a marine sesquiterpenoid hydroquinone which is chemically close related with avarol and avarone (2). This compound, which is the main metabolite of the sponge *Dysidea* sp. contains a drimane skeleton. We reported previously, the *in vitro* pharmacological evaluation of bolinaquinone on the inhibition of human synovial secretory PLA_2 and on the modulation of different human leukocyte functions (3). *In vivo*, bolinaquinone reduced the ear oedema induced by TPA after the oral administration of 3.1, 6.2, 12.5, or 25 mg/Kg (49% of oedema inhibition at 3.1 mg/Kg) as well as topically, exerting more potency than indomethacin. The chronic inflammatory response of adjuvant arthritis (by injecting *M. butyricum* 0.1 mg/0.1 ml in mineral oil into de base of the tail) was also reduced (6.2 mg/Kg, twice daily, 7 days) by bolinaquinone with a parallelism with the inhibition of PGE_2 levels in paw homogenates without affecting PGE_2 content in stomach homogenates. Additionally, bolinaquinone inhibited leukotriene B_4 release by human neutrophils stimulated with ionophore A23187 with an IC_{50} value of 2.1 μM as a consequence of a direct inhibition of 5-lipoxygenase activity (IC_{50} =1.3 μM). The present study shows the potential interest of this type of structures in the search for new anti-inflammatory drugs.



References: 1. Soriente, A. et al. (1999) Curr. Med. Chem. 6: 415-431. **2.** Ferrándiz, M.L. et al. (1994) Eur. J. Pharmacol. 253: 75-82. **3.** Giannini, C. et al. (2001) J. Nat. Prod. 64: 612-615.

B149 Cyanolipids from *Paullinia cupana* var. *sorbilis* (Mart.) Ducke

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[a] Dipartimento Farmaco-Chimico, Università,Via Orabona 4, I-70125 Bari, Italy. [b] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università, Via Monteroni, I-73100 Lecce, Italy. [c] Lab. de Farmacognosia, Jardim Botânico, 80.210-170 Curitiba, PR, Brasil.

Paullinia cupana var. sorbilis (Mart.) Ducke, commonly known as guaranà, is a plant native to the Amazonic forest, belonging to the Sapindaceae family (1). Seeds from this plant are known to contain high amounts of caffeine and are used to prepare a powder recommended as an energy reconstituent. Nevertheless, the seeds of many species of Sapindaceae are rich in oils that contain acylglycerols and an unusual class of plant lipids, the cyanolipids (2).

The chemical composition of the oil extracted from the seeds of *P. cupana* has been investigated with particular reference to the content of cyanolipids and data are reported in the present communication.

Cyanolipids amounted to 3% of the total oil from guaranà seeds. Generally, four types of cyanolipid structures, with fatty acids esterified to a mono- or di-hydroxy- nitrile moiety, have been reported as occurring in plants. ^{1}H and ^{13}C NMR analyses indicated that cyanolipids of the type I (1-cyano-2-hydroxymethylprop-2-ene-1-ol diesters) are present in the oil extract from *P. cupana*. Moreover, the GC analysis of the 4,4-dimethyloxazoline derivatives from those metabolites showed that *cis*-13-eicosenoic acid (paullinic acid) was the main fatty acid (38%) esterified to the nitrile group. Vaccenic acid (21%) and *cis*-15-eicosenoic acid (16%) were other abundant constituents. Identification of these fatty acids as the major components of the cyanolipid fraction from the guaranà seeds was also confirmed by GC/MS. To the best of our knowledge, only one paper has been previously published on the occurrence of cyanolipids in guaranà seed oils (3). Our data contribute to improve earlier findings.

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B150 Scale-up of isolation of oxindole alkaloids from *Uncaria tomentosa* by HPLC using chromatographic model

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The species *Uncaria tomentosa* (Rubiaceae), known as Cat´s Claw, is a large woody vine indigenous to the Amazon rainforest. In herbal medicine, it is employed mainly for the treatment of immunological diseases and inflammations. Pentacyclic oxindole alkaloids present in the species have been considered biochemical markers and essential to standardize the commercial herbal medicines.

Semipreparative and preparative HPLC have been used to produce high purity compounds from natural sources. In this work we have applied chromatographic models for scale-up prediction aimed to optimize the isolation of the alkaloids isopteropodine, pteropodine, uncarine F, mytraphylline, isomytraphylline and speciophylline found in *U. tomentosa*.

From the ethanol extract of the stalk bark of *U. tomentosa* an alkaloid-rich fraction was obtained through a classic acid-base partition. The parameters related to retention and separation efficiency of the oxindole alkaloids in analytical reverse-phase HPLC were determined varying the stationary phase, modifier content and temperature. The runs were performed in LiChrospher RP-18, LiChrosorb RP-18 and Shimpack MRC-ODS columns using acetonitrile-water 54:46, 46:54 and 38:62, at 30, 50 and 80 °C. The effects of the cited variations on the parameters were significant.

A model based on statistical moment analysis was used as a tool to simulate chromatograms of the studied alkaloids. Uncarine F, mytraphylline and isopteropodine were separated using a Shimpack column, under different optimal conditions, 38:62 (30 °C), 38:62 (50 °C), and 50:50 acetonitrile-water (30 °C), respectively. The predicted and experimental separations were similar revealing the applicability of the methodology.

Acknowledgement: CAPES, FAPERJ, CNPq-PIBIC, FUJB.

B151 Sanguinarine, a benzo[*c*]phenanthridine alkaloid from *Bocconia frutescens*, inhibits binding of specific ligands to the human angiotensin II AT_1 receptor

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The root of *Bocconia frutescens* L. (Papaveraceae) is used in Panamanian folk medicine to treat hypertensive conditions (1). Earlier work showed that the alcoholic extract and subfractions containing benzo[*c*]phenanthridine alkaloids inhibited the binding of [^{3}H] angiotensin II to the human angiotensin AT_1 expressed by stably transfected CHO cells (CHO-hAT_1). Sanguinarine ($C_{20}H_{14}NO_4^+$) was characterised as the most potent compound from the mixture (IC_{50} 1.9 µM). The type of interaction of sanguinarine was further evaluated in both intact cells and membranes by measuring the binding of [^{3}H] candesartan (2). The results indicate that the inhibition of [^{3}H] candesartan binding was not restricted to intact cells (IC_{50} values of 4.37 and 23.94 µM, after pre- and co-incubation respectively), but was also found on membranes (IC_{50} values of 4.37 and 23.94 µM, after pre- and co-incubation respectively). These findings suggest a receptor interaction independent of cell viability. Furthermore, saturation-binding experiments showed a reduction in the B_{max} (from 2120 to 1765 cpm) and no change in the K_D. The kinetics studies showed no reversibility of the inhibiting effect after washing off sanguinarine. These data suggest that sanguinarine interacts with the receptor in an irreversible and non-competitive manner.

Acknowledgements: FAO-Flanders (Belgium), Fundation Natura, Astra-Zeneca (Sweden), Astra (Belgium) and the Queen Elisabeth Foundation (Belgium).

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B152 Smooth muscle relaxant properties of *Achyrocline satureioides* extract and related flavonoid derivatives

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The failure of penile erection may be due to impaired relaxation of the smooth muscle of the *corpus cavernosum*. *Achyrocline satureioides* (Lam) D.C. (Asteraceae), commonly known as "marcela", is a medicinal plant widely used in Argentina and in other countries of South America as choleretic, hepatoprotective, antispasmodic, as well as against male impotency (1). In our search for compounds with smooth muscle relaxant properties, we investigated the effects of the ethanol extract of *A. satureioides*, the two main components of this extract and their methyl derivatives on the smooth muscle of the *corpus cavernosum*. The penis were obtained from Guinea pigs. Spiral strips were mounted in an organ-bath chamber with the upper wire attached to a force-displacement transducer (GRASS, model 79) at 2 g tension. After a 30 min, L-phenylephrine was used to adjust the maximal contractile tension. Ethanol extract (EE), as well as the flavonoids quercetin (Q), quercetin 3-methyl ether (Q3), quercetin 3,7,3´,4´-tetramethyl ether (Q4) and quercetin 3,5,7,3´,4´-pentamethylether (Q5) were added to the precontracted strips and the change in isometric force was measured in 5-7 min. The results showed that the EE induced at a dose of 2.5 mg/ml and 5.0 mg/ml significant responses (65.0±15.0% and 90.0±1.0% relaxation, respectively). The studied flavonoids (Q, Q3, Q4, Q5) induced an important vasorelaxation effect at the dose of 0.075 mg/ml (79.8±8.3%, 66.0±4.9%, 86.5±8.5%, 67.3±11.1%, respectively). Quercetin methyl ether derivatives have been reported to elicit relaxant activities (2,3) and our results show that the EE of *A. satureioides* and the related flavonoids are potential candidates for the treatment of the failure of penile erection by inducing relaxation of the smooth muscle of the *corpus cavernosum*.

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B153 Spasmolytic activity of *Althernathera repens* (L.) Kunze

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Alternathera repens infusions are used in México for the treatment of diarrhea, stomachache and intestinal inflammation (1,2). To determine whether the described effect is due to its activity on intestinal motility and establish the basis of this activity, in this study was evaluated the *in vitro* spasmolytic activity (3) of the aqueous extract of *A. repens* on rat isolated ileum. It was determined dose response curves of a) acetylcholine (ACh 10^{-7}-10^{-6} M); b) ACh and aqueous extract of *A. repens*; c) aqueous extract on precontracted ileum with 100 mM KCl; d) Calcium (0.35-1.63 mM) and e) Calcium in presence of aqueous extract (0.56-2.1 mg/ml). The records were obtained four times.
It was demonstrated the spasmolytic activity of the aqueous extract of the leaves of *A. repens* on rat ileum. The dose of 3.83 mg/ml of the aqueous extract reduced the spasm in a 95 % on precontracted ileum whereas with 100 mM KCl the reduction was 69%. The dose of 228 µg/ml of the aqueous extract reduced the spontaneous activity in 40% and those of 2.1 mg/ml abolished completely the peristaltic activity. The results suggest that the aqueous extract of the leaves of *A. repens* contains compounds which correspond with anti-cholinergic activity and also with calcium channel blockers.

Acknowledgements: Laboratory of Pharmacology. FES-Iztacala, UNAM. Dra. Beatríz Vázquez Cruz.

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B154 Antiulcerogenic activity of paepalantine on experimental models in mice

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Paepalantine (9,10-dihidroxy-5,7-dimethoxy-1H-naphtol(2,3c)pyran-1-one), an isocoumarin isolated from *Paepalanthus bromelioides* Silv., Eriocaulaceae (a Brazilian endemic shrub of the Serra do Cipó, MG, Brazil) was selected because it shows chemical features related to several compounds with antioxidant, antiulcerogenic and anti-inflammatory activities as coumarins, flavonoids and other phenolics. Paepalantine was isolated from capitula by Silica-gel column chromatography (yield 0,35%) and chemically defined by ^{1}H NMR, ^{13}C NMR and Infrared Spectroscopy. Antiulcerogenic activity was evaluated by three assays: ulcers induced by 0.1 ml of ethanol/animal, 7.0 ml/Kg of HCl 0.3 M in 60% ethanol and 40 mg/Kg of indomethacin plus 5 mg/Kg of bethanecol. Mice were given 100 mg/Kg of paepalantine (dissolved in tween 80) orally 1 h before administration of ulcer inducers. A control group (tween 80) and two reference groups (100 mg/Kg of cimetidine or 200 mg/Kg of carbenoxolone) were included for comparison. After each experiment, animals were killed by cervical dislocation, the stomach removed, opened along the greater curvature and fixed between two glass plates. Each stomach was scanned in a Scanner Jet HP and the image stored at 100 MB disks for use with Zip drive. A specific software (Area) was used for the measuring of each lesion point (mm^2). The results were expressed as media ± S.E.M. of the total lesion area (mm^2); relative lesion area to total stomach area (%), and ulcerative index (U.I.) Statistical significance was determined by one-way variance analysis plus Tukey for $p<0,05$. The results obtained showed that paepalantine was highly effective in the ulcers induced by ethanol and ethanol/HCl, but it was inactive in model of ulcer induced by indomethacin/bethanecol. In models of ethanol and ethanol/HCl, paepalantine inhibited in 66.8 and 71.9% the area total of ulcers, 43.2 and 51.4% the relative area of ulcer and 50.7 and 62.9% the ulcerative index, respectivelly. These results suggests that antiulcerogenic effect of paepalantine probably is due to an enhancement of the gastric mucosal defensive mechanisms.

Acknowledgements: CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Federal Government of Brazil, Proc. 200757/01-6).

B155 Evaluation of the gastroprotective activity of *Curatella americana* (Dilleniaceae), a Brazilian "Cerrado" medicinal plant

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In folk medicine bark of *C. americana* is used to bathe cuts and to treat arthritis, gastric ulcer and diabetes. When previously administered (p.o.) at doses of 250, 500 and 1000 mg kg^{-1}, the crude extract (CEB) of *C. americana* bark or cimetidine (100 mg kg^{-1}) significantly reduced ($p<0.05$) the gastric lesion index induced by HCl/ethanol solution (29, 70, 87 and 42 %, respectively). In the indomethacin/bethanechol-induced gastric-ulcer model in mice, at oral doses of 500 and 1000 mg kg^{-1}, the CEB or cimetidine significantly reduced ($p< 0.001$) the formation of gastric lesions in 42, 52 and 65 % respectively, when compared to the control group. CEB also inhibited the occurrence of gastric lesion induced by stress. In this model CEB (500 mg kg^{-1}) inhibited occurrence in 66 %, while cimetidine, the positive control, presented an 86 % increase of inhibition. We used the oral and intraduodenal route to administer CEB (500 mg kg^{-1}) to Shay's mice. In the pylorus-ligature, the CEB (p.o.) only decreased the gastric lesion index (35%) when compared with the control group ($p< 0.01$). But when the CEB was administered intraduodenally to mice, significant modifications were found such as a decrease in gastric acidity (4.12 ± 1.40 mEq ml^{-1} 4h) and increase in pH (5.14 ± 0.53) of gastric juice compared with the control group ($p<0.01$). Although the mechanism underlying this antiulcerogenic effect remains unknown, it seems to be related to presence of tanin in the bark. The good results obtained of CEB suggest the need for pharmacological research of this plant as a potential new antiulcerogenic drug.

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B156 *Qualea grandiflora* Mart. (Vochysiaceae): Evaluation of the gastroprotective activity of a Brazilian "Cerrado" medicinal plant

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Gastrointestinal disorders are among the most important causes of morbidity in populations of underdeveloped countries. An ethnopharmacological inventory of Cerrado in the central region of Brazil showed a high number of medicinal plants with uses against gastric pain and gastric disorder in general. Based on the inventory, the specie *Qualea grandiflora* was selected to study its antiulcer property. The bark of *Q. grandiflora* is used in folk medicine to treat gastric pain, inflammation and ulcers. Hydroalcoholic extracts (QHE) of *Q. grandiflora* barks were investigated for their ability to prevent ulceration of the gastric mucosa. In the HCl/ethanol gastric model, the oral administration of QHE (1000 and 500 mg/Kg) or cimetidine (100 mg/Kg) produced a significant reduction of gastric lesion index by 86, 54 and 63% respectively ($p < 0.001$). QHE (at the same doses) or cimetidine also significantly reduced the gastric lesions induced by the combination of indomethacin/ bethanechol by 70, 48 and 62 %, respectively ($p < 0.05$). The gastroprotective effect was also observed when QHE (500 mg/Kg) was administered to mice submitted to gastric lesion induced by stress (cold/restraint). QHE (500 mg/Kg) significantly protected the gastric mucosa (66%) against stress when compared with the control group. The pylorus-ligature experiment demonstrated that QHE (p.o. or intraduonally) did not change gastric juice parameters ($p > 0.05$). Although protective, the gastric lesion induced by gastric juice in pylorus-ligated mice (26.4 ± 6.54 mm)) occurred in animals treated with HE when compared with control group (42.1 ± 7.81 mm). The results suggest that the QHE of *Q. grandiflora* present a significant anti-ulcer effect when assessed in these ulcer-induced models. Although the mechanism underlying this antiulcerogenic effect remains unknown, our phytochemistry analyses showed the presence of flavonoids in these plants, which probably explained the antiulcerogenic effects.

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B157 Preventive activity of pyrrolizidine alkaloids from *Senecio brasiliensis* on gastric and duodenal ulcer induced on mice and rats

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Senecio brasiliensis is widely used in traditional medicine of South America (1). We obtained the alkaloidal extract (PAs) from *Senecio brasiliensis* inflorescences, containing a mixture of senecionine, interregimine, retrorsine, usaramine and seneciphylline and evaluated the preventive antiulcerogenic effects. NSAID-cholinomimetic (2), hypothermic-restraint (3), pylorus ligature (4) and HCl-ethanol (5) induced gastric ulcer on mouse. Cysteamine induced duodenal ulcer on rats (6). Results are presented as mean ± SD. Statistical significance was determined by ANOVA followed by Dunnet's test ($p<0.05$). In the NSAID-cholinomimetic model PAs (100 mg/kg, p.o.) showed significant activity ($p<0.0001$) corresponding to 77.8% of inhibition of ulcers. In hypothermic-restraint model, the ulcerative lesion index was reduced by 80.8% ($p<0.0001$). PAs (100 mg/kg, p.o. and i.d.) significantly decreased the gastric juice content, increased the pH values and decreased the acid output in the pylorus ligature. PAs (12.5, 25, 50 and 100 mg/kg, p.o.) showed reduction of gastric lesion index ($p<0.0001$) in the HCl/ethanol induced gastric ulcer and this activity was dose-dependent ($r=0.96$; $p<0.0001$); ED_{50} was 56.3 mg/kg. PAs (100 mg/kg, p.o.) showed significant inhibition ($p<0.0001$) of duodenal lesions (69.3 %). The results suggest that the PAs extract presents a significant anti-ulcer effect when assessed in these induced ulcer models. The mechanisms involved with the action of the alkaloid extract are in progress to determine how PAs act as anti ulcer agent.

Acknowledgements: FAPESP (grant # 98/01065-7 to JRT) and WT was the recipient of a doctoral fellowship from CAPES.

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B158 New dianthrone glucosides, anthrone C,O-diglycosides and a naphthalene glycoside from *Alvaradoa amorphoides*

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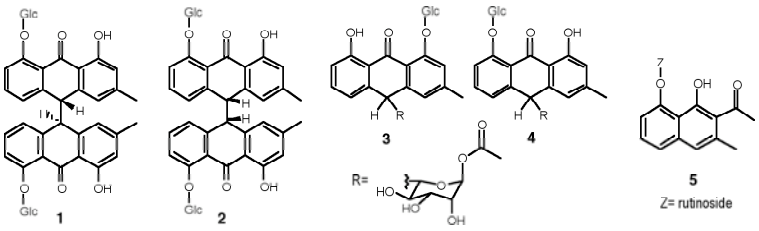
The Yucatec Maya use the leaves of *Alvaradoa amorphoides* Liebm. (Picramniaceae; local name: Belsinikche') to treat itching pimples, dermatomycosis, and psoriasis (1,2). Until now chrysophanic acid, chrysophanein, the quassinoid chaparrin and some fatty acids have been isolated from *A. amorphoides* (3,4). The only further phytochemical study on this genus described the isolation of four new anthracenone C arabinosides - alvaradoins A–D - from *A. jamaicensis*, together with the known anthraquinones chrysophanol and physcion (5).

Our current investigation on the leaves of *A. amorphoides* led to the isolation and identification of new dianthrone glucosides (**1**, **2**), anthrone C,O-diglycosides (**3**, **4**), and a new naphthalene glycoside (**5**) from the ethyl acetate extract. Structure elucidation was based on 1D and 2D NMR spectroscopy (^1H, ^{13}C, DEPT135, HSQC, COSY, HMBC and ROESY), mass spectrometry and CD spectroscopy.



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B159 Efect of erysodine on the acquisition and retention of elevated T maze task

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Autoradiographic and histochemical studies on brain tissue of patients have shown that a selective and substantial loss of acetylcholine-nicotine receptors is associated with loss of memory and learning (1). *In vivo* studies have shown that erysodine (alkaloid obtained from seeds of *Erytrina herbacea*) is a potent competitive ligand of subtype $\alpha_4\ 2_2$ brain nicotine-acetylcholine receptors, making erysodine a useful tool for the functional characterization of these receptors (2,3).
With the aim to get information on the participation of the neuronal nicotine receptors on mnemonic processes, Wistar rats were randomly assigned to independent groups (n=10) to receive intraperitoneally: a) erysodine (30 µmol/kg), b) nicotine (0.62 µmol/ kg) or c) 0.9% saline solution (1 ml/kg). The treatments were administered before the training of an avoidance T maze task.
The Friedman non parametric test showed significant differences on the retention latency of the base line (BL) and the latency of two first avoidance trials (EV_1 and EV_2) when the drugs were administered before the training. On EV_2 and EV_3 (EV_3 evaluated 24 h after the training) the differences were evident only with erysodine. The results indicate that nicotine and erysodine have not effect on the learning of avoidance, but there was an evident the loss of memory provoked by the nicotinic antagonist. Also with this T maze model was possible to show an anxiolytic effect of nicotine.

Acknowledgements: Laboratory of Psychopharmacology. FES-Iztacala. Dra. Sara Cruz Morales.

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B160 Inhibition of ^{3}H-LSD binding to 5-HT$_7$ receptors by flavones from *Scutellaria lateriflora* L.

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The aqueous extract of the flowering parts of *Scutellaria lateriflora* L. has been traditionally used by Native Americans as a nerve tonic and for its sedative and diuretic properties (1). Due to the lack of scientific studies on the plant, however, the use of skullcap has been very controversial. Recent studies on the widely used Baikal skullcap, *Scutellaria baicalensis*, have evaluated the ability of some of its flavones to bind to the benzodiazepine site of the $GABA_A$ receptor. Baicalein, baicalin and scutellarein are weak ligands of this receptor. The binding capacity of wogonin was contradictory in two studies (2,3).
In this study, a hot water extract and a 70% ethanol extract of *S. lateriflora* aerial parts were tested in a 5-HT$_7$-receptor binding assay. Both extracts were active at 100 µg/mL, showing 87.21 ± 6.20 % and 56.65 ± 1.33 % inhibition of the binding of a known ligand, ^{3}H-LSD, to the receptor. Consequently, several flavones occurring in the water extract have been identified and evaluated in the assay as well. Interestingly, the flavone-glucuronides scutellarin and ikonnoside A, showed the strongest affinity for the 5-HT$_7$-receptor with 87.16 ± 5.18 % respectively 76.59 ± 5.27 % inhibition of ^{3}H-LSD binding at 100 µg/mL. Wogonin, baicalin and baicalein were less active (69.53 ± 5.44 %, 49.89 ± 10.05 % and 46.60 ± 4.77 % inhibition).
These data are consistent with the traditional use of the plant, and suggest that *S. lateriflora* may be a promising nerve tonic and sedative plant. Further studies need to be carried out to confirm these initial findings.

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B161 The effect of *Aster squamatus* (Spreng.) Hieron (Asteraceae) extracts on gastrointestinal propulsion

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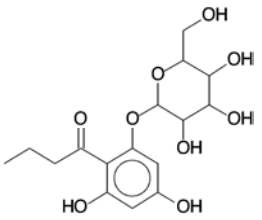
Aster squamatus (Asteraceae) is a plant that grows in South America (Brazil) where it is locally called "erva milagrosa". This plant is commonly used as an antidiarrhoeic agent, and its infusions seem to increase intestinal water and ion absorption as well as gastrointestinal propulsion. Ethanolic and aqueous crude extracts of *A. squamatus* have low acute toxicity, and the use of infusions for one month induced only minor changes on some serum biochemical parameters (1,2). Therefore, the objective of this study was to evaluate the effect of crude hydroalcoholic extracts (CHE) of the root, stalk, and leaf of this plant, as well as of fractionated extracts and of the new phenolyc compound, 1-[2,4-dihydroxy-6-(3,4,5-trihydroxy-6-hydroxymethyltetrahydro-2H-2-pyranyloxy)phenyl]-1-butanone, isolated from the ethyl acetate fraction from the stalk, on the gastrointestinal propulsion of mice. The dry ethanolic extract was partitioned between water and *n*- hexane, $CHCl_3$, ethyl acetate and *n*-butanol, respectively. A portion of the ethyl-acetate fraction was showed more activity and it was chromatographed on a silica gel column with a gradient of $CHCl_3$-MeOH to yielded **1**. The structure **1** was determined on the basis of spectroscopic data (IR, ^{1}H- and ^{13}C-NMR and MS). Gastrointestinal propulsion was investigated using a charcoal suspension according to the method of Almeida (3) and all values are expressed as the mean ± SEM percentage of the distance travelled by the charcoal with relation to the total length of the animals small intestine.



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Acknowledgements: FAPERGS, CNPq.

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B162 Antibacterial cyclopeptide alkaloids from the root bark of *Condalia buxifolia*

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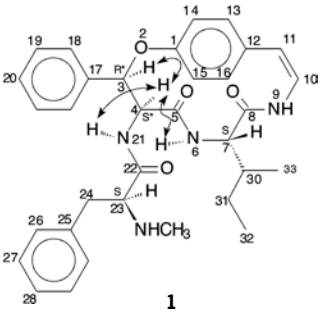
A new cyclopeptide alkaloid, named condaline-A (**1**), was isolated from the root bark (basic ether extract) of *Condalia buxifolia* Reissek (Rhamnaceae), along with the known alkaloids adouetine-Y', scutianine-B, and scutianine-C. The structures were determined by spectroscopic studies (IR, ^{1}H- and ^{13}C-NMR, MS). In this work, the antibacterial activity of each alkaloid was determined by direct bioautography (1), against *Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus* (Gram-positive), *Klebsiela pneumoniae, Salmonella setubal,* and *Escherichia coli* (Gram-negative) bacteria. Additionaly, the absolute stereochemistry of the C-7 amino acid (isoleucine) and of the *N*-methyl phenylalanine side-chain of 1 was determined by chiral phase gas chromatography (CPGC) using 3-pentyl-2,6-dimethyl-β-cyclodextrine as stationary phase (2). The *N*-trifluoroacetylated methyl ester of the D, *L*-mixture and pure *L*-form were used as CPGC standards. By comparing the R_ts of these standards with those of the corresponding amino acid from the hydrolysate of dihydrocondaline-A, it was possible to assign the absolute configuration unambiguously. In condaline-A, *N*-methyl phenylalanine and isoleucine both have the *L* (S)-configurations.



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B163 Quinoline alkaloids, coumarins and volatile constituents of *Helietta longifoliata*

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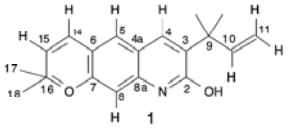
Helietta longifoliata Britt (Rutaceae), locally called "canela-de-veado", belongs to the botanical family of Rutaceae, and is a plant that grows in South America (Southern Brazil, Uruguay, Paraguay and Argentine). It has been used in Brazilian folk medicine as a natural remedy, for the treatment of various diseases (1). In continuation of our chemical studies on plants of the Rutaceae family (2-3) we now report on the isolation and structural elucidation of a new quinoline alkaloid (**1**) from the steam bark of Helietta longifoliata (chloroform extract), found together with seven other known compounds. Four of them were furoquinoline alkaloids **2-5**, and the other compounds were coumarins **6 - 8**. Compounds **2 - 6** and **8** are reported here for the first time as constituents of *H. longifoliata* and the absolute stereochemistry of compound **8** was assigned for the first time.



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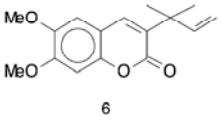

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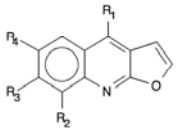
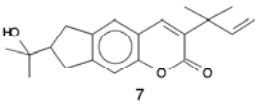


	R^1	R^2	R^3	R^4
2	OMe	OMe	H	H
3	OMe	OMe	$-CH_2OCH_2-$	
4	OMe	H	OMe	OMe
5	OMe	OMe	H	OMe



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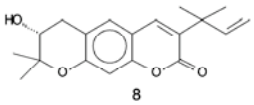

8

Acknowledgements: FAPERGS, CNPq.

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B164 Isolation, determination and antibacterial active of alkaloids from *Zanthoxylum rhoifolium*

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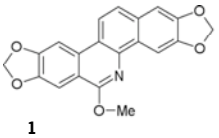
Zanthoxylum rhoifolium (Rutaceae), locally called "mamica-de-porca", is a plant that grows in South America (Brazil, Uruguay, Paraguay and Argentina). It has been used in Brazilian folk medicine against a variety of diseases. As a continuation of our chemical studies on Rutaceae plants (1), we now report on the isolation and structural elucidation of two new dihydrobenzophenanthridine alkaloids, rhoifoline-A (**1**) and B (**2**) (hexane extract) from the root bark of *Z. rhoifolium*, found together with three other known benzophenantridine alkaloids, 6-acetonyldihydronitidine (**3**) (2) {= 8-acetonyldihydronitidine (3)}, 8-acetonyldihydroavicine (**4**) (3), and zanthoxyline (**5**) (1) (chloroform extract). Spectral methods and mainly 1D and 2D NMR experiments were used to determine structures **1 - 5**.

The antibacterial studies of alkaloids **1-4** (Table 1) showed that alkaloids **3** and **4** were active against the tested Gram-positive (*S. aureus*, *S. efidermidis* and *M. luteus*) and Gram-negative (*K. pneumoniae*, *S. setubal* and *E. coli*) bacteria, as revealed by bioautography (4).



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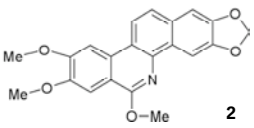

2

Alkaloids	*S. aureus*	*S. efidermidis*	*K. pneumoniae*	*S. setubal*	*E. coli*	*M. luteus*
1	NA	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA	NA
3	1.0	1.0	3.5	3.5	1.0	NA
4	1.0	3.5	1.0	3.5	3.5	NA

Table 1. Antibacterial activity: minimum amount required for inhibition on bacteria growth on TLC plates (µg).

Acknowledgements: FAPERGS, CNPq.

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B165 Anti-stress activity of *Lepidium peruvianum* Chacon

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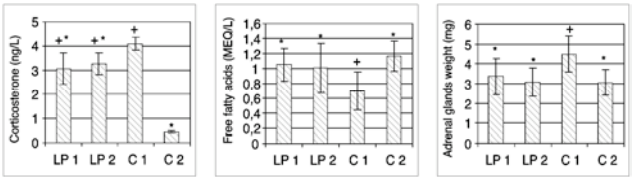
The *Lepidium peruvianum* (Maca) root has been traditionally utilized by Peruvian natives, since the Inca period, for both nutritional and ethnical medicinal purposes as an adaptogen and to enhance human and animal fertility. The aim of the present research was designed to evaluate the anti-stress activity of the methanolic extract of *L. peruvianum* roots (125 and 250 mg/kg, i.p. route) in Swiss mice using a diverse spectrum of stress-induced paradigms (determination of free fatty acids, corticosterone, glucose levels and adrenal glands weigh), gastric ulcer and forced swimming test (1-2).The drug is capable of attenuating or even eliminating variations in homeostasis produced by stress in the studied parameters (Figure 1). Reduction of glucose levels was observed too. The extract decreased the stress-induced ulcers (between 78 and 87 %) and a significative positive result has been observed in the forced-swimming test .



(a) (b) (c)

Figure 1. a) Plasma corticosterone, b) plasma free fatty acids, c) adrenal glands weight. Dose: LP 125 (LP 1), LP 250 (LP 2); Stressed control (C 1) and non-stressed control (C 2). *P<0.05 *vs* C 1; +P<0.05 *vs* C 2

Acknowledgements: The authors thank Programme CYTED.

References: 1. Porsolt, RD et al. (1977) Nature 266: 730-2. **2.** Bishayee, A. and Chatterjee, M. (1994) Int. J. Pharmacog. 32(2): 126-34.

B166 Neuropharmacological activity of *Byrsonima crassifolia* (L.) Kunth

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The bark of *Byrsonima crassifolia* (L.) Kunth has been traditionally used in South America for the treatment of digestive system disease (1) and in the treatment of dermatophytic infections (2); antifungal activity has been demonstrated (3).

Grossly observable behavioural effects after i.p. injection of the test material including autonomic, neurological and toxic reactions were observed and quantified as described by Irwin (4). The Central Nervous System (CNS) was evaluated by performing assays of its effects on spontaneous motor activity, exploratory conduct (hole-board test), body temperature (as rectal temperature) and sodium pentobarbital-induced hypnosis.

Pharmacological studies have been conducted with the dicloromethanic and hydroalcoholic extracts of *Byrsonima crassifolia* (L.) Khunth (1.25 and 0.46 g dried plant/kg weight respectively) to evaluate their effects on the CNS. The observations suggest that the hydroalcoholic extract of *B. crassifolia* produces alteration on the CNS, particularly in the general behaviour patterns; a significant reduction of spontaneous motility (60.9 % at 90 min); decrease in normal body temperature (2.54 ºC at 90 min) and in the exploratory conduct in the animals (97.1 % at 90 min) and a decreasing effect on motor coordination (93.4 % at 90 min). The dicloromethane extract did not produce activity on the CNS.

All of the above findings suggest the extract produces potent effects in CNS (depressant action) especially in the case of hydroalcoholic extract.

Acknowledgements: The authors thank Programme CYTED.

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B167 Phytochemistry and pharmacological evaluation of anticonvulsant, sedative and anxiolytic activities of *Agastache mexicana* subsp. *mexicana*

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The *Agastache mexicana* subsp. *mexicana* and the *A. mexicana* subsp. *xolocotziana* have been used in Mexican traditional Medicine for the treatment of disorders such as insomnia and anxiety.

The aim of this study was to evaluate the behavior effects in male mice produced by *A. mexicana* subsp. *mexicana*. The avoidance behavior test was employed to analyze the anxiolytic-like actions of this plant. The anticonvulsive actions were evaluated on generalized tonic-clonic seizures produced by pentilenetetrazole (PTZ), 4-amine pyridine (4AP), and bicuculine (BIC). The sedative effects were evaluated through hole board test, and this effect was confirmed, since *A. mexicana* subsp. *mexicana* prolonged the pentobarbital sleeping time.

The aqueous extract did not show anxiolytic-like effects. However, showed an important sedative effect, and exhibited an anticonvulsant activity in the seizures induced by PTZ and 4AP. In addition, the bioassay guided fractionation indicated that the anticonvulsant activity lies in the flavoniod fraction, since this fraction protected animals from tonic seizures induced by PTZ, and showed a sedative effect in the hole board test. It is worth to know three flavonoids products (acacetin, 7-O-(2"-O–acetyl)-glucosylacacetin, and 7-O-glucosyl-acacetin) were isolated from active fraction.

Acknowledgements: CONACYT grant No.4992-N, and Beatriz Piña M. for their techical assistance.

B168 Neuropharmacological evaluation of species used as central nervous system depressant in Brazilian traditional medicine.

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Several botanical species are used by different populations due to their reputed sedative/hypnotic activity, or are used against convulsive episodes. Based on their ethnopharmacological use, seven species (*Citrus aurantium*, *Cymbopogon citratus*, *Ocimum basilicum*, *Ocimum gratissimum*, *Rosmarinus officinalis*, *Passiflora alata* and *Passiflora edulis*) were selected and evaluated in order to investigate their activity upon the central nervous system (CNS). Preparations obtained from different parts of each specie, according to their traditional use, were coded as: Essential Oil (EO), 70% v/v hydroethanolic extract (HE) submitted to successive partitions resulting in hexanic (HF), dichloromethanic (DF) and final aqueous (AF) fractions. Preparations were administered orally to male Swiss mice (30-40 g) 30 min before experimental procedures. Sedative activity was evaluated by sleeping time induced by sodium pentobarbital (40 mg/kg, ip); anticonvulsant activity was determined by PTZ (pentylenetetrazole: 85mg/kg, sc) and MES (maximal eletroshock: 50 mA, 0.11 s, corneal). EO obtained from *R. officinalis*, *O. gratissimum* and *C. citratus* reduced the occurrence of tonic episodes induced by the MES test in 42%, 55%, and 78% respectively (Fisher's exact test; $p<0.05$). Sleeping time induced by sodium pentobarbital was significantly increased when compared with control group [median (IQ25-IQ75): 39 (29-60) min] in animals treated with EO from *O. gratissimum* (3.2 times), EO from *C. citratus* (3.5 times), and EO (2.1 times), HF (2.8 times) and DF (3.0 times) obtained from *C. aurantium* (Kruskall-Wallis non-parametric ANOVA followed by Dunn's multiple comparison test; $p<0.05$). No effects were observed on experimental models with preparations obtained from *O. basilicum*, *P. alata* or *P. edulis*, in spite of their traditional use as CNS depressant. The positive results obtained are according to ethnopharmacological use of the species and, after toxicological investigation, the preparations could be useful in primary medical care. In the same way, identification of compound(s) responsible for biological activity could be a source of prototypes to chemical and pharmacological studies, in order to design new safer drugs potentially useful in CNS disorders.

Acknowledgments: Financial support: FAPESP, CAPES.

B169 A β-adrenoceptor agonist isolated from the flowers of the Mexican tree *Chiranthodendron pentadactylon* Larr.

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Chiranthodendron pentadactylon Larr. is a tree growing wild in southern Mexico and cultivated in some parts of the country for ornamental purposes. Its flowers are used in Mexican traditional medicine for the treatment of heart conditions and epilepsy and, applied topically, as an antiinflammatory remedy. Since an aqueous extract of *C. pentadactylon* flowers was reported to relax the noradrenaline-contracted rat aorta (1), the plant was included in a screening program designed to identify Mexican medicinal plants with possible cardiovascular activity. A methanolic extract of the flowers was found to lower blood pressure in the anesthetized rat and to elicit vasodilatation in the perfused mesenteric vascular bed of the rat. Activity-guided fractionation of the crude extract led to isolation of a single crystalline active compound which in the chloralose-urethane anesthetized rat produced immediate and long-lasting hypotension accompanied by a slowly-developing tachycardia. In pharmacological studies in this model designed to determine the mechanism of these effects, it was found that previous selective β_1 adrenergic blockade with atenolol abolished the tachycardia but had no effect on the hypotension. In contrast, non-selective β_1 and β_2 blockade with propranolol eliminated both responses. These results are compatible with a β-adrenoceptor agonist eliciting tachycardia through β_1 cardiac receptors and hypotension through β_2 vascular receptors. Chemical characterization of the compound is in course.

Acknowledgement: Supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica, Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México. Project IN207100.

Reference: 1. Perusquía M. et al. (1995) J. Ethnopharmacol. 46: 63-69.

B170 Effects of the crude and semipurified extracts of *Paullinia cupana* var. *sorbilis* (Martius) Ducke ("guaraná") in forced swimming test

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The seeds of "guaraná" are well known as a psychostimulant drug. Previous studies carried out in this laboratory showed a potential antidepressive activity with "guaraná" seeds crude extract in forced swimming test (FST). The objective of the present study was to evaluate the role of the crude (EBPC) and semi-purified (EPA and EPB) extracts (requeried patent) obtained of the seeds of "guaraná" in its experimental model. Male Wistar rats (n=7-8) were given daily EBPC (30.0 and 60.0 mg/kg) EPA and EPB (2.0 and 4.0 mg/kg, *p.o.*), saline (S, *p.o.*) and imipramine (IMI, 10.0 mg/kg, *i.p.*) during 30-40 days. The animals were then submitted to the forced swimming test (FST) and to the open-field test (OFT). When compared to S, EBPC (30.0 mg/kg) and EPA (4.0 mg/kg) decreased immobility time at 50.7% (3.5±0.76) and 73.12% (4.0±1.8, $p<0.05$), respectively, without affecting mobility time in FST or locomotor activity in OFT. IMI decreased significantly immobility time and increased mobility time, without affecting locomotor activity. The different doses of EPB did not affect any of the parameters analyzed. These results suggest that the EBPC and EPA of the extract of the seeds of "guaraná" present a potential antidepressive activity and that the responsible compounds for this activity is still under investigation at our laboratory.

Acknowledgements: This work was partially supported by CNPq and UEM.

B171 *Guazuma ulmifolia* Lam.: microbial and chemical study

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Guazuma ulmifolia Lam., Sterculiaceae, popularly known as "mutamba" presents a wide geographical distribution, ranging from Mexico to Southern Brazil. The main interest in its microbial and chemical study is due to its use for treatment for hair loss (1). This activity can be attributed to tannins that have many pharmacological activities such anti-inflammatory, anti-oxidant, radical scavenging, anti-ulcer, anti-microbial, anti-viral and capillary protective action (2,3,4).

The material was collected in the field vicinity of Maringá, Paraná, Brazil, and after the botanical analyses, the identification confirmed the material being *Guazuma ulmifolia* Lam. var. *tomentella*. In the chemical analyses was carried out the separation of compounds from the crude extract by a column chromatography on Sephadex LH20. By the biologic point of view, observed the *in vitro* anti-bacterial activity from crude extract and semi-purified extract, by microdiluition method. In the microscopic analyses, big secretories ducts among the cells of the parenquimal tissue were detected. The result for activity against *Staphylococcus aureus* and *Bacillus subtilis* was showed by the aqueous fraction of crude extract (MIC 31.25 µg/ml, and MBC 250 µg/ml; MIC 125 µg/ml and MBC 500 µg/ml, respectively). Still, the ethyl acetate fraction presented some activity against *Escherichia coli* (MIC 250 µg/ml). Phytochemical approach proved the presence of tannins, flavonoids and saponins. From the fractionation of a crude extract of *G. ulmifolia* stem bark, with the ethyl acetate fraction, led to the isolation of two monomers (epicatechin and catechin) and one dimmer of catechin, whole characterized by spectroscopic methods by NMR (^{1}H, ^{13}C), MS and comparison with literature values.

Aknowledgements: Brazilian Sponsors: Capes, CNPq, UEM, UNESP.

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B172 Chemical study and microbiology and acute toxicology evaluation of the seeds extracts of *Paullinia cupana* var. *sorbilis* (Martius) Ducke, Sapindaceae (guaraná).

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Paullinia cupana var. *sorbilis*, popularly known as "guaraná", is a Brazilian plant, growing in the Amazonian area. This plant is used by the native population as a stimulant of the cerebral functions. It has been also used as component in the food and bevarage industry. The seeds of "guaraná" has a high concentration of tannins and methylxantines. From the crude extract EBPC of the seeds of "guaraná" was obtained a semi-purified extracts (EPA) and aqueous fraction (FAQ) (requeried patent). The EPA was chromatographed by CC (Sephadex–LH20) and 24 fractions were obtained. The fractions F2 and F5 were rechromatographed by CC and were obtained 3 compounds: caffeine, catechin and epicatechin. There were identified by NMR and MS spectroscopy and by comparison with the literature. The antibacterial activity of the EBPC, FAQ and EPA was carried out with *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6623), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 15442), through the microdilution assay with the determinate of the Minimum Inhibitory Concentration (MIC). All the extracts presented a negative effect at the concentration lower than 1000 µg/ml. The acute toxicological test was carried out with Swiss male mice. The doses of 5.0, 2.5 and 1.0 mg/kg (*p.o.*) and several doses *i.p.* (2.5, 1.5, 1.0, 0.5 and 0.1 mg/kg) (n=10/group) was administrated. The control group received water. After administration the animals were observed at the first hours and during 15 days daily. It was evaluated the week ponderal evolution and the final weight of the following organs: heart, liver, lung, kidney and spleen, after sacrifice. The LD50 were 1.659 g/kg (*p.o.*) and 0.792 g/kg (*i.p.*). In the acute test by *i.p.* was found a significative decrease of the lung weight of the animals treated with the dose of 0.5 g/kg (treatment = 0.30 ± 0.07; control = 0.40 ± 0.07). This event did not occur in any other of the tested doses. The continuation of the work is necessary to corroborate the results obtained until moment with the acute toxicological test and the determination of the responsible compound for it.

Acknowledgements: This work was partially supported by CNPq and UEM.

B173 Antiviral activity of plants extracts to polio- and herpexvirus

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Crude extract (CE), aqueous and ethyl acetate fractions (AqF, EtoAc) from *Stryphnodendron adstringens* (Martius) Coville, Leguminosae, "barbatimão" and *Guazuma ulmifolia* Lam., Sterculiaceae, "mutamba" stem barks, and partially purified fractions I and II from *Heteropteris aphrodisiaca* O. Mach., Malpighiaceae, "nó-de-cachorro" roots, were assayed for *in vitro* antiviral activity to polio- and bovine herpexvirus. The inhibition of virus replication, in Hep-2 cell cultures, was monitored by plaque assay, reduction of virus titer ($TCID_{50}$) and, in some cases, by immunofluorescence assay. Barbatimão's CE, AqF and EtoAc inhibited more than 90.0% the replication of polivirus and its CE inhibited herpexvirus in 74.3%. On the other hand, mutamba's CE and AqF inhibited poliovirus in approximately 90%, while EtoAc in 65.0%. Both AqF and EtoAc from mutamba inhibited the replication of herpexvirus in 99.0%, however no inhibition was observed with the correspondent CE. As far as mutamba's AqF and EtoAc are concerned, we demonstrated a reduction from 80.0 to 84.0% in the number of poliovirus-infected and treated cells presenting virus-specific fluorescent foci, in comparison to control infected and non treated cells. Fractions I and II of "nó-de-cachorro" roots presented activity for poliovirus inhibiting its replication in more than 90.0%. Moreover, fraction I inhibited the number of poliovirus-infected cells presenting virus-specific fluorescent foci at the concentration of 50 µg/mL. In conclusion, we demonstrated that "barbatimão" and "mutamba" extracts, as well as, "nó-de-cachorro" partially purified fractions presented antiviral activity for polio- and bovine herpexvirus. Studies to find out the mechanisms of action of these extracts were not carried out, for the time being.

Aknowledgements: This work was partially supported by CNPq, UEM, UEL, UNESP and Laboratórios Biosintética Ltda.

B174 Pharmacognostic study and antibacterial activity of the stem bark extract *Stryphnodendron obovatum* Benth. (Leguminosae)

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Stryphnodendron obovatum Benth. (Leguminosae), "barbatimão de folha miúda", is a native species from Brazilian "cerrado" (1), containing higher concentration of phenolic substances in stem bark. Popularly stem bark decoctions are use to seat bath, inflammations, infections and, as a scarless. The following pharmacopeic tests were carried out with the collected stem bark (Feb/2001) (2): a) loss on drying: 12.8%; b) extractives content: 28.63%; c) total tannin content: 15.03%. The turbo extract acetone: water (7:3; V/V – FAA) was partitioned with ethyl acetate (EtOAc), leaving over the water fraction (FW). The EtOAc was fractionated through the column chromatography (Sephadex®-LH20) being evaluated with the TLC. The subfraction F8 was rechromatografied in the same support and, the isolated substances were acetilated and submitted to NMR 1D (^{1}H, ^{13}C), 2D (COSY), MS analyses and the comparison with the literature were identified as gallocatechin and epigallocatechin (3). The FAA, EtOAc, and the FW, were submitted of the antibacterial activity test through the microdilution method with the aim to determinate of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericide Concentration (MBC) (4) to the samples of *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC6623), *Pseudomonas aeruginosa* (ATCC15442) and *Escherichia coli* (ATCC25922). Then, the fractions FAA, EtOAc and FW presented MIC of 125, 125 and 125 µg/mL and MBC of 250, 500 and 500 µg/mL to *S. aureus*, while the others samples had MIC and MBC higher than 1000 µg/mL. The results obtained through the quality control allowed us to draw profile of the plant collected during the Brazilian summer. The preliminary chemical study shows the presence of the flavan-3-ols and the EtOAc presents activity against *S. aureus* in the concentration of 125 µg/mL (MIC) and 500 µg/mL (MBC).

Aknowledgements: Sponsors of the Brazilian Council: Capes, CNPq, UEM, UNESP.

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B175 Anti-inflammatory activity of *Bursera simaruba*

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The Burseraceae family of plants is represented in Mexico by 20 genera and over 600 species. These plants are characterized by their exudates and resins which are rich in essential oils used with medicinal and industrial purposes. A potential insecticidal activity has been also described for some species. From *Bursera simaruba*, phenolic and terpenoid compounds have been isolated. On the other hand the anti-inflammatory activity of the hexanic extract obtained from the leaves of this species has been also described (1). On the basis of the previous discussion in this work the methanolic and acetonic extracts obtained from the leaves of a Mexican population of *B. simaruba* were tested, for its anti-inflammatory activity, in an assay of 12-O-tetradecanoyl-phorbol-acetate (TPA, 2.5 µg), induced ear edema in mice, and after 10 min loading dose 0.31 mg extract was topically applied. The results indicate that the acetonic extract produces 40.89 % of inhibition of the induced edema (p = 0.0431). An inhibition of 20.86% was obtained for the methanolic extract (p = 0.0569). Biodirected fractionation of the acetonic extract looking for the bioactive compounds is now in progress.

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B176 Anti-inflammatory activity of two *Dyssodia* species (Asteraceae)

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Dyssodia is a New World genus. The plants belonging to this genus are distributed in the tropics and subtropics of North and Central America (1). In Mexico two species are used as medicinal plants, *D. porophylla* and *D. pappposa*. Their folk uses indicate a probable anti-inflammatory activity (2) that has been investigated in this work. Hexane and methanol extracts were prepared from the parts of the plants used in Traditional Medicine, stems and leaves of *D. porophylla* and stems and inflorescence of *D. papposa*. From these extracts the anti-inflammatory activity was determined by the mouse ear edema test induced with TPA (12-O-tetradecanoyl-phorbol-13-acetate), 2.5 µg TPA / ear topically applied and after 10 minutes 0.31 mg extract / ear, also topically applied.
Anti-inflammatory activity was found in the methanol extract of *D. papposa* inflorescence, 22.09 % inhibition of the ear edema (p 0.01), and in the methanol extract of *D. porophylla* stems, 26.57% inhibition of the ear edema (p 0.05). The results indicate that the plants have an anti-inflammatory activity which is in accordance with their use in the Traditional Medicine.

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B177 Antiinflammatory activity of *Siphoneugena reitzii* (Myrtaceae) and some isolated volatile compounds on chemotaxis of polymorphonuclear leucocytes

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Siphoneugena reitzii belongs to the Myrtaceae family and it is widely distributed in the Rio Grande do Sul State (Brazil). The volatile oil of this species and *S*-α-pinene, β-caryophyllene, α-bisabolol and its synthetic derivatives were used in the chemotaxis experiment to evaluate their influence in the locomotion of polymorphonuclears leukocytes (PMN). The oil of *S. reitzii* was obtained from the fresh leaves by hydrodistillation and analyzed by GC and GC/MS, using DB-5 fused silica capillary column and Supelco B-CDEX 120 column coated with beta-cyclodextrin for quiral separation. All the samples were tested in the chemotaxis assay in a concentration of 100 µg/ml. *S*-α-pinene is present in the *S. reitzii* oil with a percentage of 12.08% and it showed to be active on inhibition of PMN migration (91.93% of inhibition) (1). Another compound of this species is β-cariophyllene and it was also active (86.85% of inhibition). Other sesquiterpene, α-bisabolol was submitted to selective oxidation reactions, using chromium and MCPBA (2), as oxidants, and the five derivative oxygenated obtained were tested in the chemotaxis assay. Bisabolol diepoxide and 1-oxo-bisabolol were able to inhibit the locomotion of PMN presenting 92.20% and 91.25% of inhibition, respectively. *Epi*-hernandulcine did not present activity and bisabolol oxide B and bisabolol acetate demonstrated cytotoxic activity, occurring the breaking of the wall cell.

Acknowledgements: CNPQ/FAPERGS.

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B178 Segregation of Southern Brazilian *Myrceugenia cucullata* and *Myrceugenia mesomischa* (Myrtaceae) essential oil composition and antimicrobial activity

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Myrceugenia cucullata D. Legrand and *Myrœugenia mesomischa* D. Legrand & Kausel, two southern Brazilian species until now merger under the first name, are evaluated for their chemical, morphological and ecological features and proposed here to be considered as distinct entities. The essential oil was obtained from fresh leaves by hydrodistillation and analyzed GC and GC/MS using both Durabond-DB5 (polidimethyldiphenylsiloxano) and Supelco B-CDEX120 (β-ciclodextrin). The oil composition of *M. cucullata* presented a great amount of *trans*-nerolidol (92 - 94 %), while that of *M. mesomischa* was rich in (–)-α- (20 – 28 %) and (–)-β-pinene (18 – 22 %). The two species may be distinguished morphologically by dimensions of leaves and pedicels, and ecologically they present distinct behaviors at the collections sites, where *M. cucullata* has a scattered distribution while *M. mesomischa* grows in dense groupings of individuals, suggesting a possible allelopathic action. The antimicrobial activity of the essential oils was assayed against *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae*, by plate agar diffusion method. The results are showed in the Table 1.

Table 1. Antimicrobial activity of oils from *M. cucullata* and *M. mesomischa*.

	S. aureus	*S. epidermidis*	*E. coli*	*M.luteus*	*C. albicans*	*S. cerevisiae*
M. mesomischa	16.17	8.73	R	9.93	9.68	IT
M. cucullata	9.78	9.12	8.54	14.25	R	NT
Chloramphenicol	19.12	23.06	13.96	25.98	-	-
Nystatin	-	-	-	-	12.10	13.27

The values are the medium of three measurements (mm). NT: not tested; IT: total inhibition.

M. mesomischa showed high inhibition against *S. cerevisiae* and *S. aureus*, low activity against *S. epidermidis*, *M. luteus* and *C. albicans*. *M. cucullata* showed better activity against *M. luteus* and lower against *S. aureus*, *S. epidermidis* and *E. coli*.

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B179 Alkaloids of *Hippeastrum* (Amaryllidaceae) from the South of Brazil

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The Amaryllidaceae comprises about 13 tribes, 58 genera and 870 species distributed in tropical and subtropical regions of the world (1). *Hippeastrum* is one of the 11 genera of the American tribe Hippeastreae (2), with about 60 species ranging from Mexico to Argentina. Amaryllidaceae alkaloids have shown a wide range of biological activities including antitumor, antiviral, antimalarial and immunostimulant. In Rio Grande do Sul, Brazil, there exist about 8 species, 2 of which were collected and surveyed until now, *Hippeastrum glaucescens* (Mart.) Herb. and *H. vittatum* (L'Hérit.) Herb. Using classical methods of total alkaloid extraction, bulbs and aerial parts were analyzed. The yield varied from 0.03% to 0.5% in the bulbs and 0.02 to 0.1% in aerial parts, for *H. glaucescens* and *H. vittatum*, respectively. Four alkaloids were isolated from the bulbs of *H. glaucescens*: lycorine, pretazetine, tazetine and an unidentified one with a tazetine-type nucleus. As far as we know, till now there are no reports on chemical or pharmacological studies of this species. From the bulbs of *H. vittatum* the alkaloid montanine, not previously reported for this species, was isolated. The total alkaloid extract from bulbs and leaves of *H. glaucescens* showed cytotoxicity to lung (H460) and colorectal (HT29) tumour cells, whereas those from *H. vittatum* showed important antitumoral activity in colorectal (HT29) and glioma (U373) cell lines at concentrations of 0,4 µg/ml.

Acknowledgements: This work had financial support from CNPQ and FAPERGS.

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B180 Nutritional elements and antioxidative properties of mate (*Ilex paraguariensis*)

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In large regions of South America leaves of the mate tree are traditionally used to prepare stimulating teas. Mate was already consumed some hundred years ago by the Indians of South America and many tales and stories have been reported about nutritional and health protective features of mate.

Recently, we reported on variability of phytochemical constituents of mate which are useful markers in quality control. The content of caffeine and caffeoyl-quinic acids was shown to be defined at the level of individual plants. Earlier literature reports on high levels of ascorbic acid could not be verified (1). Now, we investigated the variability of nutritional elements in mate samples from different years. Antioxidative properties of mate were measured and samples in parallel characterized phytochemically (caffeine, rutin, caffeoyl-quinic acids by HPLC; pigments photometrically).

Levels of K, Ca, Mg, Mn, Fe, Zn, Na and Cu in green mate were determined by means of atomic absorption spectrometry. Generally, the variability of content for each element was low. Especially interesting was the remarkable content of Mn (1.8 to 2.5 g/kg dry weight). Samples of toasted mate showed no significant deviations.

Antioxidative properties of extracts and of purified compounds from mate were estimated by means of an iron-katalyzed TBA-based assay. Preparations of green mate were shown to have substantial antioxidative properties. In concentrations of about 40 µg mate per ml extract inhibition by different samples was 40 to 60% compared to the reference compound linolenic acid. Analysis of purified compounds revealed that antioxidative capacity of dicaffeoyl-quinic acids (50% inhibition) is higher than of monocaffeoyl-quinic acids (30% inhibition).

Antioxidative properties of mate have already been reported in literature (2,3). Because of the large variability of caffeoyl-quinic acids in mate we recommend that it is always necessary to analyze the phytochemical composition of mate samples when they are assayed on health protective features which could be due to antioxidative properties.

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B181 Complement modulating activity of plants from Guatemala

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Fifty eight extracts from different parts of thirteen plants from Guatemala were investigated for their influence on complement-mediated hemolysis. Classical (CP) and alternative (AP) complement pathways activities were determined in human serum (1). The plants (*Acalypha guatemalensis*, *Byrsonima crassifolia*, *Gliricidia sepium*, *Guazuma ulmifolia*, *Lippia graveolens*, *Neurolaena lobata*, *Ocimum micranthum*, *Petiveria alliacea*, *Quassia amara*, *Simarouba glauca*, *Smilax lanceolata*, *Tridax procumbens* and *Wigandia urens*) were selected on the base of their ethnomedicinal use in Guatemala.

Eight plant extracts showed potent inhibitory activity on CP (IC_{50} < 5 µg/ml): *T. procumbens* (chloroform/methanol from aerial parts, IC_{50} = 0.77 µg/ml), *W. urens* (chloroform from flowers, IC_{50} = 1.62 µg/ml), *O. micranthum* (alcoholic from leaves, IC_{50} = 1.65 µg/ml), *S. lanceolata* (chloroform from rhizome, IC_{50} = 2.14 µg/ml), *P. allia - cea* (alcoholic from leaves, IC_{50} = 2.40 µg/ml), *A. guatemalensis* (chloroform from leaves, IC_{50} = 2.84 µg/ml), *N. lobata* (alcoholic from leaves, IC_{50} = 3.89 µg/ml) and *G. sepium* (alcoholic from leaves, IC_{50} = 3.91 µg/ml). Quercetine was used as a positive control (IC_{50} = 33.7 µg/ml).

Only two extracts, the aqueous from bark and leaves of *G. sepium* exhibited interesting activity on AP (IC_{50} of 80.48 µg/ml and 38.64 µg/ml, respectively). An aqueous extract from leaves of *Azadirachta indica* was used as a positive control (IC_{50} = 226.4 µg/ml)

Acknowledgements: CYTED, project X-3 and Farmaya for providing the plants. Volunteers for providing the blood samples.

References: 1. Klerx et al. (1983) J. Immunol. Methods 63: 215-220.

B182 Activity of "sangre de drago" and a fucoarabinogalactan from *Croton urucurana* Baill. on complement system and lymphocyte proliferation

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Croton urucurana Baill. (Euphorbiaceae), known as Urukurá and Uruchnum, is a common species in Paraguay, Northern Argentina, Southern Brazil, and Uruguay. Incision in the bark of the trunk and branches produces an immediate excision of a blood red latex, known as "sangre de drago". Once the "bleeding" has stopped, the gum then exudes over the same lesion and may be collected solidified a few days later. Both products are used in folk medicine. The gum is mainly constitued by a high molecular weight fucoarabinogalactan (CU-1) (1). The latex is mainly constitued by catechins and proanthocyanidins, such as the SP-303 (2). In order to investigate the possible immunomodulatory activity of the latex and the fucoarabinogalactan, activities on classical (CP) and alternative (AP) complement pathways in human serum (3), and on proliferation of murine lymphocytes by [^{3}H]thymidine uptake (4) were investigated.

The polysaccharide CU-1 exhibited a strong activity on AP of complement system (IC_{50} = 28.8 µg/ml) resulting in consumption of complement factors. CU-1 also stimulated the normal splenocytes proliferation (at 100 µg/ml) and lymphoid leukaemia cells growth (at 10 µg/ml to 100 µg/ml).

Latex exhibited a potent inhibitory activity on CP (IC_{50} = 6.2 µg/ml) and AP (IC_{50} = 119.7 µg/ml) of complement system and inhibited the proliferation of activated and non activated splenocytes and lymphoid leukaemia cells growth, at 100 µg/ml.

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B183 Cytotoxic activity of *Lithraea molleoides* against human tumor cell lines

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Lithraea molleoides (Vell.) Engl. (Anacardiaceae), trivial named "chichita" or "molle de Córdoba", grows in South America and have long been use among the native population as antirheumatic, diuretic and in respiratory disorders.

The dichloromethane extract of aerial parts of *L. molleoides* shows cytotoxic activity against Hep G2 (1), for this reason a bioguided fractionation was done to isolate the active constituents. A Sephadex LH20 column was used, with dichloromethane:methanol in different proportions, to obtain 6 fractions; the most active was purificated with HPLC where a catechol was identificated.

The cytotoxic effect of the dichloromethane extract, fractions 3 and 6 and the catechol isolated from *L. molleoides* was evaluated against three human tumor cell lines (Hep G2, SK-Mel-28, H292) and three transformed, but not tumor, cell lines (Vero, MDBK, MDCK).

The residue of each sample was suspended in DMSO:H_2O (1:5), it was centrifuged and the supernatant was used. In the in vitro cytotoxic activity assay, cells in the exponential phase of growth were incubated 48 h at 37°C with serial dilutions of the different samples. Cell proliferation was evaluated with MTT and the IC_{50} was determined (2).

As a result of the cytotoxic bioguided assay against Hep G2 a catechol was isolated from *L. molleoides*. This compound also presented cytotoxic activity against the other five cell lines tested, with different IC_{50} values: 17.0±1.4 µg/ml (SK-Mel-28), 18.0±1.4 µg/ml (H292), 21.7±3.8 µg/ml (Hep G2), 73.5±9.2 µg/ml (Vero), 59.5±7.8 µg/ml (MDBK) and 72.1± 4.8 µg/ml (MDCK).

The result of our investigation shows a poor selective cytotoxic activity of the catechol against the human tumor cell lines; but the same effect was seen with the positive control vinblastine (an anticancer drug in clinical use) (3). Nowadays, studies are carried out to elucidate the mechanism of action of the isolated compound.

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B184 Bioactive compounds from Atlantic Forest species *Alchornea glandulosa* and *A. sisifolia* (Euphorbiaceae)

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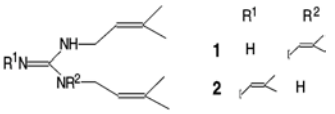
Plants of the family Euphorbiaceae have been used as traditional medicines in several parts of the world. In Africa, *Alchornea cordifolia* has been used as anti-parasitic, mainly to treat amoebiasis and malaria. As part of our continuing efforts to search for new antioxidant and DNA-damaging agents from Brazilian plant species, specially from Atlantic Forest, we have investigated the hydroalcoholic extract of leaves from *Alchornea sisifolia* and the chloroformic extract of leaves from *A. glandulosa* collected in São Paulo State. The guided-fractionation of these extracts using β-carotene (1) and DPPH (2) tests to detect antioxidant agents led to the isolation of the ellagic tannin coriolagin and the flavonol glucoside astilbin from *A. sisifolia*, and 3-*O*-glucosyl-kaempferol from *A. glandulosa*. The use of mutant strains of yeast *Saccharomyces cerevisiae* to select DNA-damaging agents led to the isolation of four guanidine-type alkaloids from *A. glandulosa* extract: pterogynine, pterogynidine besides the two new derivatives **1** and **2**. The structures of the bioactive compounds were established on the basis of spectroscopic data, mainly 1D and 2D NMR and MS. In the plant kingdom, guanidine alkaloids are rare and restrict to the families Euphorbiaceae and Leguminosae. Pterogynine and pterogynidine have previously been isolated from *Pterogyne nitens* (Leguminosae) and proved to be potential antitumoral agents (3).



Acknowledgements: This work was sponsored by the program BIOTA-FAPESP, CAPES and CNPq.

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B185 Cytotoxic evaluation of diterpenes from *Croton malambo*

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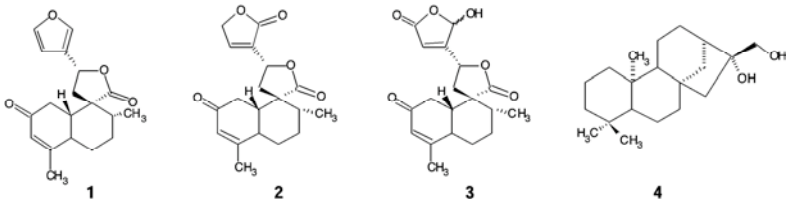
Croton malambo Karst (Euphorbiaceae) is small tree growing in the north-east region of Venezuela, where is called "palomatias" (1). Just as other species of the *Croton* genus it is widely used in traditional Venezuelan medicine. An aqueous decoction of the bark is employed as remedy for arthritis as antiinflammatoy, analgesic and also in the treatment of diabetes and gastric ulcers. In the course of this research, we have previously investigated the antinociceptive and antiinflamatory activity of an aqueous extract. Analysis of the dichloromethane extract of the aerials parts of *C. malambo*, led to the isolation and identification of the four diterpenes: *t*-dehydrocrotonin (**1**), cajucarinolide (**2**) isocajucarinolide (**3**), together with 16α, 17β-kauranediol (**4**) (2,3). The structural elucidation was determined by spectroscopy data. In this work we present the results of the evaluation of these extracts *in vitro* against several tumoral cell lines.



1 **2** **3** **4**

Acknowledgments: Fonacit-CNR Grant PI-2001000347.

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B186 Pharmacological activity and in vitro toxicity of extracts from *Tynanthus panurensis* (Bur.) Sandw. "clavo huasca" (Bignoniaceae) barks

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Four extracts from *Tynanthus panurensis* (Bur.) Sandw. (Bignoniaceae) bark were tested to determinate their possible cytotoxic effects, bacterial growth inhibition potential, and antinociceptive and antiinflammatory activities. Barks were collected in Iquitos (Peru) and neutral, moderately polar, basic and polar extracts were prepared according to a general procedure for extracting plant tissues and fractionating into different classes according to polarity (1). Cytotoxicity was evaluated by MTT assay over human HeLa, HT29 and PC3 cells and hamster CHO cells. The extracts were tested against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *S. typhymurium*, *A. niger* and *C. albicans* growth by the microdilution method, the results being expressed by Minimal Inhibitory Concentration (MIC). Antinociception was assessed by acetic acid writhing in male OF1 mice, and the antiinflammatory activity was determined with the carrageenan-induced hind-paw edema method in male Sprague-Dawley rats. The only significant cytotoxic effect was found to be moderate and was exhibited by the basic extract against HeLa and CHO cells (IC_{50} values were 4,95 and 6,72 µg/mL respectively); incubation with the highest concentration of the polar extract was devoid of any effect against the four cellular lines tested. All extracts showed high MIC values in antimicrobial tests (MIC>50 µg/mL) and then should not be considered significant growth inhibitors. The polar extract exhibited a considerable degree of antinociceptive activity, and both the neutral and polar extracts caused edema reductions comparable (and even superior in the case of polar extract) to indomethacin (5 mg/kg). Further studies of the active principles contained in the polar extract would be then advisable due to the positive benefit/risk ratio depicted by the combined pharmacotoxicological studies; quaternary alkaloids and N-oxides are found to be present in this extract and could be responsible for the described effects.

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B187 Structure-citotoxicity relationships of 6-methoxy flavonols isolated from *Paepalanthus hilairei* Koern (Eriocaulaceae)

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It is well know that some structural features are important determinants for medical properties of flavonoids. Although potential protective effects have been attributed to phenolic compounds they also were reported to be toxic to eukaryotic cells. In this study we investigated *in vitro* structural parameters that could affect the cytotoxicity of four 6-methoxy flavonols (1) isolated from *Paepalanthus hilairei* on viability of McCoy cell line by the neutral red assay (2). The cytotoxic potential of the studied flavonols was, in order of decreasing effectiveness, 6-methoxyquercetin (538.48 ± 106.74 µg/ml) > 3-O-β-D-glucopyranosyl-6-methoxyquercetin (336.31 ± 41.40 µg/ml) > 6-methoxykaempferol (223.21 ± 8.48 µg/ml) > 3-O-β-D-glucopyranosyl-6-methoxykaempferol (139.09 ± 2.46 µg/ml). The results showed that the presence of cathecolic structure (3',4'-di-OH) in the quercetin derivatives reduce the toxic activity of these compounds. On the other hand, substitution of position 3 by a sugar increased the toxic activity of both quercetin and kaempferol derivatives.

Acknowledgements: CNPq, PADC/FCF-UNESP.

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B188 Antimicrobial, cytotoxicity and immunoeffects of vioxanthin from *Paepalanthus bromelioides*

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Chemical substances obtained from plants have been for the pharmaceutical industry one of the most important sources of new products. Actually, there is strong tendency to screen natural products for antimicrobial properties and their involvement in immunological system. Appropriate elimination of bacteria requires both the effectiveness of antimicrobial drug against microorganisms and a very well functioning defense system of host. Macrophages are the first cells to participate in the immunological response and they can be activated by a variety of stimuli. The hydrogen peroxide (H_2O_2), nitric oxide (NO) and tumoral necrosis factor (TNF-α) are effector molecules for the microbicidal and cytotoxic response of macrophages. In this study, we evaluate *in vitro* the antimicrobial activity (1) of vioxanthin, an isocoumarin isolated from *P. bromelioides*, and its cytotoxicity (2) and some immunoeffects on murine peritoneal macrophages (3, 4, 5). The results showed that the vioxanthin exhibited an strong activity against some gram-positive bacteria (0.98-1.98 µg/ml). On the other hand, this compound was inactive against *E. coli* at 500 µg/ml. Vioxanthin demonstrated to be toxic to macrophages displaying cytotoxic index of 44.68 ± 3.42 µg/ml and it is not been able to stimulate H_2O_2 and NO production by macrophages. The macrophages treated with 40 µg/ml of vioxanthin significantly increased TNF-α production (323.9 ± 94.0 U/ml).

Acknowledgements: FAPESP.

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B189 Effects of *Achyrocline satureioides* on mean blood pressure and heart rate of the rat.

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Achyrocline satureioides (Lam) D. C., Asteraceae, (v.n. "marcela", "marcelita", "falso yateí-caá") is a medicinal plant whose aqueous extracts of the aerial parts are widely used in folk medicine in Argentina and other countries of South America for the treatment of several human ailments, particularly those related to gastrointestinal dysfunction as choleretic, hepatoprotective and antispasmodic. Previous pharmacological *in vivo* studies have reported anti-inflammatory, analgesic, sedative, inmunostimulating and antioxidant properties. The aim of the present study was to determine the cardiovascular effects of the aqueous extract of *A. satureioides* in the anesthetized rat.

A. satureioides was collected in Buenos Aires province. Aqueous extract was prepared as infusion 5% P/V (1). 200 g of plant material (aerial part) dried and groundered were stored 20 minutes after addition of boiling water and then filtered. The filtrate was freeze-dried and the resulting powder was considered as the aqueous extract. Dilutions of 1% (M1), 2% (M2), 4% (M4) and 8% (M8) were made and intravenously administered to Wistar rats weighing 220-250 g, which were previously anaesthetized with pentobarbital (40 mg/kg). The femoral vein and the carotid artery of the rats were canulated for extract administration and blood pressure measurement respectively. A Statham transducer and a Grass polygraph were used for this purpose.

Marcela extracts induced a concentration dependent decrease in mean blood pressure when compared with the administration of saline solution (SS): M1: –9.5±2.7* mm Hg, M2: –15.2±2.8** mm Hg, M4: –23.6±4.4** mm Hg, M8: –38.5±4.3** mm Hg vs. SS: 1.7±0.3 mm Hg; *p<0.05, **p<0.01, n=7. The 8% dilution of the extract also decreased the heart rate (M8: –26±5.8 beats/min vs. SS: –3.8±2.9 beats/min, p<0.01, n=7). Methyl atropine (MA: 1.2 mg/kg iv) slightly antagonized the decrease in blood pressure (MA + M8: –25.1±2.2 mm Hg, n=4 vs. M8: –38.5±4.3 mm Hg, n=7, p<0.05).

The aqueous extract of *A. satureioides* induced significant changes on mean blood pressure and heart rate of the rat. The activation of muscarinic receptors seems to be only partially involved in these effects.

Acknowledgements: This work was supportes by grant SECYT-UBA B 079.

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B190 Bois Bandé, a popular aphrodisiac in the light of science

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The Caribbean island of Grenada furnishes the popular aphrodisiac drug Bois Bandé, which consists of the stem bark and the roots of a native tree growing in the island's rain forest. Contrary to Grenadian sources (1) the drug does not stem from *Roupala montana* Aubl. (Proteaceae) but *Chione venosa* (SW.) Urban (Rubiaceae), a plant known under the same vernacular name in other islands of the West Indies (2). The drugs morphological and anatomical features are presented. Anatomical characteristics are conspicuous agglomerations of stone cells and fibers in the cortical parts of the plant.

Folk medicine recommends to leave a piece of the crude drug in white rum over a period of one week. Accordingly, samples of stem bark and roots collected in Grenada were powdered and extracted with methanol 40%. The resulting extract was fractionated by CC on silica gel 60 using chloroform, chloroform-methanol and chloroform-methanol-water mixtures as mobile phases. The polar fractions yielded free sugars as glucose, mannose, fructose and saccharose which were identified by TLC and GC-MS after derivatization. Purification of the more apolar fractions by HPLC on RP 8 material with methanol-water as mobile phase yielded glycosylated terpenoids.

This is the first time that anatomical and phytochemical characteristics of this drug are presented.

Acknowledgements: We thank David William Taylor, The University of Michigan, USA, for identifying the herbarium samples and Telfor Bedeau, nature guide in Grenada, for guiding us to the drug-furnishing trees.

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B191 Tyrosinase inhibitors from Brazilian medicinal plants

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Tyrosinase (EC 1.14.18.1), also known a polyphenol oxidase (PPO), is a copper-containing enzyme found in microorganisms, animals and plants. Tyrosinase inhibitors have become increasingly imporant in cosmetic and medicinal products in relation to hiperpigmentation. The development of tyrosinase inhibitors is necessary (1,2) and the brazilian plants are a rich source of bioactive compounds. Species from Asteraceae (*Vanillosmopsis*, *Lychnophora*, *Baccharis* and *Solidago*), Anacardiaceae (*Anacardium*), Gentianaceae (*Lisianthus*, *Dejanira*) and Rhamnaceae (*Ampelozizyphus* and *Zizyphus*) were collected and identified. Different extracts (*n*-hexane, ethyl acetate, etanol and *n*-butanol) were prepared with each plant. After drying, the extract were assayed to tyrosinase inbibition. The mushroom tyrosinase used for the bioassay was purchase from Sigma Chemical Co. (St. Louis, MO). Although mushroom tyrosinase differs somewhat from other sources, it was used for experiment because it is readily avaiable. All the samples were dissolved in DMSO and used for the experiment at 30 times dilution. The enzyme activity was monitored by dopachrome formation at 475 nm up to the appropriate time (not longer as 10 min). The active extracts were fracionated in silicagel and C-18RP chromatographic column in order to isolate the active compounds. The ethanol and ethyl acetate extracts from *Baccahris trimera* showed a significant inhibitory activity. Fractionation guided study from these extracts lead to a flavonoid rich fraction (aglycon and glycosides), with showed a ID_{50} of 700 µg/mL. Extracts from the other plants shower lower activity in comparison with *B. trimera*.

Acknowledgements: FAPEMIG (Belo Horizonte/ Brazil).

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B192 Plants used to treat fevers and malaria in Brazil

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Several plants are used in traditional medicine of Latin America to treat fevers and malaria (1,2). In order to know the plants more used in Brazil, we have performed a extensive revision of plants indicated to treat fevers and malaria in the Brazilian etnomedical bibliography. A total of 108 bibliographical sources (technician and popular books, articles from national periodic and annals of congress) have been consulted. Each bibliographic reference received a weight (10, 2 or 0.4), according to the publicated information. A book which describes a ethnobotanical survey on a endemic area of malaria of Amazon, for example, received weigth 10. Another which consisted on a simple revision of plants used in Brazil received weight 0.4. In each reference we have noted data as family, scientific and popular names, part used and indications of the plants. A total of 197 different species are indicated as useful for the treatment of fevers and malaria. The calculation of their citation frequency *versus* weight of each reference lead to the different values of scores for each one. The species *Senna occidentalis* (L.) Roxb. and *Momordica charantia* L. received the highest scores (139.6 and 125.6, respectively), followed by *Carapa guianensis* Aubl. (64.4), *Geissospermum sericerum* Benth & Hook ex Miers. (64.0), *Aspidospemum nitidum* Benth ex Muell. (62.4), *Myrtus brasiliana* L. (59.6), *Piper umbelata* (*Pothomorphe umbellata*) L. (57.6), *Croton cajuçara* Benth (50.0), *Solanum paniculatum* L. (47.6), *Coutarea hexandra* (Jacq.) K. Schum. (46.4) and *Casearia sylvestris* SW (40.4). The results demonstrate that several plants are used to treat fever and malaria in Brazil and that more used should be explored.

Acknowledgements: CNPq (Brasília), FAPEMIG (Belo Horizonte), IFS (Stockholm).

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B193 Screening of some Mexican medicinal plants for antibacterial activity

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Medicinal plants may offer a source of antibacterial agents for us, in this sense, in the present work we selected 15 Mexican plants traditionally used in the treatment of diarrhoea. Methanol and aqueous extracts were tested by quantitative testing of antibacterial activity. The following pathogenic bacterial cultures were used: *Escherichia coli*, two *Shigella sonnei* strains, two *Shigella flexneri* strains, three *Salmonella* strains, and *Vibrio cholerae*. The testing of the antibacterial activity was performed according to the microdilution method described by Galvan and Barry (1). The aqueous and methanol extracts from *Geranium mexicanum*, and *Punica granatum* were the most active against *S. flexneri* species and *V. cholerae* with MIC values ranging from 1 to 4 mg/ml. The methanol extract from *Thymus vulgaris* showed 100% inhibition towards all species tested when evaluated at 8 mg/ml. In general, the methanolic extracts were more active than aqueous extracts. These preliminary results from the evaluation of bacterial activity gave evidence of the probable presence of compounds of biological interest in the methanol extracts from *G. mexicanum*, *P. granatum*, and *T. vulgaris*.

Acknowledgements: Silvia González Arrroyo. The investigation was supported by CONACYT grant 38030M, and IMSS-FOFOI (FP-2001/053).

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B194 Is the starch from *Solanum lycocarpum* St. Hill. fruits a hypoglicemic?

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Several Brazilian plants are used in the traditional medicine to treat diabetes (1). The starch obtained from the unripe fruits of *Solanum lycocarpum* St. Hill. (Solanaceae) has been widely used as a hypoglycemic in Brazil (2). *Per os* administration of the starch (1000 or 2000 mg/kg, twice daily for 7 days) did not change glycemia of non-diabetic mice evaluated on the 7th day. In streptozotocin-induced diabetic mice, chronic treatment with the starch (1000 or 2000 mg/Kg, twice daily for 7 days) did not change the elevated glycemia in the 7th day, 3 after the last dose. In animals fasted for 15 h, *per os* administration of glucose (600 mg/Kg) significantly increased glycemia 1 h later. Previous (–30 min) treatment of the animals with the starch (1000 or 2000 mg/Kg) did not change the increase of glycemia. *Per os* administration of the starch (1000 or 2000 mg/Kg. day, twice daily for 7 days) did not induce body weight gain or loss. In interviews with 56 diabetic patients, a total of 29 medicinal plants were reported as useful in the treatment of diabetes and *S. lycocarpum* was the sixth most mentioned. All the interviewed patients reported that they also used insulin or oral hypoglicemic drugs. The results do not offer any evidence of a hypoglycemic effect induced by the polysaccharide fraction of *S. lycocarpum*, both in normal and hyperglycemic mice. The results of the present study clearly represent an example of the need of an adequate pharmacological investigation of the natural products largely used by the population.

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B195 Antimycobacterial screening of crude extracts obtained from medicinal plants growing in Northeast Mexico, using a native resistant strain

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The resurgence of tuberculosis as a major disease in many parts of the world is prompting the search for novel compounds, active against *Mycobacterium tuberculosis*. According to the WHO, there were 8.4 million new cases of TB in 1999 (1). According to data obtained from the IMSS (northeast delegation), 25% of the cases appearing in Mexico came from this region. The problem is becoming a major concern on account of the prevalence of resistant strains from *M.t.* More than 50% of the strains isolated from patients in our region were resistant to at least two of the antibiotics commonly used for TB treatment (2). In a first effort to identify sources for bioassay-directed isolation of novel compounds active against resistant strains of *Mycobacterium tuberculosis*, extracts of 20 plant species growing in northeast Mexico were screened. Plants were selected on basis of ethnopharmacological criteria. Methanolic and aqueous extracts were obtained from each part of the plants under test. Antimycobacterial activity was performed by means of the redox-dye Alamar Blue test (3) using $H_{37}RV$ and a native strain resistant to five antibiotics (CIBIN/umf 28:99) isolated and characterized in our laboratory. Positive assays were retested by means of the Bactec 460. From this first screening we obtained the following results a) Alamar Blue has a good prediction capability, being cheaper, more simple, and rapid, as many extracts can be tested in the same microtitre plate, b) DMSO was found to potentiate the activity from extracts, c) methanolic extracts obtained from roots and leaves of *Leucophyllum frutescens* (cenizo) showed the greatest activity in the resistant strain by both methods employed and was considered as a source for a following bioassay-directed fractionation.

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B196 New chromanone acids with antibacterial activity from *Calophyllum brasiliense*

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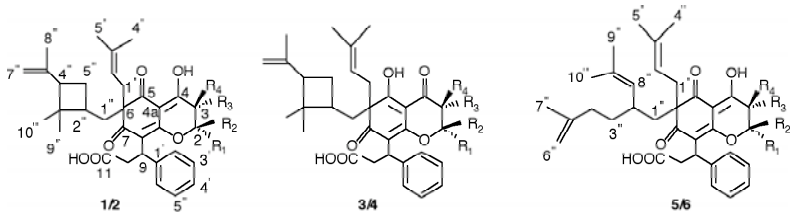
The bark latex of *Calophyllum brasiliense* (Clusiaceae) is used in the traditional medicine of the Popoluca (Mexico) to treat toothache, and to prevent wound infections by microorganisms (1). Using antibacterial activity against gram-positive bacteria as a lead, bioactivity-guided fractionation of the n-hexane and ethyl acetate extract of the bark of *C. brasiliense* afforded six new chromanone acids (**1/2**, **3/4** and **5/6**) as three inseparable mixtures, together with the three known triterpenes, friedelin, friedelan-3-ol, and betulinic acid. The structures of the isolates were elucidated on the basis of extensive 1D and 2D NMR experiments, as well as high resolution mass spectrometry. Compounds **1/2** showed a MIC value of 1 µg/ml against *B. cereus* and were significantly more active than the reference compound chloramphenicol (MIC of 4 µg/ml), whereas **3/4** and **5/6** exhibited MIC values of 8 and 16 µg/ml, respectively. All compounds were not cytotoxic against KB cancer cells (ATCC CCL 17) up to 20 µg/ml.



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B197 Screening of antibacterial active extracts obtained from Amazon rain forest plants

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Seven hundred and five organic and aqueous extracts obtained from 429 plants belonging to 70 different families native to the Amazon rain forest were submitted to a screening against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. Plants were collected according to a phytochemical and chemosystematic approach. They were dried and ground and macerated with methanol : dichloromethane (1:1) in order to obtain the organic extract. A following water maceration was done so as to result two extracts from each plant material. Extracts were prepared to 20 times the desired test concentration (2 mg/mL) in water or DMSO 50%. Broth microdilution method was performed to evaluate the antimicrobial activity of the extracts. The bacterial inoculum of each ATCC strain were obtained from fresh colonies in blood agar plates. They were initially prepared to a concentration of 1.5 x 10^8 CFU/mL and were then diluted to 1,5 x 10^2 CFU/mL. From these diluted bacteria suspensions, 190 µL were transferred to each well of the microplates. Ten µL of the extract solutions were added to the wells and the microplates were then incubated at 35º C, for 18 to 20 hours. Results were visually analyzed.
Two out of the 705 extracts showed activity against *E. faecalis*: organic extracts obtained from aerial parts of *Rapanea* sp. and organic extract obtained from aerial parts of *Smilax* sp. One extract showed activity against *S. aureus*: organic extract obtained from stem of *Ruizterania* sp. MICs were determined (<100 µg/mL each extract).

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B198 Alphitolic acid: an unusual triterpenoid from leaves of *Bixa orellana* and evaluation of its antifungal activity

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Bixa orellana L. is a reputed traditional remedy used for several purposes by native people in Latinoamerica. The main of these is the aplication of “annato”, a kind of paste obtained with the aril of the seeds, as a dermatological protection and treatment of skin diseases.
In a previous screening (1) antifungal activity was detected on dichloromethane and methanol extracts from leaves of *B. orellana* using agar disk diffusion assay. With the aim of isolating the active compounds, dichloromethane extract was obtained in a Soxhlet apparatus and submitted to a biossay-guided fractionation. It was separated by MPLC on Si60 eluting with a gradient of hexane-AcOEt-MeOH (1:0:0 to 0:1:0 to 0:0:1). Fraction VI inhibited the growth of *Microsporum gypseum* CECT 2908 and *Trichophyton mentagrophytes* CECT 2795 in an agar overlay bioautographic method. HSCCC and CC on Sephadex® LH-20 were used to isolate the active compound from fraction VI. Its structure was elucidated by standard spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR, DEPT, H,H-COSY, HSQC, HMBC, EI-MS, CI-MS and IR) and by comparison with our own triterpene database. The final elucidation of the active compound was performed by comparison of its NMR data with those of the related compounds betulinic and goreishic acid and identified as alphitolic acid (2α,3β-dyhidroxy-20(29)-lupen-28-oic acid), a pentaciclic triterpene.
Alphytolic acid occurs rarely in nature. It was previously isolated from *Zizyphus joazeiro* and *Licania heteromorpha* and its antifungal activity was established against *Candida albicans* (2).

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B199 Antifungal sesquiterpene from the root of *Vernonia tweedieana*

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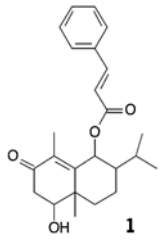
With the aim of searching new antifungal compounds, and etnopharmacological survey was carried out in Paraguay. Several species selected from an interview taken *in situ*, were screened in order to establish their antifungal activity against yeasts, dermatophytes and/or filamentous fungi (1). The dicloromethane extract from *Vernonia tweedieana* Baker root inhibited the growth of 2 of the 11 strains tested, in agar disk diffusion assay (1). The biossay-guided fractionation of the extract using an agar overlay bioautographic method allowed the isolation of the active compound.



1

The dicloromethane extract was fractionated on MPLC Si60 eluted with a gradient of hexane: $-Cl_2CH_2-$MeOH (1:0:0 to 0:1:0 to 0:0:1). Fraction 4A was active against *Cryptococcus neoformans* CECT 1075, *Microsporum gypseum* CECT 2908 and *Trichophyton mentagrophytes* CECT 2795. The active compound (**1**) was isolated from fraction 4A by MPLC and CC on Si60, CC on Sephadex® LH-20 and HPLC on Nucleosil® 100 column.

The structure of **1** was elucidated by standard spectroscopic techniques (^{1}H-RMN, ^{13}C-RMN, DEPT, H,H-COSY, HSQC, HMBC, EI-MS, CI-MS and IR) and identified as 6-cinnamoyl-1-hydroxy-eudesm-4-en-3-one, a new antifungal compound only previously described in *Ambrosia artemisioides* (2). Its minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) against yeasts and dermatophytes were between 4-16 µg/ml.

Acknowledgements: Iberoamerican Program CYTED (Project X-7) and Generalitat de Catalunya (ACI), A.P. was granted by the University of Barcelona.

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B200 Antiprotozoal activity and chemical investigation of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Mexico

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As a part of our effort to discover natural products with potential use as antiprotozoal agents, 25 Mexican medicinal plants were screened for their ability to inhibit the growth of trophozoites of *Entamoeba histolytica* and *Giardia lamblia* (1, 2). Accordingly, after the initial observation of the significant activity displayed by some species, *Rubus coriifolius* Focke (Rosaceae), *Teloxys graveolens* Willd (*Chenopodiaceae*), and *Lepidium virginicum* L. (*Cruciferae*) were selected for the activity-guided fractionation. The extract of the aerial parts of *Rubus coriifolius* gave (–)-epicatechin, (+)-catechin, nigaishigoside F1, hyperine, gallic acid, and ellagic acid while that of the aerial parts of *T. graveolens* afforded melilotoside, rutin, narcissin, pinocembrine, pinostrobin, and chrysine, and that from the roots of *L. virginicum* yielded glucotropaeolin and β-sitosterol. Epicatechin, melilotoside, and glucotropaeolin had the lowest IC_{50} values among the pure compounds at < 20.4 µg/ml for *E. histolytica* and at < 16.8 µg/ml toward *G. lamblia*. Epicatechin was the most potent inhibitor with IC_{50} values of 1.92 for *E. histolytica* and of 1.64 µg/ml against *G. lamblia*, its activity was comparable to emetin, but no exceeded that of metronidazole. The results of the present study lend some support to use of these species in traditional medicine for the treatment of dysentery.

Acknowledgements: The investigation was supported by CONACYT grant 38030M.

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B201 Anti-trypanosoma activity of extract obtained from plant *Piper umbellatum*

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The use of medicinal plants in the world, especially in South America, contributes significantly to primary health care. Many plants are used in Brazil in the form of crude extracts, infusions or plasters to treat common infections without any scientific evidence of efficacy. Chagas' disease affects about 18 million people and is responsible for the death of 45,000 patients every year (1). For the treatment of Chagas' disease, alternative drugs are necessary with more trypanosomicidal power and less incidence of toxic side effects. In this study extract of plant "Pariparoba" (*Piper umbellatum*) was screened for its anti-protozoan activity in epimastigote form of *Trypanosoma cruzi* "Y" strain. For this purpose the cells were cultivated in LIT medium (2) containing 10% foetal bovine serum at 28ºC with 10 to 1000 µg/ml of crude extract. Cell growth was determined by counting the parasites with a Neubauer hemocytometer. A dose dependent inhibition of the protozoan proliferation was observed. After 120 h of incubation, growth inhibition percentages of the cells were 28.8%, 85.3%, 94.2%, and 98.4% in the concentrations 10.0, 100.0, 500.0, and 1000.0 µg/ml of crude extract of "Pariparoba", respectively. These results demonstrated that this plant contains active principles against *T. cruzi* justifying the search for the study of plants extracts used in folk medicine of the treatment of tropical disease caused by protozoa.

Acknowledgments: CNPq, CAPES, Programa de Pós-graduação em Ciências Farmacêuticas/UEM, Fundação Araucária, and PPG/UEM

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B202 Effect of crude extract and fractions of "barbatimão" (*Stryphnodendron adstringens*) on growth and ultrastructure of *Herpetomonas samuelpessoai*

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Stryphnodendron adstringens (Martius) Coville, popularly known as "barbatimão", is a medicinal plant used in the treatment of leukorrhoea, diarrhoea, and also as an anti-inflammatory and cicatrizant agent (1). We report the influence of crude extract and fractions of "barbatimão" on growth of *H. samuelpessoai* cultivated in a defined medium at 28ºC. For this purpose, 100.0 to 5000.0 µg/ml of crude extract or 1000.0 µg/ml (fractions F3.1 to F3.12) were added to the medium. Cell growth was estimated by counting in a Neubauer's chamber. For study of the influence of "barbatimão" in protozoan's ultrastructure, cells treated with crude extract were fixed in 2.5% glutaraldehyde. Postfixation was carried out in 1% osmium tetroxide plus 0.8% potassium ferrocyanide and 5 mM $CaCl_2$, dehydrated in acetone, and samples were embedded in Epon. Ultrathin sections were observed in a Zeiss CEM-900 electron microscope. After 72 h of incubation, growth inhibition percentages of the cells were 17.8%, 48.3%, 73.3%, and 99.7% in the concentrations 100.0, 500.0, 1000.0, and 5000.0 µg/ml of crude extract of "barbatimão", respectively. The fractions F3.9 and F3.12 showed significant higher inhibition activity when compared with crude extract in the same concentration, with 96.7% and 97.8% growth inhibition, respectively. If compared with untreated cells of *H. samuelpessoai*, the cells treated with "barbatimão" extract showed several morphological changes in the parasite's ultrastructure. In these cells, a markedly mitochondrial swelling were observed. These results indicate that crude extract and fractions of "barbatimão" have a progressive inhibitory activity on the growth of *H. samuelpessoai* and determine some ultrastructural mitochondrial alteration.

Acknowledgements: CNPq, CAPES, Programa de Pós-graduação em Ciências Farmacêuticas/UEM, Fundação Araucária and PPG/UEM.

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B203 Biological activity of *Erythrina* alkaloids

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In previous works we have described some of the achievements with *Erythrina* alkaloids, e.g. physiology, toxicity or pharmacology (1). Now in this work we try to deep in their toxicology, insecticidal evaluation and also explore their herbicidal activity. β-Erythroidine and its semisynthetic derivative dihidro-β-erythroidine were evaluated for acute toxicity (2) administrated intraperitoneally on mice: LD_{50} were 27.3 mg/kg and 7.3 mg/kg respectively. For the toxicity test (3) it was evaluated the free alkaloids (FA) and liberated alkaloids (LA) on *D. magna* and *P. redivivus*; the LA fraction showed a moderate toxicity. Insecticidal activity was evaluated with two of the main alkaloids present in many *Erythrina* species: β-erythroidine and erysovine. The test showed a 60% of mortality on *Culex quinquefasciatus* a LC_{50} of 225 and 394 ppm respectively. Finally the herbicidal activity (4) was evaluated in FA and LA fractions of alkaloids on the inhibition of germination of *Phaseolus vulgaris* and *Zea mays* and the photochemical activity on isolated chloroplasts of *P. sativum*. The tested fractions did not affect the germination in *P. vulgaris* and *Z. mays*, but in contrast concentrations lesser than 2.5 mg/mL of free alkaloids inhibited the ATP synthesis and proton pump. The liberated alkaloids fraction (<5 mg/mL) stimulated the ATP synthesis but did not affect the proton pump.

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B204 Biological activities of alkamides from species of the tribe Anthemidea (Asteraceae) endemic to Mexico

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Alkamides are the result of the condensation of a fatty acid with an amine, may be an amino acid with concomitant decarboxylation. This functional group is frequent in the constitution of proteins but not that frequent in natural products. Even when alkamides are restricted in species, these have been reported in eight plant families. Species containing alkamides are often found used in traditional medicine. Families containing most of the species with alkamides are *Piperaceae*, *Rutaceae* and *Asteraceae*.

The bioactivities observed in alkamides range from fungicides and bactericides to organoleptic stimuli. There may or may not be specific receptor for this small molecules. Some of them interact with receptor involved in the detection of noxious temperatures in mammals. Some of these molecules are as toxic to insects as pyrethrins. However, the mechanism of toxicity in lower organisms is different to that observed in insects.

The chemical structures are in some way characteristic of the genus or even restricted to species. The acyl moiety frequently presents a carboxyl conjugated 2*E* double bound, related to the specific activity. The presence of acetylenic and conjugated double bonds and aromatic moieties are organ specific. The distribution of the structures offers a taxonomic tool at the genus and specific level. *Acmella* and *Heliopsis* are the better-studied genera, containing small number of species and characteristic amides with specific tissue distribution. Traditional uses and potential applications in the field of pharmacology and agriculture are discussed.

B205 Biological activity of crude alkaloid extracts from *Aspidosperma ramiflorum* and *Aspidosperma tomentosum*.

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Aspidosperma species are known for its high indole alkaloid contents. Many of these alkaloids have important pharmacological properties, such as antitumoral and antimicrobial activities. Crude alkaloid extracts from *Aspidosperma tomentosum* (leaves and twigs, seeds and seed wings) and from *A. ramiflorum* (seeds and seed wings) were assayed for antibacterial activity by agar diffusion method. In addition, the crude alkaloid extracts from *A. tomentosum* leaves and branches and *A. ramiflorum* seeds and seed envelopes were assayed for a potential antitumor activity using two mutant strains of *Saccharomyces cerevisiae* (RAD 52Y and RS 321) with a deficiency in the DNA repair system (1). Neither alkaloid extract from *A. tomentosum* nor from *A. ramiflorum* presented antibacterial activity at the concentration assayed (120 µg). However, crude alkaloid extracts from of *A. ramiflorum* seed wings (0.2 mg/mL) and from *A. tomentosum* twigs (2.0 mg/mL) showed a potential antitumor activity.

Acknowledgements: FAPESP, CNPq.

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B206 Establishment of a *Pilocarpus pennatifolius* cell culture

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Natives living from South America used *Pilocarpus* species (vernacular name jaborandi) against some diseases, specially some kind of fevers, with the aim to induce copious sweating. The name jaborandi is a general term used for some Rutaceae and Piperaceae (1, 2).

Pilocarpine is the most important alkaloid found in *Pilocarpus* species due to its employment in glaucoma treatment. There are also descriptions of its use in baldness treatment, dropsy and xerostomy (3). All the pilocarpine used as medicine is isolated from natural source despite of the lower contents in jaborandi leaves. At the moment, there is no efficient synthetic route known (4, 5, 6).

The main goal of our work was to establishment a *Pilocarpus pennatifolius* cell culture with the aim to produce pilocarpine in a scale comparable to the natural source. Starting from leaves veins, we were able to establish a callus culture in MS medium, supplemented with MS vitamins, antioxidant solution having 2,4-D (0,55 mg/L), Picloram (0,03 mg/L), IAA (0,20 mg/L) and NAA (0,50 mg/L), as auxins and BAP (0,12 mg/L) as the only cytokinin. Initially, the calli obtained were hard but, adjusting the growth regulator balance, we were able to obtain friable calli.

At the moment, we were not able to detect the presence of alkaloid pilocarpine in the cultures.

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B207 Berberine production by *in vitro* culture of *Berberis buxifolia*

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Berberine is a quaternary isoquinoline alkaloid, which is used as an antibacterial agent. The aim of this work was to establish *in vitro* cultures of *Berberis buxifolia* (native species of Patagonia Austral) producing berberine and to study the antimicrobial activity from *in vivo* and *in vitro* cultures of *B. buxifolia* extracts.
B. buxifolia rhizomes were the initial explants for *in vitro* culture (1). *In vitro* plants were micropropagated on MS medium (2) supplemented with BAP (1 mg/L). For callus induction MS medium was supplemented with 2iP (1, 5 and 10 mg/L). In order to increase alkaloid production these calli were transferred to MS liquid medium supplemented with BAP (1 mg/L). Extracts of *B. buxifolia* from *in vivo* shoots and *in vitro* cultures medium containing berberine were tested in order to estimate the antimicrobial activity against *Staphylococcus aureus* ATCC25923 and *Escherichia coli* ATCC 25922.
Callus culture of *B. buxifolia* was established in MS medium using 2iP at all concentrations tested. Before BAP treatment, an intermediate growth step with TDZ or PIC was used. Both growth regulators were able to induce alkaloid releasing into the culture medium (0.6 g/L). The level of berberine obtained reached up to 0.8 g/L when BAP was added to the culture medium. All the extracts tested had a higher MIC value than the berberine standard. The results obtained show that the *in vivo* and *in vitro* extracts of *B. buxifolia* could contain some antimicrobial compounds that may have a synergical action between them.

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B208 Plants used in Mexico for the treatment of gastrointestinal disorders. 1. Antimicrobial screening

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Ethnobotanical surveys and literature reviews showed that 70 plants from 23 families are used in Morelos (Mexico) for the treatment of gastrointestinal disorders (1). In this study, thirty four crude extracts (hexanic, $CHCl_3$-MeOH (1:1) and methanolic) of different sections of eleven plants used in Mexican traditional medicine for the treatment of gastrointestinal illness were evaluated for their antimicrobial activities. Seven bacteria (*Enterococcus faecalis*, *Salmonella typhi, Aeromonas hydrophila* β-hemolytic, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus cereus*) were used as pathogenic targets. The selected species were *Crescentia alata* (bark), *Caesalpinia pulcherrima* (stem, aerial parts and leaves), *Acacia bilimeckii* (bark), *Acacia farneciana* (bark), *Spondias mombin* (bark), *Gliricidia sepium* (bark and leaves), *Pithecellobium dulce* (bark), *Vitex mollis* (leaves and bark), *Mastichodendron capiri* (bark), *Acacia angustissima* (bark) and *Cytocarpa procera* (bark). The selection of the sections of the plants to be tested was performed based on traditional practices carried out by herbalist in the State of Morelos. The crude extracts were evaluated for qualitative antimicrobial activity using the Mitscher method (2).
Results indicate that 29 (85 %) plant extracts inhibit one or more of the microorganism tested. The most inhibited bacteria was *A. hydrophila* β-hemolytic (65 %) and the most resistant was *E. coli* (6 %). The plants which exhibited the best antibacterial activity were: Chloroformic-methanolic extracts from the bark of *C. alata*; methanolic extracts from aerial parts of *C. pulcherrima*; methanolic extracts from bark of *P. dulce*, chloroformic-methanolic extracts from the bark of *M. capiri* and methanolic extracts from the leaves of *V. mollis*. The present study indicates that the use of traditional medicine is supported by the antimicrobial effects observed.

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B209 Plants used in Mexico for the treatment of gastrointestinal disorders. 2. Toxicity to brine shrimp (*Artemia salina* Leach) screening

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Continuing with earlier work on medicinal plants used in Mexico for the treatment of gastrointestinal disorders, we screened eleven medicinal plant species from Morelos State (Mexico) that could contain useful compounds. The plant selection was based on existing ethnobotanic information and literature reviews. The selected species were *Crescentia alata* (bark), *Caesalpinia pulcherrima* (stem, aerial parts and leaves), *Acacia bilimeckii* (bark), *Acacia farneciana* (bark), *Spondias mombin* (bark), *Gliricidia sepium* (bark and leaves), *Pithecellobium dulce* (bark), *Vitex mollis* (leaves and bark), *Mastichodendron capiri* (bark), *Acacia angustissima* (bark) and *Cytocarpa procera* (bark). Traditional practices by herbalist in the State of Morelos were used as a guide for the choice of the plant section to be tested.

Forty-five crude extracts (hexane, $CHCl_3$-MeOH (1:1) and methanolic) of different sections of the plants were screened using the Brine Shrimp Lethality Test, (BSLT) (1). From 11 species evaluated, eight (72%) produced extracts that displayed LC_{50} 1000 ppm, which are considered active. The hexanic extracts from, *Caesalpinia pulcherrima* (aerial parts), *Cytocarpa procera* (bark) and *Acacia farneciana* (bark) were the most active, with LC_{50} of 0.1199 ppm, 0.2113 ppm and 0.3114 ppm, respectively. These plants are popularly used in Morelos for diarrhea and digestive complaints (2).

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B210 New species and uses for the catalogue of Aymara medicinal plants (Bolivia and Peru)

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The Aymara culture extends on the Andean highlands of Peru, Bolivia and Chile. A 78 % of the Aymara population is in Bolivia, 19 % in Peru and 3 % in Chile. The Ayamara descent of the Tihuanaco culture, and during the Inca period they conformed the "Qollasuyu" district, meaning region of healers (Qolla = medicine, Suyu = region or district).

The well known "Kallawaya" wandering Andean healers are now Qechua speaking but their original name derives from the Aymara "Qollawayu" (Qolla = medicine, Wallu = bearing bags). Therefore we found extremely interesting to produce a critical review of the available literature concerning the lists of medicinal plants and their local uses in Aymara territories.

Hundreds of medicinal plants have been recorded with their Aymara uses for instance (1,2). European and American species are used. Most of them are locally grown. Some relevant species are the Andres Waylla (*Cestrum parqui* L'Hérit), Anu Ch'api (*Xanthium spinosum* L.), Aqhana (*Werneria nubigena* H.B.K); ChukuChuku (*Hieracium padcayense* Sleumer), Kuka (*Erythroxylon coca* Lam.), Kimsa K'uchu (*Baccharis genistellioides* (Lam.) Pers.), Manka P'aki (*Eupatorium sternbergianum* DC), Manzanilla (*Matricaria recutita* L.), Matico (*Piper acutifolium* Ruiz & Pav.), etc. We have recorded present day uses in Bolivian highlands involving species not reported as medicinal so far (*Calceolaria* sp. pl.) or unregistered uses as those for *Satureja boliviana* (Benth.) Briq.

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B211 Morphology and volatiles of glandular trichomes of *Montanoa tomentosa*

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Glandular trichomes are widely distributed over the aerial reproductive and vegetative organs of many plants (1). They are the primary secretory organs of these plants and their structures can vary highly among species. The essential oil produced by glandular trichomes may act as defense compounds in the aerial parts of the plant against herbivores and pathogens (2). The morphology and volatile components of glandular trichomes of leaves and flowers of the medicinal plant *Montanoa tomentosa* Cerv. (Astereaceae) were analyzed by scanning electronic microscopy (SEM) and gas cromatography-mass spectrometry (GC-MS) after extraction by solid phase microextraction (SPME). Glandular trichomes peltate and capitate types were observed in leaves and flowers. On the abaxial side of leaves peltate type predominated, however, the adaxial surface only presented non-glandular trichomes. By SPME-GC-MS sixteen and seventeen volatiles were totally characterized in flowers and leaves, respectively. However, three compounds were the most abundant in leaves and flowers, sabinene, α-pinene and α-thujene, which accounted for almost 70 % of the whole extract. It is worthwhile to mention that the rest of compounds found in the extracts were all sesquiterpenes such as α-gurjunene, caryophyllene and germacrene D. Finally, the total percent of volatiles identified was 83.7 for flowers and 86.81 for leaves.

Acknowledgements: To Francisco Solorio from the Instituto de Investigaciones Metalúrgicas of the Universidad Michoacana de San Nicolás de Hidalgo and M. C. Dolores Elena Alvarez Gasca of the Facultad de Arquitectura of the Universidad de Guanajuato for their technical support in SEM. To the CONACYT by scholarship 86454 to R.E.R.Z.

References: 1. Serrato-Valenti G. et al. (1997) Ann. Bot. 79: 329–336. **2.** Werker, E. (1993) Flavour Fragrance J., 8: 249-255.

B212 Identification problems of various Catuaba samples traded in Brazil and Europe

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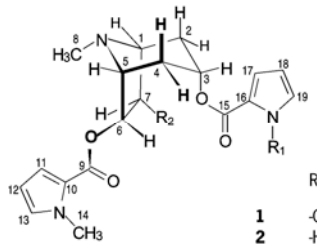
The popular bark Catuaba comes from Brazil and is known as a tonic, stimulant and aphrodisiac. Identifying the traded drugs creates problems, because the vernacular name Catuaba is attributed to various species belonging to different genera such as *Anemopaegma, Erythroxylum, Ilex, Micropholis, Secondatia, Trichilia* and *Tetragastris* (1,2). The existence of one of the most frequently cited species *Erythroxylum catuaba* is even doubtful, because the species identification only bases on a *nomen nudum* generated by A.J. da Silva in 1904 in Brazil. Anatomical and chemical examinations of various Catuaba samples available in health shops, via internet and via personal contacts in Brazil showed heterogeneity. Some of our samples contained a bark similar to *Trichilia catigua* (Meliaceae), others consisted of crude drugs which could not be assigned to species related with Catuaba due to the lack of reference material. From such Catuaba samples traded in Brazil akaloids of the tropanol ester type (**1**,**2**) were isolated and structurally elucidated (3). In a TLC screening the respective alkaloids were not detectable in all examined Catuaba samples.



	R_1	R_2
1	-CH3	-OH
2	-H	-H

Acknowledgements: We thank Mag. M. Kaniak and Mag. W. Stindl for their isolation work as well as Prof. Luis Carlos Marques, University of Maringá, Parana, Brazil, for providing bark samples of *Trichilia catigua* A. Juss.

References: 1. Daly, D.C. (1990) Kew Bulletin 45 (1): 179-194. **2.** Marques, L.C. (1998) Espec. de Capa, Mar/Abr. 8-11. **3.** Stindl, W. (2001) Master's thesis, University of Vienna.

B213 Volatile constituents of *Aristolochia argentina*

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The essential oils from leaves, stems and underground organs of *Aristolochia argentina* Gris. were obtained by hydrodistillation and analyzed by GC and GC/MS. This species is rich in argentilactone (0.7 and 6 g/kg fresh weight in aerial and underground parts of the plant, respectively), a volatile compound which can be hydrodistilled or extracted with organic solvents. In addition, forty-three components were identified in the oils. All parts of the plant afforded volatile oils characterized by high levels of argentilactone (57-89 %) and the presence of undecatriene isomers (0.3-4.0 %). These last constituents mainly contribute to the characteristic odor of the plant. Analysis of the volatile compounds of *A. argentina* extracted with solvents showed the same profile of the hydrodistilled oil. A commercial tincture of this native species, named "Charrúa" and produced from its subterranean organs, is claimed (internally) to have astringent, antihemorrhoidal and emmenagogue properties in popular medicine. The analysis of the volatile compounds of this tincture also afforded argentilactone, an unsaturated δ lactone with eventually toxic properties. Taking in account these results, and the prohibition of commercializing plant preparations based on this genus bearing aristolochic acids, a more strengthened control should be encouraged to avoid the dispensation of this specially harmful species.

B214 Influence of seasonality and leaf position on leaf biometry and essential oil yield of *Lippia alba*

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Lippia alba (Mill.) N.E.Br. ex Britt. & Wilson (Verbenaecae), commonly called "falsa melissa", is a widespread plant in Brazil whose fresh leaves are used in folk medicine such as antispasmodic, sedative, and for the treatment stomach diseases. Phytochemical studies with *L. alba* revealed the presence of γ-terpineol, β-caryophyllene, citral, β-myrcene, *p*-cymene, undecanone, cadinene and α-humulene in the essential oil (1,2). Chlorophyll parenchyma, whose development is influenced by light conditions, is the principal site for producing essential oil. This work relates variations in leaf biometricy and in yield of essential oil of *L. alba* as a function of the season of the year and position of the leaves on the branch. Thus, the parameters of seasonal variation (spring, summer, autumn, and winter) were considered concerning location of leaves in three parts of the branch (apical, median, and basal). Extractions of essential oil were accomplished through hydrodistillation. For biometric analysis of the leaves, samples were collected from of the third median of the blade. The highest yield of oil was obtained in summer, in leaves located in the apical part of the branch; in these leaves during the same season, the total height of the blade was greater, due to a of greater development of the parenchyma palisade and epidermal cells of the adaxial face.

References: 1. Correa, C.B.V. (1992) Rev. Bras. Farmacia,v, 73: 57-64. **2.** Gomes, E.C. et al. (1993) Rev. Bras. Farmacia, v, 74: 29-32.

B215 Four new 2-acyl-3-hydroxycyclohex-2-en-1-ones from the essential oils from *Piper amalago*

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As part of our ongoing investigations on the essential oils from *Piper* sp. we now report the chemical composition of the oils from leaves, branches, stems and spikes of *Piper amalago* L. from Panama. Qualitative and quantitative analyses of the oils obtained by hydrodistillation following the method described in the European Pharmacopoeia (1) were carried out by GC and GC-MS using two different capillary columns: Supelcowax™ 10 and methylsilicone. Identification of the components was achieved from their retention indices on both columns and by comparison of their mass spectral fragmentation patterns with those stored in our own data base and with literature data (2). Identity of major compounds was also confirmed by ^{13}C-NMR analysis of the total samples (3).

In total, thirty-nine components were identified meaning more than 95% of each sample. Main constituents were a series of four new 2-acylhydroxycyclohexenones (more than 50% of the oils), whose structures were elucidated from their ^{13}C-NMR and MS data as 2-hexanoyl-3-hydroxycyclohex-2-en-1-one, 2-octanoyl-3-hydroxycyclohex-2-en-1-one, 2-decanoyl-3-hydroxycyclohex-2-en-1-one and 2-dodecanoyl-3-hydroxycyclohex-2-en-1-one. DEPT pulse sequences from 2-octanoyl-3-hydroxycyclohex-2-en-1-one, isolated from the leaf essential oil by CC over silicagel eluting with hexane and petroleum ether, allowed the determination of the hydrogenation pattern of each carbon. Sesquiterpene hydrocarbons were also characterized in the oil from leaves, such as α-selinene and β-bisabolene, while monoterpene hydrocarbons, mainly α- and β-pinene, predominated in the oils from spikes, branches and stems.

Acknowledgements: ACI Program of the Generalitat de Catalunya, Iberoamerican Program CYTED (Subprogram X).

References: 1. Conseil de l'Europe (1996) Pharmacopée Européenne. Maisonneuve S.A. Sainte Ruffine. **2.** McLafferty, F.W. (1993) Registry of Mass Spectral Data. John Wiley & Sons. New York. **3.** Tomi, F. et al. (1995) J. Magn. Res. Anal. 1: 25-34.

B216 New glutarimide alkaloids from *Croton cuneatus*

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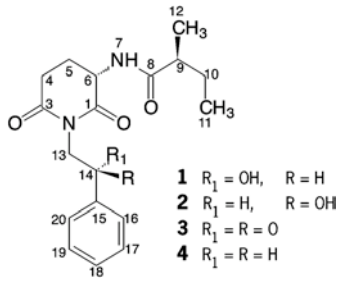

1 R_1 = OH, R = H
2 R_1 = H, R = OH
3 R_1 = R = O
4 R_1 = R = H

Croton cuneatus (Euphorbiaceae) is a plant found in the Venezuelan amazon. The aqueous extract is widely used by the natives to treat inflammations, wound healing and against gastric problems. Analysis of the dichloromethane extract of the aerials parts of *Croton cuneatus*, led to the isolation and identification of the new glutarimide alkaloids (*S*)-14-hydroxy-2[N-(2-methylbutanoyl)]-N-phenylethylglutharimide (**1**), (*R*)-14-hydroxy-2-[N-(2-methylbutanoyl)]-N-phenyl-ethylglutharimide (**2**) and 14-oxo-2-[N-(2-methylbutanoyl)]-N-phenylethylglutharimide (**3**), together with the known julocrotonine (**4**) (1,2). These structures were elucidated by spectroscopic methods, particularly by using high field NMR spectroscopy. The configurations of the stereocenter (C-14) were assigned as (*S*) for **1** and (*R*) for **2** after the hydrolysis of glutarimide and by analogy with (*S*) and (*R*)-2-amino-1-phenylethanol (3,4).

Acknowledgements: Fonacit-CNR Grant PI-2001000347.

References: 1. Aboagye F. et al. (2000) Fitoterapia. 71: 461. **2.** Nakano T. et al. (1961) J. Org. Chem. 26: 1184. **3.** Ziegler T. et al. (1990) Synhesis. 7: 575. **4.** Meyers A. and Slade J. (1980) J. Org. Chem. 45: 2785.

B217 Andirol A and B, two unique rotenoid-type flavonoids from *Andira inermis*

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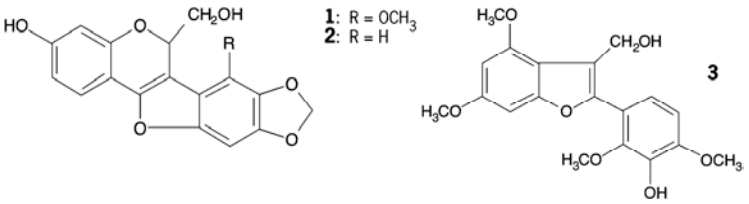
Andira inermis (W. Wright) H.B.K. (Fabaceae) is a Middle American plant remedy, its stem bark is used against fever. It is native from Mexico to Southern America, but also often occurs as ornamental in Tropical West Africa. Recently, we reported the isolation of 2-arylbenzofuran-3-carbaldehydes (andinermals) from the leaves of a Panamanian sample of *A. inermis* (1); now we have investigated the leaves of this species collected in Ghana. From a methanolic extract we isolated andirol A and B (**1**, **2**), two compounds with a novel type of rotenoid-related skeleton and andinermol (**3**), a new 2-aryl-3-hydroxymethyl-benzofuran. Their structures were elucidated on the basis of spectral data (EIMS, ^{1}H-NMR, ^{13}C-NMR, HMBC, and NOE).



In an *in vitro* antiplasmodial assay against *Plasmodium falciparum* **1** showed no remarkable antiplasmodial activity (IC_{50} values: 15.0 µg/ml [PoW]; 42.5 µg/ml [Dd2]). Andinermol (**3**) exhibited moderate activity (IC_{50} values: 8.6 µg/ml [PoW]; 13.7 µg/ml [Dd2]). However, **3** was less active than the related carbaldehyde andinermal A (1).

References: 1. Kraft, C. et al. (2001) Phytochemistry 58: 769-774.

B218 Cornutins C-G, novel neoclerodane-type diterpenoids from *Cornutia grandifolia* var. *intermedia*

K. Jenett-Siems [a], I. Köhler [a], C. Kraft [a], K. Siems [b], M.P. Gupta [c], U. Bienzle [d]

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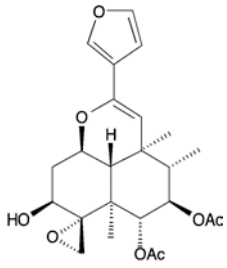

Cornutin F

Cornutia grandifolia var. *intermedia* Moldenke (Verbenaceae) is a shrub or small tree native in Central American rainforests from Guatemala to Panama. The plant is used as a remedy against malaria by Panamanian Indians. Earlier investigations of *C. grandifolia* yielded the Cornutins A and B, neoclerodane-type diterpenoids with repellent activity against leafcutter ants (1).

During our ongoing investigations on antiplasmodial plants from Central America, we isolated five new cornutins, which we named cornutin C-G, from the aerial parts of *C. grandifolia*.

The structures of these diterpenoids have been elucidated by spectroscopic means (EIMS, HREIMS, ^{1}H NMR, ^{13}C NMR, H-H COSY, HMQC, HMBC, NOESY).

The enolether sub-structure of cornutin F is unique among neoclerodane-type diterpenoids. Of the isolated compounds, only cornutins C and D exhibited moderate activity in an *in vitro* bioassay against *Plasmodium falciparum*.

References: 1. Chen T.B. et al. (1992) J. Org. Chem. 57: 862-866.

B219 Unusual sesquiterpene lactones from *Montanoa hibiscifolia*

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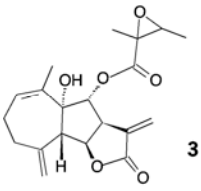
Montanoa hibiscifolia (Benth.) Sch. Bip. (Compositae, tribe Heliantheae, subtribe Montanoinae) has been studied previously leading to the isolation of several trans,trans-germacranolides as well as of montahibisciolide which exibited a new skeletal type (1). We here report on the reinvestigation of its aerial parts collected in Costa Rica. Five sesquiterpene lactones (SLs) were isolated and identified by means of one and two dimensional NMR as well as ESI and CI-MS analysis. Two SLs, 8α-(2',3'-epoxy-2'-methylbutyryloxy)-9-oxo-germacra-4*E*,1(10)*Z*-dien-6β,12-olide (**1**) and 8α-(2',3'-epoxy-2'-methylbutyryloxy)-1α-methoxy-9-oxo-10αH-germacra-4*E*-en-6β,12-olide (**2**), have already been known from this species. SL **3** was identified as the new 11,13-dehydroderivative of 8α-(2',3'-epoxy-2'-methylbutyryloxy)-9α-hydroxy-montahibisciolide. Compounds **4** and **5** possessed both an eudesmanolide skeletal with an endoperoxide structural element which has been rarely found in nature. One of these new SLs was esterified with 2',3'-epoxy-2'-methylbutyric acid, the other one with its 2',3' epimeric acid.



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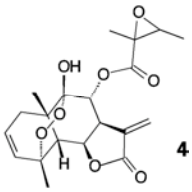

4

SLs **1** and **2** were studied for their inhibitory activity on DNA binding of the transcription factor NF-κB using Jurkat T cells as well as RAW 264.7 cells. DNA binding of this central mediator of the human immune response was inhibited at micromolar concentrations.

References: 1. Bohlmann, F.J. et al. (1984) Nat. Prod. 47: 663-672.

B220 New dammarane glycosides from stems of *Anomospermum grandifolium*

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In our ongoing research for new bioactive compounds from medicinal plants of Peruvian rainforest, the stems of Icu (*Anomospermum grandifolium* Eichler) have been studied. Icu is a native liana which belongs to the family of Menispermaceae and grows in the Amazonian riversides. It is traditionally used as an ingredient of curare (1). Previous studies in leaves and (or) stems have reported the presence of alkaloids (2) and the extracts of stems have also shown a curare action (3) .

Because of the important action of the curare and the well known activities of other plants of the same family (4), a phytochemical study has been performed in order to isolate the secondary metabolites of the stems.

A sequential extraction at room temperature was performed on powdered stems of the plant using solvents of increasing polarity. The methanol extract was fractionated on column chromatography on Sephadex LH-20 and semipreparative HPLC on reversed phase C-18. By this means the two news saponins jujubogenin 3-O-α-L-arabinofuranosyl (1→2)-[β-D-glucopyranosyl (1→6) β-D-glucopyranosyl (1→3)]-α-L-arabinopyranoside and lup-20(29)-en-27,28-dioic acid 28-O-β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl (1→3)]-β-D-xylopyranosyl (1→2)-β-D-glucopyranoside ester, along with the know jujubogenin 3-O-α-L-arabinofuranosyl (1→2)-[β-D-glucopyranosyl (1→3)]-α-L-arabinopyranoside and lup-20(29)-en-27,28-dioic acid, were isolated.

The structure of the saponins were elucidated based on 1D (^{1}H, TOCSY and ^{13}C) and 2D (DQF-COSY, HSQC and HMBC) NMR spectral data.

References: 1. Brack, A. (1999) Diccionario Enciclopédico de Plantas Útiles del Perú. Programa de las Naciones Unidas para el Desarrollo y Centro de Estudios Regionales Andinos Bartolomé de las Casas, Cusco Perú. **2.** Da Rocha, A. et al (1984) Acta amazónica 14 (1-2), 244-254. **3.** King, H. (1948) J. Chem. Soc., 1945-1949. **4.** Ciccia, G. et al. (2000) J. Ethnopharmacol. 72, 185-189.

B221 Characterisation of C-glycosidic flavonoids from Brazilian *Passiflora* species using LC-MS exact mass measurement

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Passiflora species are used as phytomedicines in Brazil due to the sedative properties that are related to the presence of flavonoids in leaves. Due to the importance of flavonoids and their glycosides to these species, the identification and/or structural determination of such compounds occurring in leaves play an important role. As the analysis of natural products from crude plant extracts using LC-MS and LC-MS-MS is becoming more routine, a study of the parameters required using an orthogonal acceleration time of flight mass spectrometer to provide an efficient route to the dereplication of *C*-glycosidic flavonoids extracted from Brazilian *Passiflora* species has been performed.

Hydroethanolic extracts of the leaves of *P. incarnata*, *P. alata*, *P. edulis* and *P. caerulea* were all analysed using Oa-TOF LC-MS. Using the LCT (Micromass) equipped with a dual ESI source, the presence of 6-C and 8-C flavonoid glycoside isomers (vitexin / isovitexin and orientin / isoorientin) have been possible using exact measurement and elemental composition calculation. This further allows for the specific identification of the species from which the flavonoids have been extracted.

Using the functionality of Oa-TOF where low level analyte detection can achieved when acquiring data over a wide mass range, exact mass measurement has been used as a tool for unequivocal identification of flavonoid isomers. It has been possible to distinguish and correctly assign the flavonoids of interest from degradation products due to the presence of other flavonoids, which also have the same luteolin-type or apigenin-type of skeletal structure.

B222 Characterization of the UV radiation filter hexadecenoic (C 16:1) fatty acid in seed oil of *Gevuina ave - llana* Mol. clones

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The genetic and production improvement programme of *Gevuina avellana* Mol. (Proteaceae), a native tree of Chile, had developed high productive and quality clones of edible nuts (1). High content of monounsaturated fatty acids characterize the seed oil of *Gevuina* with interesting aspects in nutrition and pharmacological possibilities. After oil Soxhlet extraction, fatty acid methyl esters (FAME) were prepared by reacting the oil in *n*-hexane with a methanolic solution. FAME of nine selected clones were analyzed in a Hewlett Packard 5890 (II) gas chromatograph, equipped with a flame ionization detector. There were seed oil differences among years in total fat and changes in monounsaturared and polyunsaturated fatty acids. The potential UV radiation filter value of *Gevuina* seed oil is discussed on the basis of its composition. Unusual positional isomers (2), of monounsaturated fatty acid (hexadecenoic, palmitoleic) $C_{16:1}\Delta^{11}$ (21 to 24%), $C_{16:1}\Delta^{9}$ (0.1 to 0.5 %) and minor quantities of others non determinated, were in different concentration among clones ($P< 0.05$ LSD). Results of a similar research with the shell oil content are perented and will be important in the next future of *Gevuina* genetic improvement.

Acknowledgements: Fruvax Ltda., Universidad Austral de Chile.

References: 1. Medel, F. (2001) Acta Hort. 556: 521-528. 2 Bertoli, C. et al (1998) JAOCS 75 (8): 1037-1040.

B223 Tropane alkaloids from the bark of *Erythroxylum catuaba* (Erythroxylaceae)

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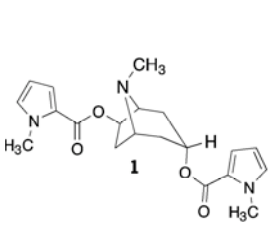
In our ongoing search for new bioactive compounds from higher plants, the bark of Catuaba (*Erythroxylum catua - ba* A.J. da Silva), a tree native to the northern part of Brazil, was investigated. Catuaba, long valued by local populations as an aphrodisiac and a central nervous system stimulant (1), has recently been the focus of great public interest because of use of the bark as a remedy for erectile dysfunction. In order to evaluate its activities and because of the well-known effects of other plants of the same family, it was decided to study the alkaloid extract. The LC/DAD/APCI-MS^n and the LC/UV/NMR of the alkaloid extract showed the presence of many products having the same tropan skeleton esterified by 1-methyl-pyrrole-2-carboxylic acid or 4-hydroxy-3,5-dimethoxybenzoic acid. Fourteen novel tropane alkaloids were isolated, including methyl-catuabine C (**1**) and demethyl-catuabine A (**2**). The structures were established by spectroscopic methods, including multiple stage mass spectrometry (MS^n) and 2D-NMR heteronuclear correlation experiments (2, 3). The absolute configurations of the tropane products obtained by alkaline hydrolysis were established by the Mosher method (4).



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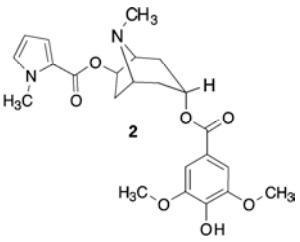

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B224 A new saponin from *Vochysia pacifica* stem bark

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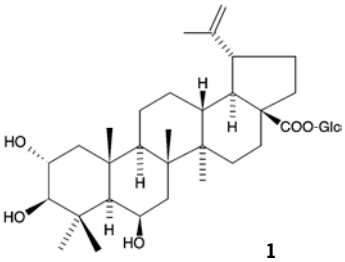
As part of our continuing interest in antiprotozoal natural products (1), we investigated the stem bark of a Colombian tree, *Vochysia pacifica* Cuatrec. (Vochysiaceae), which had shown activity in preliminary leishmanicidal assays. By means of silica gel and Sephadex LH 20 column chromatography techniques, we isolated 2α,3β,6β-trihydroxy-lup-20-en-28-oic acid glucosyl ester (**1**), a new lupan type saponin along with 4 known triterpenoids.

The structure of the isolated compounds was determined by spectrometric methods, in particular HR-FAB-MS together with mono- and bidimensional NMR experiments. The biological evaluation of the pure compounds is in progress.



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Reference: 1. Weniger B. et al. (2001) J. Ethnopharmacol. 78: 193 - 200.

B225 Constituents of fruit juice of *Euterpe oleracea* Mart. (açai palm)

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The aim of this work was to contribute to the phytochemical characterisation of the constituents of the fruit juice (açai) of *Euterpe oleracea* Mart. (açai palm). This palm is widely diffused and cultivated in Brazil and other Amazon regions due to its fruit and palm heart. The fruit is a globose black berry at complete maturity and has an unusual flavour similar to raspberries or blackberries but with a nutty taste. Especially in the Parà state, the pulp of the fruit has a large consumption (about 180 t/year): it is eaten fresh or fermented into beverage and used to prepare desserts and ice creams. The analysis of the fruit juice was performed on ethanol extraction of the pulp and on the extract obtained by the traditional preparation of the pulp by water maceration. The fruit pulp extract was first purified by liquid-solid extraction (LSE) and liquid-liquid extraction (LLE) procedure and submitted to HPLC analysis. A qualitative and quantitative analytical HPLC-DAD-MS method of the constituents of pulp was developed and constituents were identified by the combination of UV and MS data. Several polyphenols were identified, i.e. the flavonoid derivatives of quercetin, taxifolin and apigenin and anthocyanins such as cyanidin glucoside and cyanidin rutinoside. The percentage of total anthocyanins was near 5.3 mg/g of dried weight. The presence of these constituents having antioxidant properties could suggest its use as a dietary supplement. In addition, the similarities in the anthocyanin profile with other herbal drugs such as blackberry could encourage its use as an ingredient of cosmetic preparations and herbal medicinal products. This fact could be important for the development of the economy of the Amazon regions.

Acknowledgements: This work was supported by MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Rome).

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B226 Cultivation and breeding of Cowslip (*Primula veris*) for producing medicinal drugs

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Primula veris is a perennial, herbaceous plant with a short root stock. Each of about 10 stalks (from 2 to 20 cm high) per plant are possessing a multi-blossom umble (1). *Primula veris* were used as traditional medicinal plants. The yellow blossoms (*Primulae flos*) contains flavonoids and the roots (*Primulae radix*) is rich of saponines (2). The extracts of blossoms and roots are used against catarrh of the respiratory tract and as addition drug for cough-teas.

Until now, *P. veris* is collected at wild habitats in South-east Europe, but this way of utilization is endangering the survival of *P. veris*. Therefore, a cultivation of *P. veris* combined with a controlled collection from wild habitats is necessary for a sustainable use.

The task of the project was to collect and examine different *Primula veris*-accessions from the Mediterranean region (Greece and Croatia) and Central Europe (Southern Germany) to start commercial cultivation. The primary breeding goals are the yield of blossoms and the content of secondary components. Further breeding goals are minimal gradient of blooming, a high germination capacity (stimulation of the germination is necessary), a limited retiring of leaves during the summer, the seed viability and the net reproductive rate (3).

First results of yield from the different habitats are indicating a clear gradient from the north (Southern Germany) to the south (Southern Greece).

The results of thin-layer-chromatography (TLC) shows higher contents of the marker substances gossypin and rutin at the Croatian habitats and in general the chemical polymorphism in the flavonoid fingerprints of cowslip from Central and South-East Europe.

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B227 Influence of Zn and Ti ions on growth and selected element accumulation in *Centella asiatica* plantlets cultured *in vitro*

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Centella asiatica from Apiaceae family, a valuable Asian origin medicinal plant, is widely used as nervine tonic, for treatment of asthma, bronchitis, skin disease, kidney troubles, and showing antibacterial, antifeedant, antistress activities, wound-healing and other properties. Plantlets were cultured on Nitsch and Nitsch (NN) or Schenk and Hildebrandt (SH) modified media with various growth regulators.
The aim of the work was to find out how abiotic stress factors, such as Zn and Ti ions, are influencing on growth factor and the accumulation of other elements in *C. asiatica* plantlets. Zn^{2+} is routinely added to media in concentrations sufficient for biomass growth. In our experiments NN medium were additionally supplemented with $ZnSO_4$ from 0.0144 to 0.287 g/l (50-1000 µM). Plantlets were grown on this medium 3, 5 and 7 weeks. Ti ions were appied as solution of Tytanit – foliar fertilizer (titanium is the main element of the formula). Plantlets were treated by soaking in Tytanit (0.04%) only or with subsequent rinsing with water. Growth of plantlets on NN medium was observed after 6 and 10 weeks, then were transferred into soil in room-temperature conditions. The control material to each experiment was collected. The best plant growth was noticed on medium supplemented with 0.0287 g/l $ZnSO_4$ after 7 weeks. Among plantlets treated by Ti ions and rinsed with water the best growth was noticed after 6 weeks of culture, but after 10 weeks – plantlets soaked only in Tytanit were bigger. The concentrations of Cu, Mn, Zn, Fe, Ca and Mg (determined by ASA using Spectrophotometer SP 1900 Pye Unicam) in either "free"" (soluble in water) or "bound" (in celll structures) form were evaluated. The content of analysed elements was dependent not only on Zn concentration in the medium but also on the form of Ti applied and on the duration of the experiment. The content of free Zn was the highest after 3 weeks - 33.3%, the lowest -11.5% after 7 weeks and in control 22.5%.

Acknowledgements: Part of the work was supported by the Medical University in Warsaw theme no FW/21/W-1/2001. We thank Prof. Dr. Marziah Mahmood from University Putra Malaysia University, for kind gift of *C. asiatica* plantlets for starting of tissue culture.

B228 Changes in the essential oil content and mineral composition of medicinal plants caused by metal ions application

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We describe the reaction of Umbelliferae family plants caused by Ti, Mn and Cu ions application. The studied material was: *Crithmum maritimum* L after fertilization with 0.04% Tytanit (Ti main component) formula on the leaves, *Pastinaca sativa* L. stressed with Cu and Cu + Mn and Tytanit (0.04% and 0.4%) for 24, 48 and 72 h, *Peucedanum verticillare* Koch. stressed with Cu and Cu + Ti (24, 48, 72 h). Total content of essential oil in the samples was determined by steam water distillation. Quantitative analysis of essential oil components (α- and β-pinene, *p*-cymene, limonene, γ-terpinene) was performed by gas chromatography. The concentrations of selected elements ("free" and "bound" forms) were determined by ASA. Used stress factors evoke important changes in the quality and quantity of volatile oils as well as in the mineral composition of studied plants. After fertilization with Tytanit, *C. maritimum* collected in the first vegetation season was significantly more rich in volatile oil and showed higher percentages of the five evaluated monoterpenes than the controls. Next year the differences were less striking. The highest amount of limonene was in essential oil obtained from the *P. sativa* leaves stressed with 0.04% Tytanit solution, the lowest amount of this monoterpene was in oil of plants' fruits stressed with Cu + Mn solution for 72 hours. Also in *P. verticillare* the changes in oil content and composition were important, but to the small extent concerning the analysed five monoterpenes. The application of Ti, Mn and Cu ions influences the concentration of Zn, Mn, Fe, Cu free and bound forms in tested plants. The reactions of particular species were various.

B229 Encapsulation as a method for micropropagation of three Asian plant species used in therapy

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The aim of the work was to elaborate the process of the encapsulation of three species meristematic tissues and different organs in the form of beads and the comparison of their preservation at lower temperature and development in different light conditions on solid and in liquid media. Small buds or organs were mixed with sodium alginate and dropped individually into a $CaCl_2$ solution. After hardening, the "somatic seeds" produced were stored in 4ºC for 2 weeks to 6 months and then transferred to solid or liquid media.
Tinospora cordifolia Miers. (Menispermaceae) is a climbing shrub, growing in south Asia, its stems have been used in Ayurvedic preparations for treatment of diabetes, skin diseases and possessed anti-inflamatory, anti-allergic and immunomodulating properties. The tissue culture was started from sterilised fragments of stems collected in the Botanical Garden in Bombay; several callus lines were obtained and subcultured on DCR medium with NAA. Two lines differentiated on medium Nitsch and Nitsch (NN) with IBA and BAP into shoots. These structures were used for encapsulation.
Centella asiatica L. from Apiaceae family is widely used as nervine tonic, for treatment of asthma, bronchitis, skin disease, kidney troubles, and showing antibacterial, antifeedant, antistress activities, wound-healing and other properties. Plantlets were cultured on NN or Schenk and Hildebrandt (SH) modified media with various growth regulators. For encapsulation, leaf-borne thin roots were selected.
Withania somnifera (L.) Dun. (Solanaceae) contains several compounds with anti-bacterial, immunostimulating, immunosupressive, cytostatic and others activities. Sterile plantlets raised from shoot tips of seedlings were cultivated on Murashige and Skoog (MS) medium. Apical and axillary meristems were obtained on NN with BAP and IBA.
The development of "somatic seeds" after 6 months storage in 4ºC was: - 80 % of *W. somnifera* buds, 35 % of *C. asiatica* encapsulated leaf-borne roots and 20 % of *T. cordifolia* structures.

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B230 Chemical and genetical response of *Trigonella foenum-graecum* L. to certain mutants

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DNA based molecular markers have become important tools for developing improved cultivars and for studying phylogenetic relationships. Phytochemical investigation and randomly amplified polymorphic DNA (RAPD) fingerprints were used to determine variations produced in *Trigonella foenum-graecum* L. as a response of presawing seeds to chemical mutants (di-ethylamino-ethyl dextran), three different doses of γ-radiation (300, 500 and 700 rad.). Isolation of genomic DNA and RAPD analysis were carried out according to CTAB method of Doyle and Doyle (1). RAPD using OPA07 and OPA15 as the primers (Figures 1 and 2) showed genetic variations in the treatment chemically affected more than γ-radiation treatments, (similarity coefficient in the first treatment is less than 0.5 but in the three doses of γ-radiation treatments they are (more than 0.5), 0.62, 0.65 and 0.7 respectively. Phytochemical investigation revealed that the amounts of total alkaloids (2,3) produced from the control and the four treatments (chemical and three doses of radiation) were 0.960, 0.704, 1.072, 1.280 and 1.488 mg / 100 g) respectively, calculated as trigonelline base. Similar results were obtained for the dry weight of seeds produced from the first generation (0.159 control, 0.130 chem, 0.160, 0.186 and 0.220 radiation treatments g / 10 dry seeds). This leads to the conclusion that chemical mutation produces decrease in alkaloid concentration and dry weight production while small doses of radiation produces an increase in alkaloid concentration and dry weight production. The amounts of steroidal sapogenins showed that the diosgenin content decreased in the chemically mutated plants while increased in γ-radiation treatments (0.14% control, 0.09% chemical mutant sample, 0.16%, 0.17%, 0.2% γ-radiation treatments).

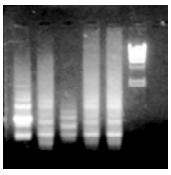

Figure 1

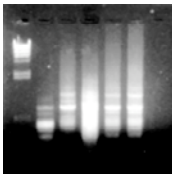

Figure 2

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B231 Clonal propagation of the Himalayan medicinal plant *Picrorhiza kurroa* (Scrophulariaceae)

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The perennial species *Picrorhiza kurroa* Royle ex Benth. is distributed in the alpine Himalayas at 3300 – 4300 m altitude. The main active compounds in the rhizomes (iridoid glycosides, cucurbitacins, and acetophenone derivatives) have been shown to possess various activities, e.g. hepatoprotective, choleretic, immunomodulatory and antiasthmatic (1). Due to ruthless exploitation of the natural population and lack of cultivation programmes the plant is endangered in several areas. *In vitro*-propagation might be helpful in conserving the species and for the production of large amounts of genotypically well-defined plantlets for further field culture.

Shoot explants obtained from aseptically germinated seeds were inoculated on semisolid MS medium supplemented with factorial combinations of zeatin and IAA. The highest multiplication factor (14.8) with low callusing and hyperhydration was obtained with 10 µM zeatin alone. Shoots were rooted using medium with 1 µM IBA and acclimatized to greenhouse conditions as described earlier (2). Plantlets transferred to a test plot in autumn 2001 survived the winter season without major losses.

HPLC and HPLC/MS analyses of roots and aerial parts obtained from *in vitro* propagation showed the presence of the major compound classes: iridoids, cucurbitacins and acetophenones. In comparison with root material collected in the field the constituent pattern was almost identical, whereas significant quantitative differences could be observed.

Our results indicate that large numbers of genetically homogenous plants for field culture can be produced through clonal propagation *in vitro*. Moreover, the procedure allows the selection and mass propagation of genotypes with an optimal pattern of secondary metabolites.

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B232 *In vitro* clonal propagation of *Charybdis* sp. through nodule culture in liquid medium

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Bufadienolides found in various species of the genus *Charybdis* (syn. *Urginea*, Hyacynthaceae) have recently gained renewed interest due to pronounced immunoregulatory activities (1). Furthermore, the compound scillirosidе has a distinctive rodenticidal effect (2, 3). Because of lack of field cultures and as an alternative to the slow conventional propagation a micropropagation method for the mass production of elite plantlets was developed. The advantages of this protocol which is based on liquid medium over labour and cost intensive procedures using agar medium are presented in this study.

The formation of a special, highly regenerative tissue (nodules) was induced on leaf explants cultivated in liquid medium in the dark. Nodule multiplication was performed by regular subculture under the same conditions. Shoot regeneration is basically triggered by exposure of the cultures to light. Under these conditions (continuous light at 60 $\mu M \cdot m^{-2} \cdot s^{-1}$, MS (4) medium with 1% sucrose) two liquid culture systems (shake culture and temporary immersion culture) were compared to the standard system on semisolid medium.

The highest shoot regeneration rate (number of shoots per gramme) was observed in liquid shake culture (58.8±8.3), but the majority of the shoots was severely hyperhydrated. Shoot formation in the temporary immersion system (28.5±3.1) was only a little higher that on semisolid medium (20.8±5.1) but resulted in healthy shoots. Despite of the lower regeneration rate when compared to shake culture, one temporary immersion unit still yielded nearly 300 shoots. The use of liquid medium makes possible interesting features like automation and scale-up for the quick and cheap production of genetically uniform elite *Charybdis* plantlets for further field culture.

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B233 Cloning and heterologuous expression of a terpene synthase gene from *Marrubium vulgare* L. (Lamiaceae)

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Marrubium vulgare is a medicinal plant which is not rich in essential oils but accumulates large amount of furanic labdane diterpenes. The major constituent of extracts from *M. vulgare* is the bitter tasting diterpene lactone marrubiin which is deduced from the genuine precursor premarrubiin. We have shown that isoprenic units of the diterpene marrubiin are built via the 2-C-methyl-D-erytritol 4-phosphate (MEP) pathway (1). The first specific step in biosynthesis of most isoprenoids is the cyclisation of linear diphosphates. In order to get an insight into the molecular biochemistry of isoprenoid biosynthesis and its regulation in *M. vulgare* we looked for terpene synthases using a PCR-based strategy.

Degenerated primers for PCR were deduced from highly conserved regions of known mono-, sesqui- and diterpene synthase sequences of angiosperms (2). The complete genomic and cDNA-sequence of a putative terpene synthase (*mvtps1*) could be established by means of different PCR- and extension-techniques. The cDNA was characterised by an open reading frame of 1641 bp encoding 546 amino acids. The sequence showed highest identity and similarity to known sesquiterpene synthases. The corresponding sequence of genomic DNA (3976 bp) is organized in six introns and seven exons, which were defined by comparison with literature data.

The *mvtps1*gene was heterologously expressed in *E. coli*. The resulting protein was shown to catalyse *in vitro* the cyclisation of geranyl diphosphate and farnesyl diphosphate. The main product was identified to be a selinen/cadinen like sesquiterpene (C15H24) by GC-MS.

References: 1. Knöss, W. et al. (1997) Biochem. J. 326, 449-454. **2.** Wildung, M.R. et al. (1996) J. Biol. Chem. 271, 9201-9204.

B234 Chemotype analysis in *Thymus pulegioides* – ecological and biosynthetical considerations

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Thymus pulegioides (Lamiaceae) is a medicinal plant which is rich in essential oil compounds. Occurrence of different chemotypes was analyzed within a population of *T. pulegioides* in a botanically well characterized natural reservation area at Hämmersberg in the Eifel region (Germany). Essential oil compounds from samples of about 100 cushions of *T. pulegioides* were extracted and analyzed by GC. Sample preparation and chromatographic analysis were developed in order to consume only a minimum of plant material from the natural reservation area.

Cluster analysis of the monoterpene composition of the samples revealed the existence of at least six different chemotypes, five of which have already been reported in literature (1,2). Most abundant was the so-called La-chemotype (58%), which is characterized by linalyl acetate as the main constituent. Less samples were found from chemotypes dominated by either linalool (1%), geraniol/citral (13%), thymol (7%) or carvacrol (2%).

In order to investigate possible allelopathic effects of monoterpenes from *T. pulegioides* distance mapping of plants growing close to cushions of *T. pulegioides* was performed. Statistical evaluation of the data showed that in contrast to reports on mediterranean habitats no defined allelopathic effect could be assigned to specific chemotypes. This results demonstrate that biological effects, which could be expected from measurements under laboratory conditions need not always be of biological relevance under field conditions.

The pattern of major monoterpenes from all chemotypes could be hypothetically explained by inhibition of specific steps in monoterpene biosynthesis. Especially interesting was the occurrence of chemotype X (17%), which has not been previously reported. This chemotype is characterized by a very low absolute content of monoterpenes while concentration of sesquiterpenes is comparable to the other chemotypes. This phenomenon might be caused by a block in a very early step of monoterpene biosynthesis or a regulatory/channeling mechanism.

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B235 ADR-4® Energy Stimulator – new elicitor inducing secondary metabolites in *in vitro* culture of *Ammi majus*

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Ammi majus, family Apiaceae, is considered to be the richest natural source of coumarins and linear furanocoumarins, which have been successfully used as photosensibilising substances (1). Callus cultures were induced from 4 weeks old hairy roots obtained after transformation of *A. majus* by Agrobacterium rhizogenes strain A4 (2). The efficient growth of callus was obtained on agar (0.75%) solidified Murashige Skooge medium containing 2.5 mg/l 1-naphthylacetic acid, 1.0 mg/l 6-benzylaminopurine and 3% sucrose. Water used for preparation of the media in first set of experiments was exposed to the influence of ADR-4® Energy Stimulator plates, which modified the intermolecular structure of water molecules (3), modified ADR-4®- plates with vial consisting of mineral substances equivalent to those found in living organisms and plates with magnets for 15 minutes with vigorous shaking. Callus cultures were grown in phytotron at a temperature of 20-22°C and illumination of 1200 lux. In the second set of experiments callus cultures were exposed to the influence of ADR-4® plates and modified ADR-4® for 30 days. After this time callus cultures were harvested, dried and the level of coumarins and furanocoumarins was determined. Callus samples were extracted exhaustively with petrol ether, chloroform and methanol using Soxhlet apparatus. Coumarins and furanocoumarins were determined using RP-HPLC system in the optimised multilinear gradient elution program: Tetrahydrofuran (THF)-Water, from 4 to 75 volume % of THF (Column: 250×4 mm i.d., Lichrospher RP 18 5 mikrometer, flow 1.5 ml/min). Due to many impurities in the analyzed extracts the UV-VIS DAD detector was used. No significant differences in growth rate between the tested callus samples were observed. However, in the elicited callus cultures, the level of umbelliferone was two times higher than in the not elicited one. Simultaneously, the accumulation of the compounds not previously detected in *A. majus* callus was observed (i. e. bergapten - 33.3 µg/g DW and scopoletin - 17.0 µg/g DW).

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B236 Cocultivation as a new way to obtain coumarins and fuanocoumarins from two plant species: *Ammi majus* and *Ruta graveolens*

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Ammi majus L. seeds are a good source of umbelliferone, while *Ruta's* tissues are rich in furanocoumarins: xanthotoxin, psolaren and bergapten. Umbelliferone is used as a substrate in the production of furanocumarins. For this reason, an attempt to cultivate both species *A. majus* and *Ruta graveolens* L. in one Erlenmeyer flask as a coculture was undertaken. Two variants of the coculture were tested. The first one was a coculture of *A. majus* hairy roots with *R. graveolens* cell suspension while the second one was a coculture of *A. majus* hairy roots with *R. graveolens* shoots. The cocultures were grown in a growth chamber with monitored conditions: photoperiod 16:8 (day:night), temperature 22ºC or 24 h night at the same temperature. 25 days old cocultures were harvested dried and the level of furanocoumarins was determined. Samples of the cocultures were extracted exhaustively with petrol ether, chloroform and methanol using Soxhlet apparatus. Coumarins (xanthotoxin, bergapten, imperatorin) were analysed quantitatively by solid-phase extraction (SPE) and RP-HPLC. A Hewlett- Packard (Palo Alto, CA, USA) model 1050 Liquid chromatograph, equipped with a 20 µl sample injector (Rheodyne, Cotati, CA, USA) and a variable wavelength UV-VIS detector was used. All compounds were detected at λ = 254 nm. In the first set of experiment (*A. majus* hairy roots with *R. graveolens* cell suspension) very slow growth of cocultures was observed, probably due to the inhibition of hairy root growth by Ruta's exudates. In the second set of experiment (*A. majus* hairy roots with *R. graveolens* shoots) the growth was satisfactory. Higher rate growth was observed when coculture was grown in the darkness than in the light (respectively 0.86 g FW/day and 0.58 g FW/day). However, the production of furanocoumarins was higher in the coculture growing in the photoperiod. RP–HPLC analyses of the coculture extracts indicated that the level of xanthotoxin was 3 times higher in Ruta's tissues growing in the coculture (24.34 mg/g DM) than in single culture (8.21 mg/g DM). The production of bergapten in Ruta's tissues in the coculture was about two times higher than in single culture.

B237 Feasibility study of the long-term cultivation of transformed hairy root cultures of *Ocimum basilicum* (Lamiaceae) in a novel spray-bioreactor system

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Rosmarinic acid (RA) ist a caffeic acid ester with antimicrobial and antioxidant activity. The compound can be found in various Lamiaceae and Boraginaceae species. Suspension cultures of *Coleus blumei* have been investigated for years as a potential source for RA without leading to commercial production.
Alternatively, sweet basil (*Ocimum basilicum* L.) hairy root cultures (HRC) have shown to contain high yields of RA with maximal concentrations of up to 14 % of dry weight (1).
In a feasibility study, we transformed hairy root cultures of sweet basil via co-cultivation with various *Agrobacterium rhizogenes* strains. The hairy root cultures were scaled-up in shake flasks for inoculation in a novel 80 Liter spray bioreactor. One cell-line was grown to about 1.5 kg (fresh weight) and maintained for a total of 4 month. Following the harvest, spot-like samples were taken and analysed to investigate the variation of RA-content between inner and outer layers of the dense root mesh. Samples were also taken for further subculture-cycles. After 4 month, the transformed roots contained 3 - 4 % (of dry weight) RA, irrespective of where the sample had been taken. This concentration was about 40 % of the RA-content found in juvenile HRC of the same cell-line.
In conclusion, we could show that our bioreactor system allowed cultivation of these HRC over a prolonged period of time without loosing vitality and that the variation of the RA-content within the biomass was relatively low.

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B238 Saponin production in root cultures of *Panax ginseng* C. A. Meyer

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Panax ginseng C.A. Meyer (Araliaceae) is a herbaceous plant, which in oriental medicine has a strong reputation since ancient times for being tonic, regenerating, and rejuvenating, even though its pharmacological activity is difficult to pin down. It was reported (e.g. 1) that ginsenosides and polyacetylenes isolated from ginseng roots have cytotoxic activity too. Native ginseng plants need 5 – 7 years prior to harvest and the content of ginsenosides is low. *In vitro* large-scale cultivation of roots seems to be promising to solve these problems. Roots were isolated from plantlets regenerated from somatic embryos and cultivated separately in liquid media in different types of bioreactors. Based on HPLC analyses, the total content of ginsenosides (Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg_1) reached 0.5% of dry weight according to media composition, bioreactor design and cultivation conditions. The best ginsenoside production were achieved in “temporary immersion system” bioreactor (RITA flask) in SH (Schenk and Hildebrandt) media supplemented with 5 mg/l IBA, with immersion time 5 minutes every 60 minutes. Production and ratio of individual compounds were comparable with their content in native root.

Acknowledgements: This work was supported by 521/02/P064, COST 843.10 and Z4 055 905 projects.

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B239 Quaternary ammonium compounds found in salt stressed plants

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Betaines and their tertiary sulphonium analogues have been shown to facilitate adaptation to saline and dry environments. For this role, however, the concentration must be high, as in the Malvaceae (1), but in many betaine-containing plants, the levels of these compounds are low as in the Bromeliaceae (2). In recent years it has been demonstrated that betaines have a role in aiding plants to resist attack by pathogens (3).

In this communication, a detailed study of 50 flowering plant species distributed in 43 genera and 22 families was undertaken. The betaines were extracted from dry plant material with 80% methanol. The extracts were passed through a column of cation exchange resin and the concentrate of ammonia eluate, then examined by thin layer chromatography (TLC). The Dragendorff-positive compounds were isolated by preparative TLC and identified by ^{1}H NMR spectroscopy and FAB mass spectrometry. The content of each betaine present in the extract of each species was estimated using a ^{1}H NMR procedure (4).

Betaines were found in the majority of species tested. In some (all members of the Chenopodiaceae, *Avicennia marina*, *Sesuvium portulacastrum* and *Inula crithmoides*), the content was sufficiently high to have a significant effect as a compatible osmolyte. The most commonly found betaine was glycinebetaine. However, β-alaninebetaine and choline-O-sulphate were isolated along with glycinebetaine from *Armeria maritima*, *Limonium humile* and *L. vulgare*. Prolinebetaine and trans-4-hydroxyprolinebetaine were extracted from *Sesuvium portulacastrum* and prolinebetaine, as well as glycinebetaine from *Honkenya peploides*. The majority of species tested had low quantities of betaines (0,001 – 0,10 %), the role of which is thought to be associated with various defence mechanism. The qualitative distribution of betaines within different species of any one genus was consistently the same.

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B240 Diterpenoids in hairy root culture of *Salvia sclarea* L.

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Salvia sclarea L., the clary sage, is a biennial plant naturally occurring in the Mediterraean region, in the southern part of Europe, the northern Africa and in Iran (1). The roots of the plant used in folk medicine for the treatment of various complaints and diseases are a rich source of abietane diterpenoids (2). This type of compounds has been reported as having antibacterial, anti-inflammatory, cytotoxic and analgesic activities (3). In the study presented here hairy root cultures of *Salvia sclarea* were established and their potential for producing diterpenoids were investigated.

Hairy roots of *S. sclarea* were induced from axenic shoots by direct infection with LBA 9402 *Agrobacterium rhizogenes* strain. Hairy roots culture was established on $^{1}/_{2}$ Gamborg's B5 liquid medium (macro and microelements according to B5 in half strength) (4) supplemented with 30 g/l sucrose. They were grown in continuous light (40 μm · s^{-1} · m^{-2}) or in the dark. Evidence of transformation was given by opine assay and PCR method. After two years of continuous subculturing analysis of diterpenoids in hairy root culture was carried out. The dry roots (ca 70 g) were extracted with acetone. The extract was purified by column chromatography on silica gel. The column was eluted with dichloromethane, mixtures of dichloromethane and ethyl acetate (9:1 → 1:1 v/v) as well as ethyl acetate and methanol (8:2 v/v), respectively, to give 8 fractions. Further separation of diterpenoids was achieved by preparative TLC. In this way three fractions, eluted with dichloromethane, were investigated to yield 4 compounds. They were identified on basis of their ^{1}H- ^{13}C- NMR and MS data as aethiopinone, 1-oxoaethiopinone, ferruginol and salvipisone. Five further fractions are being analysed now. We also found that hairy roots cultured in darkness and in the light showed similar diterpene pattern of spots on TLC.

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B241 Taxol: acquisition, functional expression, and characterization of the acyl/aroyl transferases in taxol biosynthesis

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The diterpenoid taxol, a potent anticancer drug, is produced by *Taxus* plants and derived cell cultures. A reliance on these biological systems for the semi-synthesis or complete synthesis of taxol will likely continue for the foreseeable future. Therefore, identification of the genes and characterization of the corresponding enzymes involved in taxol biosynthesis are required for the purpose of engineering suitable hosts to facilitate drug production and address the continued increase in demand. The taxol biosynthetic pathway comprises five acyl transfer steps, each adding a functional group that contributes to the overall structural pharmacophore. Acquisition of these five acyl transferases required reverse-genetics and a homology-based PCR cloning strategy to obtain a set of acyl transferase probes to screen a cDNA library made from *Taxus* cells cultures induced with methyl jasmonate for taxol production. Nine full-length acyl transferase clones were acquired by this method and expressed in *E. coli* (1). Extracts containing the corresponding operationally soluble recombinant enzymes were screened for function with appropriate taxane and acyl-CoA co-substrates. From this set of nine clones, all five full-length acyl transferase cDNA clones specific to taxol biosynthesis were identified (2-4). The function and properties are described for these recombinant transferases including acetyl-CoA: taxadien-5α-ol-*O*-acetyl transferase (2), acetyl-CoA: 10-deacetylbaccatin III-10β-*O*-acetyl transferase (2), a benzoyl-CoA: taxane 2α-O-benzoyl transferase (2), β-phenylalanoyl-CoA: baccatin III 13α-*O*-β-phenylalanoyl transferase (4) and benzoyl-CoA: *N*-debenzoyl-2'-deoxytaxol *N*-benzoyl transferase (3). Each taxol biosynthetic pathway acyl transferase possesses a K_m value with the respective co-substrates of between 1 µM and 1 mM, a pH optimum between ~7 and 9, and contains several signature conserved catalytic and structural sequence motifs found in other acyl transferases (5).

Acknowledgements: Robert Williams (Colorado State University).

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B242 The alkaloid production of genetically transformed and non-transformed cultures of *Lobelia inflata* L.

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Lobelia inflata L. plant (Lobeliaceae) is indigenous in North-America. The herb contains more than 20 piperidine alkaloids. The main alkaloid is the pharmacologically active lobeline, which has stimulative effect on the respiratory centre and it has been applied in cases of asthma, collapse, gas- and narcotic poisoning. Lobeline also causes nausea. In large doses its effect resembles to that of the nicotine, so that it is used in preparations against smoking.

We have studied the effect of growth regulators and alkaloid precursor amino acids (Lys, Phe) on the growth and alkaloid production of cell suspension-, callus-, organized- and hairy root cultures of *L. inflata* L. The investigations showed that these cultures are able to synthesize the characteristic alkaloids of the intact plant.

The synthetic regulator Sz/11 combined with Phe increased the total alkaloid content considerably in callus- and organized cultures. In callus cultures growing on medium supplemented with Sz/11 and 2,4-D, as well as 10^{-4} M phenylalanine the total alkaloid content was 7.5 times higher than in the control. Regulator Sz/28 especially increased the lobeline content (as a result of Lys and Phe application in organized culturesand callus tissues, respectively).

Growth regulators (NAA, IAA) added to the B5 media alone or in combination with amino acids increased the biomass formation of the genetically transformed hairy root cultures. In hairy roots cultivated on hormone-free medium containing Phe the lobeline level was maximum (36 µg/g). The lobeline production of hairy roots increased on hormone-free medium containing only Phe or Lys. Low concentrations of NAA and IAA (0.2 mg/l) increased the lobeline level of the cultures as compared to the control. By the addition of alkaloid precursor amino acids and hormones it succeeded to increase the lobelin production.

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B243 Isolation and culture of *Cordyceps sinesis* (Berk.) Sacc. (Caterpillar Mushroom) from Himalayan region of Nepal

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Cordyceps sinensis (Berk.) Sacc. is one of the very rare and endangered medicinal herbs of the ergot family. In Nepal, it is reported to found at higher altitude in the sub-alpine to alpine regions (4000-5000 m.) near the snow line (1). This fungus grows parasitically on insects and attacks a caterpillar. It is thus composed of the fruiting body of the fungus and its host larva. It has long been used in traditional medicine as a tonic. It shows a wide range of biological and pharmacological activities in renal, hepatic, cardiovascular, anticancer and nervous as well as immunologic systems (2). However, as the source and collection is very rare and difficult, the natural *Cordyceps* can not fulfill the market demand. Hence, this study was carried out with an objective to isolate the pure mycelial cultures to conserve this rare and endangered medicinal herb.
The samples of *C. sinensis* were collected from Dolpa district of Nepal during rainy season. The outer surface of its fruiting body was sterilized with 70% ethanol (v/v) and the inner parts were then cultured in different culture media at 28°C. After three weeks, the pure cultures were isolated by a series of transfer of different colonies using hyphal tip method. The pure mycelial culture of *C. sinensis* was identified as *Paecilomyces* sp. on the basis of asexual spores produced. This would eventually help to conserve this rare and endangered herb and also promote further research on this fungus living at very high altitudes.

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B244 Somatic embryogenesis in suspension cultures of *Podophyllum hexandrum*

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This study describes the establishment of embryogenic cell suspensions of *Podophyllum hexandrum* (Berberidaceae), plant which contains podophyllotoxin-type lignans, some of which have anti-inflammatory properties. Podophyllotoxin is used against certain virus and skin cancer diseases(1). Friable root-derived callus (4 months old, approx. 1.5 g f. wt.) was dispersed in 40 ml liquid UM medium containing 2.0 mg/l 2,4-D and 0.25 mg/l Kinetin. Cultures were incubated on a rotary shaker in the dark at 25 ± 2°C and diluted after 14 d with 5 ml of new UM medium. After 14 d of culture, 30 ml aliquots of cell suspension from individual flasks were transferred to 50 ml of new UM medium to produce stock cultures. The cell cultures were either cream or brown in colour. Differentiation of the cells commenced after 90 d, with cultures being composed of single, spherical and elongated cells, cells with dense cytoplasm forming aggregates, and somatic embryos. The latter were present at the globular, heart-shaped and torpedo-shaped stages in the same cultures in UM medium. The suspensions retained their embryogenic potential for 6 months over 24 transfers. The viability of cell suspensions, assessed 4 d after subculture, was 94% using fluorescein diacetate staining. Light microscopy showed that somatic embryos developed from somatic cells, with mitotic division of embryogenic cells initially giving rise to two celled proembryos. These embryogenic cell cultures offer possibilities for the *in vitro* propagation and/or genetic manipulation of *Podophyllum hexandrum*.

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B245 Ariltetralin lignans from callus and suspension cultures of *Linum campanulatum* L., *Linum narbonense* L. and *Linum strictum* L. *in vitro*

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In continuation of our search for podophyllotoxin (PTOX) containing cell cultures we have established several callus and subsequently suspension cultures from single sterile seedlings of *L. campanulatum*, *L. narbonense*, *L.* and *L. strictum* as described in (1) and checked for the occurrence of lignans. These lines were used to examine cell growth and production of lignans during a culture period of 14 days. Major cellular growth was obtained in MS medium supplemented with 2 mg/l kinetin, 0.1 mg/l indolacetic acid and 0.2 mg/l 2,4-dichlorophenoxyacetic acid with 2% sucrose. Maximum biomass was achieved after 10 days together with maximal uptake of sucrose. A higher lignan content and better growth was found when the cells were cultivated in darkness instead of light (0.12 and 0.06 % of dw, respectively). The production of lignans was monitored by HPLC. Our data indicate that podophyllotoxin and deoxypodophyllotoxin are the main lignans in suspension cultures of *L. campanulatum*, *L. narbonense* and *L. strictum* provide an interesting system for the production and may serve as a tool to study the biosynthesis of lignans.

A screening for antitumor activity of methanolic extracts prepared from sus-pen-sions, using the MTT-dye reduction assay with SKW-3 human leukemic cells, HDMYZ48, MCF772 and HL-60 demonstrated that the most active material was the one with the highest concentration of podophyllotoxin.

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B246 Seasonal variations in the amount of secondary metabolites in field cultivated *Gentiana lutea* L.

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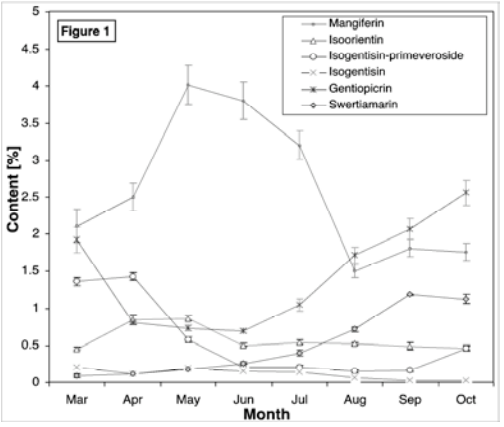
In both folk and modern medicine, the roots of *Gentiana lutea* (Gentianaceae) are very popular. Because of their bitter taste, extracts are also used in the production of vines and liquors. In addition, recent studies pointed out to a very inte-resting chemical composition of the aerial parts (1). A large demand for the drug resulted in a drastic decrease of the spontaneous resources in nature. Considering those facts, we established an experimental field (16 x 20 m, consisted of 16 parts of 4 x 5 m) on mountain Suvobor (750 m) using GA_3 germinated seeds of *G. lutea*. The amount of xanthones (mangiferin, isogenti-sin/gentisin, isoge-ntisin-primveroside/gentisin-pri-mveroside), flavonoids (isoorientin) and secoiri-doids (gentiopicrin, swertiamarin) was measured in the aerial parts and roots during 8 months (March-October) in the fourth year of cultivation. Quantification was done by HPLC (1). In the springtime, mangiferin dominated in the aerial parts (4.02% in May) while gentiopicrin was the most abundant compound in autumn (2.56% in October), (Figure 1). In the roots, two maximums of accumulation of gentiopicrin were recorded (April and October), similar as in the plants growing on natural locality (1).



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B247 Molecular and chemical analyses of *Aconitum* species

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The genus *Aconitum* is a complex systematic group and modern studies show different taxonomic positions with regard to the criteria adopted to distinguish species and subspecies (1-3). In this work we have therefore examined populations characterised by yellowish (*A. vulparia*) and blue-violet flowers (*A. paniculatum*, *A. napellus* subsp. *tauricum* (Wulfen) Gáyer, and *A. napellus* subsp. *neomontanum* (Wulfen) Gáyer) present in Northern Italy, in order to evaluate the potential of some molecular (obtained using RAPD analysis) and chemical (flavonoid compounds) markers as diagnostic features. For the RAPD analysis fourteen primers were selected, providing fifty one polymorphic bands. The phenogram based on UPGMA clustering of Jaccard coefficient revealed a clear division between yellowish and blue *Aconitum* plants and inside this second group *A. paniculatum* is clearly separated from all populations belonging to *A. napellus* group.

The flavonol compounds identified are 3 and/or 7-*O*-glycosides, represented by derivatives of kaempferol and quercetin and the sugar moieties include glucose, rhamnose, arabinose, and galactose, and disaccharides based on these sugars. Each species and subspecies analysed showed a characteristic flavonoid profile.

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B248 Myricetin, quercetin and kaempferol during the physiological development of guava (*Psidium guaja - va* L.)

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Studies about the medicinal properties of guava leaf infusions (*Psidium guajava* L. Myrtaceae), support its antimicrobial and spasmolytic effect and the relation of these activities with the presence of flavonols as quercetin, kaempferol or myricetin (1). The dynamics of their accumulation was followed during one year.

Myricetin, quercetin and kaempferol were quantified in leaves and fruits of different physiological phases by HPLC (2). The results showed that the leaves presented the highest concentrations of these flavonols: 4612 mg/Kg in September and 2804 mg/Kg in November, followed by the buds with concentrations ranging from 2380 mg/Kg to 368 mg/Kg. In the set fruit, the range varied from 1608 mg/Kg to 964 mg/Kg and in the last stage of fruit growth the range varied from 914 to 1004 mg/Kg.

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B249 Quality of medicinal plant products prepared by supercritical fluid extraction

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Supercritical fluid extraction is a technique which is now widely applied to prepare extracts of vegetable origin. The standardization of these extracts is essential for the beneficial and safety use in medicine and food industry. The effect of sample preparation, extraction conditions and separation circumstances was examined on the yields and composition of bioactive constituents. The raw materials were extracted with carbon dioxide in a high pressure apparatus equipped with 5 L volume extractor vessel. The effect of two factors (pressure and temperature) were investigated in the ranges of 150-450 bar and 40-60ºC by designed experiments in the 3^2 full factorial design. Model plants were: *Taraxacum officinale* Wiggers et Weber, *Cnicus benedictus* L., *Chrysanthemum parthenium* L. (Beruh), *Vitex agnus castus* L., *Origanum vulgare* L., *Thymus serpyllum* L. and *Thymus vulgaris* L. Terpenoids were analysed and identified by TLC, TLC-densitometry, HPLC and comparison with authentic standards.

It was concluded that the products gained by supercritical fluid extraction have to be considered as special raw materials of plant origin. Using optimal pressure and temperature as parameters the sesquiterpene-γ-lactone parthenolide, the triterpenoids: taraxasterol and β-amyrin, as well as the phytosterols were quantitatively extracted and selectively enriched in supercritical extracts. We could produce extracts containg 16.0-24.5 % β-amyrin from dandelion, 9.1 % triterpenes from chaste tree, 1.1-2.0 % parthenolide from feverfew and products rich in phytosterols from chaste tree, thyme and dandelion. The yields of these bioactive constituents in the supercritical extracts were several times higher than those corresponding extracts wich were prepared by traditional methods. To characterise the extractability of the individual compounds an index was introduced.

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B250 Co-extraction of herbal substances: an ancient but successful procedure brought into focus

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A great number of marketed herbal medicinal products consists of combinations of two to four plants. Many of them are established medicines with experimentally and clinically proven efficacy and safety. In the last decades a scientific theory took hold, that such phytopharmaceuticals should and could be reliably produced only by mixing individual extracts. However, the time proven way of preparing such medicines by simultaneous extraction results in reasonably uniform preparations and has in its favour the experience of generations of pharmacists in Europe as well as in Asia (1). There are technical advantages in the successful method of co-extraction. For example, turbidity and precipitation problems in liquid preparations can be circumvented (2).The proper labeling, stating the solvent, the drug to extract ratio and the internal mass-relation of the drugs specified, is certainly a basic requirement. In the declaration of a co-extract the drug/extract ratio (DER) has in addition to consider the total mass of all drugs extracted in relation to the extract equivalent, thus including the internal composition of the drugs. For example: “1 tablet contains x mg dry co-extract (DER y:1) of drug A, drug B and drug C (a:b:c), extraction solvent…” respectively “100 ml solution contains z ml fluid co-extract (DER 1:u) of drug A, drug B and drug C (a:b:c), extraction solvent…”. The quality of each drug should be controlled according to a pharmacopoeial-type specification before extraction and in the resulting co-extract. The identity and purity of the co-extract is checked by usual analytical techniques such as fingerprint-chromatograms. In the final herbal medicinal product one marker substance for the active ingredient, which is by definition the co-extract, suffices to quantify the correct amount of material (batch-specific recovery). The use of co-extraction is nothing new. Good examples are the co-extraction of herbal medicinal tea mixtures and the very detailed and rigidly defined procedures of the Chinese pharmacopoeia in the preparation of its extracts. A close look at formulations of the RF and DRF still in use also strengthens the argument of using co-extraction rather than mixing individual extracts in combinations.

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B251 Study on the influence of some operational parameters on the extraction of the total phenolics and arbutin of the leaves of *Arctostaphylos uva-ursi* L.

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The Uva-ursi, *Arctostaphylos uva-ursi* (L.), Spreng. (Ericaceae) has a place not only in the old herbals but also in the modern pharmacopoeias. Due to its powerful astringency as well as to the diuretic and urinary antiseptic effects, it has a traditionally value for kidney diseases and treatments of inflammatory disorders of the urinary tract (1). The wide range of uva-ursi leaves' active constituents is well-documented (2).
As stated, the highest hydroquinone contents of the uva-ursi leaves was shown following 12 hours cold extraction, while the four hour long cold extraction gave the lowest tannin content (3). However, there is very little information on the optimised extraction procedure. In order to obtain an optimised aqueous extract of the uva-ursi leaves, with regards to its contents of total phenolics and arbutin, it has been studied as a function of the operational parameters: hydromodule (solid: liquid) from 1:20 to 1:400; extraction temperature (from 25°C to 90°C); time of extraction (from 1 to 3 h) and particle size (from 150 to 750 μm) during the extraction process. The efficiency of the total phenolics and arbutin extractions were followed by Folin-Ciocalteu and Pharm. Eur. 3 methods respectively. The establishment of the main and of the interactive influence of the studied operational parameters on the system: Uva-ursi leaves and aqueous medium performed using mathematical statistic design - 2^4 full factorial experiment. The analysis of linear regression equations obtained, ascertained that the most influential examined factor of the extraction of the total phenolics is the particle size of the sample. The distinct influences of the temperature on the extraction of the total phenolics and arbutin have been shown only during the longer phase contact. However, the hydromodule (solid:liquid) shows only a slight influence. These results will be used for the process optimization using the Box-Wilson method.

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B252 Experimental modelling and optimisation of phenolic acids extraction from *Thymus moesiacus* subsp. *moesiacus*

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Sample extraction is often the time consuming step in a chemical analysis of a plant material. Now, the experimental modelling of the extraction process, as a more rapid and efficient way of optimising the procedure of analyte extraction, is being paid a considerable attention.
Phenol acids (PA), particularly the benzoic and cinnamic acids, are widely distributed in *Thymus* L. species as free acids or as esters or ethers (1). We have established an optimal condition for PA ultrasonic extraction in a nitrogen atmosphere, from an unmodified matrix (aerial part of *Thymus moesiacus* subsp. *moesiacus*) using 30 % aqueous methanol as an extraction medium. The process of PA extraction was studied as a function of the following operational parameters: hydromodule (solid:liquid) of 1:20 to 1:100; extraction temperature (from 25°C to 75°C); time of extraction (from 30 to 60 min) and particle size (from 150 to 750 μm). The efficiency of the total PA extraction was followed by potentiometric determination of acidity expressed as contents of the caffeic acid. The first stage was determination of the main and of the interactive influence of the studied operational parameters in the system *T. moesiacus* subsp. *moesiacus* aerial part and 30% methanol, and it was performed using mathematical statistic design (2^4 full factorial experiments). The second stage was optimisation of the experimental modelling of PA extraction, performed using the Box-Willson method. The analysis of the results of the experimental modelling ascertained that the 1:33 hydromodule in the 30% methanolic aqueous medium during 38 min of extraction at 36°C with the particle size of 150 μm is the optimised condition for PA extraction from the examined sample.

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B253 Contribution of medicinal plants to the daily intake of various toxic elements in Catalonia, Spain

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The objective of this study is to determine the levels of arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) in the most consumed species of medicinal plants in Catalonia, Spain, to calculate the daily intake of these elements and to assess potential health risk. The concentrations of As, Cd, Hg and Pb were determined by ICP-MS in 115 samples of 15 different species. The Hg levels in all samples were under the detection limit (1,2).

Daily intakes of As, Cd and Pb were calculated according a consumption of two herbal teas of 2 g per day (4 g/day) and assuming that the whole content of metal from plants is ingested with the herbal tea (3,4).

The median and range intakes (µg/day) through medicinal plants consumption were: As 0.56 (0.36-303.8), Cd 0.2 (0.16-6.84) and Pb 2.72 (0.12-39.44). The daily intake of As, Cd and Pb through medicinal plants was found to be about 0.2%, 1% and 5% of dietary daily intake of these elements, respectively.

The risk assessment was based on the figure of total daily intake of metals (through diet + herbal teas) in the worst scenario (assuming that the total content of metals from plants was in the herbal tea and taking the most contaminated sample). According to the FAO/WHO provisional tolerable weekly intake (PTWI), the total daily intake of As, Cd and Pb should not mean potential toxic effects. However due to the high variability of the found data, a systematic control might be advisable.

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B254 Development of a biosensor on cyanogenic glycosides: production of recombinant cyanidase from *Pseudomonas stuzeri*

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Because of their content of cyanogenic glycosides, many medicinal and food plants are toxic for man (1, 2). If plant material containing cyanogenic glycosides is disintegrated, cyanide will be liberated by the action of different enzymes. Especially in developing countries, chronic poisoning by cyanogenic plants is a serious problem. Since probably more than 2500 plant species and also some insects contain cyanogenic glycosides, a rapid and precise method for the determination of these compounds should be developed. A biosensoric system based on a potentiometric electrode and the enzyme cyanidase [EC 3.5.5.1] seems to be an effective analytical method for this class of substances and a promising alternative to an ion-selective cyanide electrode. This biosensor should be used for screening purposes as well as for the quality control of cyanogenic medicinal and food plants.

The key-step in the development of such a sensor is the selection of a suitable cyanidase, which has been previously reported for bacteria. A gene encoding cyanidase could be located in the bacteria *Pseudomonas stuzeri* and was overexpressed in *E. coli*. To allow a sufficient purification by affinity chromatography, the protein was modified by a hexa-histidine tag. The recombinant enzyme was fully characterized. V_{max} was found at 500 µmol CN-/mg/min and the temperature optimum was at 30°C. A broad pH optimum between pH 7 and pH 8 makes the enzyme highly suitable for biosensoric application. The construction of a biosensor based on a miniaturized semiconductor-structure is under current investigation.

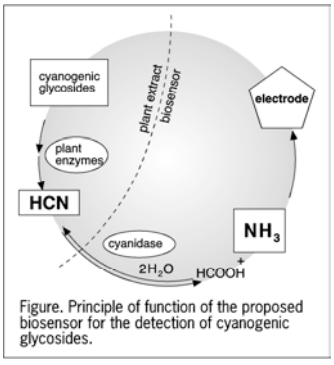

Figure. Principle of function of the proposed biosensor for the detection of cyanogenic glycosides.

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B255 Is water activity a good indicator of degradation in *Echinacea purpurea* L. root glycerites?

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Echinacea purpurea is one of the best-selling phytomedicines in the United States (1). It is used as an immunostimulant and appears to act by stimulating T-cell production, phagocytosis, lymphocytic activity, cellular respiration, and inhibiting hyaluronidase activity (2). Caffeic acid derivatives, polysaccharides and alkamides are thought to be among the compounds responsible for this complex mode of action (3).

Recent work has shown that caffeic acid derivatives are highly susceptible to enzymatic degradation and oxidation in glycerite, aqueous and hydroalcoholic solutions (4-7). Bauer (8) reported a rapid decline in cichoric acid content in 50% hydroalcoholic preparation after 5 days. As the occurrence of degradation is related to the amount of water present in the extract, might it be possible to predict the rate of degradation using the water activity value?

We performed an experiment with glycerin extracts of *Echinacea purpurea* roots with different levels of water (20, 40, 50, 60, 80, 100%) and measured the caffeic acid derivatives as well as dodeca-(2*E*,6*Z*,8*E*,10*E*)-tetraenoic acid isobutylamide, the major alkamide, over 3 weeks. The results showed that in extracts with water activity (a_w) levels between a_w=0.500-0.700, corresponding to 60-80% glycerin, the cichoric acid and the dodeca-(2*E*,6*Z*,8*E*,10*E*)-tetraenoic acid isobutylamide started to degrade. This degradation increased with the water content. Caffeic and caftaric acid increased as a result of the hydrolysis of cichoric acid.

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B256 Microbial decontamination by γ-irradiation method decreases the mucilage content in *Althaea radix*

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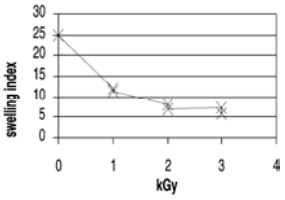
Althaeae radix (obtained from *Althaea officinalis* L., Malvaceae) contains mucilages (about 25-35%) with galacturonic acid, glucuronic acid and rhamnose as major components (1). Due to its high mucilage content, Althaeae radix is often subject to microbial contamination. γ-irradiation is a very effective, economic, fast and practical method for decontamination of herbal medicines, allowed by the WHO and Ph.Eur. (2).

We tested eight samples of Althaeae radix having a determined microbial contamination. All samples had a maximal swelling index of 25. First two samples were not treated with γ-rays and represented controls, whereas samples 3 and 4 were treated with 1 kGy, samples 5 and 6 with 2 kGy and samples 7 and 8 with 3 kGy, respectively. Cobalt-60 served as a source of γ-irradiation. After the irradiation, the same samples were analysed for microbiological decontamination and thereafter, they were also tested for the swelling index according to the methods and requirements of the Ph.Eur. 4th 2002.

The γ-irradiation dosis of 1 and 2 kGy degraded mucilages that were analysed by the method of swelling index. The results show that the swelling number decreases after the irradiation treatment, while the herbal drug is not being microbiologically decontaminated. The decontamination was achieved by the irradiation dosis of 3 kGy. However, the swelling index still decreases below 10, which is the Pharmacopean requirement for Althaeae radix. The difference between 3 kGy irradiated Althaeae radix and the control samples was visualised histochemically by staining the mucilages with the methylene blue. Since it has a destructive effect on the mucilage content of radix, the γ-irradiation method can not be prescribed for decontamination of this herbal drug.



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B257 Solubilization of St. John's wort constituents by micelles and their characterization by DOSY experiments

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Preparations based on extracts of St. John's wort are widely marketed for treating mild to moderately severe depressive disorders and other health conditions such as anxiety and sleep disorders (1). In this study the optimisation of technological and pharmaceutical aspects of dried commercial extract of St. John's wort and some purified fractions enriched in active constituents were evaluated by the formation of micellar systems.

Flavonols and naphthodianthrones are quite polar derivatives but their water solubility is very scarce; phloroglucinols are lipophilic and completely not water-soluble constituents. In addition, hypericins and hyperforins are not stable with regard to heat and light (2). Micellar solutions of sodium dodecyl sulfate (SDS), Tween 80 and 6-O-ascorbyl-octanoate (ASC8) (3) were chosen because they are not toxic, accepted food additives and not expensive. In addition ASC8 has the same antioxidant properties of vitamin C and acts as a powerful radical scavenger in aqueous as well as non-aqueous media. Solubility of flavonoids was increased about two times by ASC8, three times by SDS and two times by Tween 80. Solubility of hyperforins was increased about seven times by ASC8, seven times by SDS and four times by Tween 80. Solubility of hypericins was increased about two times by ASC8, four times by SDS and two times by Tween 80. Characterization of micellar systems was performed by DOSY (4). This technique involves the accumulation of several multidimensional NMR spectra in which one dimension displays the apparent diffusion coefficient. Information from DOSY complements those available from other tools such as quasi-elastic light scattering and small-angle neutron-scattering used for studying aggregated systems and for giving information on the sizes of the aggregate.

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B258 Improvement of the technological and biopharmaceutical characteristics of kava-kava preparations by cyclodextrins

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Kava-kava (*Piper methysticum* G. Forster) has been widely used to improve stress disorders, nervous tension and restlessness (1). In the last three years unexpected, high liver toxicity has been reported in several patients and it was related to kavalactones mainly depending on their dosage. Thus, to improve the technological and biopharmaceutical characteristics of preparations based on kava-kava and decrease the dosage of kavalactones, the interaction of constituents with some cyclodextrins (CDs) were evaluated using isolated kavalactones and a commercial extract. CDs are cyclic oligosaccharides that can interact with a wide variety of drugs and the formation of such a supramolecular complex can influence the dissolution rate and the drug's aqueous solubility and therefore, improve their bioavailability profile (2). In this attempt, β-cyclodextrin and γ-cyclodextrin were selected for the dimensions of the internal cavity because they fit to accommodate kavalactones. Complexes were obtained by freeze-drying using 1:1 ratio and characterized using different physico-chemical methods based on differential scanning calorimetry (DSC) and NMR spectroscopy. The formation of supramolecular complexes was related to the degree of unsaturation of the different kavalactones and to the size of the cyclodextrin cavity. Thus, among kavalactones, yangonin (a full unsaturated kavalactone) showed the highest percentage (82%) of interaction with γ-cyclodextrin while 7,8-dihydrokavain (a full saturated kavalactone) the highest percentage (96%) of interaction with β-cyclodextrin.

Acknowledgements: This work was supported by MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Rome).

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B259 Formulation, preparation and physicochemical quality control of vaginal suppositories containing volatile oils of *Allium sativum* and *Zataria multiflora*

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Vulvo-vaginal candidiasis is a kind of vaginitis, caused most commonly by *Candida albicans*. Due to the high incidence of this infection and also because of the antimicrobial activity of *Allium sativum* L. and *Zataria multiflora* Boiss, the preparation of vaginal suppositories containing their volatile oils, has been considered as the main objective of this study.

In the first step of this investigation, volatile oils of *A. sativum* (A.s.) and *Z. multiflora* (Z.m.) were prepared by hydro-steam and steam distillation techniques, respectively, and their constituents were then separated and identified by gas chromatography mass spectrometry. The results showed the presence of diallyldisulfide (30.1%), diallylsulfide (10.1%), diallyltetrasulfide (3.8%) and allylmethyltrisulfide (3.5%) in A.s. oil and thymol (51.5%), carvacrol (30.4%) in Z.m. oil. The initial studies indicated approximately equivalent antimicrobial effects for A.s. and Z.m. oils. However, in order to mask the unpleasant odor of A.s. oil, vaginal suppositories were prepared by Z.m. oil and a mixture of A.s. and Z.m. oils in the ratio of 4:1. The minimum inhibitory concentration of each oil was determined by the methods of paper disk plate and cup plate. The results showed a range of 1.8 – 8.0 µl of oil per ml of solvent or suppository base, depending upon the method and the presence of either one or both volatile oils.

In the second step, physicochemical quality control tests including appearance, content uniformity, hardness, disintegration time, assay (using biological method) and weight variation, were performed on various formulations of polyethylene glycol based vaginal suppositories, containing 30 µl of Z.m. oil per mL of the base and 15 µl of A.s. oil and Z.m. oil mixture per mL of the base. From the stability point of view, it was found that storage at temperatures higher than 4ºC could cause a decrease in the concentration of the active ingredients.

In conclusion, vaginal suppositories with appropriate antimicrobial activities and desired stability could be prepared with low amounts of either Z.m. oil alone or a mixture of A.s. and Z.m. oils.