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Book of Abstracts

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Monday, 26th August, 2018 - Pavilion - 12:00 - 17:30

Young Researchers' Workshop

Anti-Influenza Virus Activity of Lanostane Triterpenes from Polypores

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The constant increase of viral resistance and the limited number of effective antiviral agents emphasizes that the current portfolio of anti-influenza drugs needs extension^[1]. To identify new anti-influenza agents from nature, locally grown European polypores belonging to the polyphyletic group of Agaricomycetes (Basidiomycota) were collected and identified via rDNA ITS phylogenetic analyses^[2]. Fruit bodies from ten species were collected and mycochemically investigated, i.e. *Fomes fomentarius*, *Fomitopsis pinicola*, *Ganoderma lucidum*, *G. applanatum*, *Gloeophyllum odoratum*, *Ischnoderma benzoinum*, *Laetiporus sulphureus*, *Phellinus robustus*, *Piptoporus betulinus*, and *Trametes gibbosa*. The generated ethanol extracts were screened in a cytopathic effect (CPE) inhibition assay against H3N2 influenza virus A/Hong Kong/68 (HK/68) in MDCK cells. Especially the extract of *G. odoratum* dose-dependently inhibited the CPE with an IC₅₀ of 15.0 µg/mL^[3]. A fluorescence-based neuraminidase (NA) inhibition assay excluded that the antiviral activity was based on the inhibition of the surface protein NA. HRESIMS, 1D and 2D NMR spectroscopy were used for the identification of eight isolated lanostane triterpenes with two novel natural compounds and three undescribed so far for *G. odoratum*. The most potent activity was determined for trametenolic acid B against HK/68 and the 2009 pandemic H1N1 strain A/Jena/8178/09 with IC₅₀s of 14.1 and 11.3 µM, respectively. Additionally, this compound was able to bind to cell-free viruses and to neutralize their infectivity in a plaque reduction assay.

[1] U. Grienke, M. Schmidtke, S. von Grafenstein, J. Kirchmair, K. R. Liedl, J. M. Rollinger, *Nat Prod Rep* 2012, 29, 11-36.

[2] P. Dresch, D. A. MN, K. Rosam, U. Grienke, J. M. Rollinger, U. Peintner, *AMB Express* 2015, 5, 4.

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A metabolomic approach to investigate metabolization of a medicinal plant extract in the gastrointestinal tract in-vitro

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Introduction

Metabolization of medicinal plant extracts in the gastrointestinal tract may lead to the formation of new active compounds and could therefore be highly relevant for the explanation of the mode of action. STW-5 is a well-known liquid fixed 9-herb combination, which is effective in treating functional dyspepsia and irritable bowel syndrome (IBS) ^[1]. It was pre-digested by a static in-vitro digestion method and thereafter incubated with human gut bacteria to mimic human digestion and microbial fermentation in the gastrointestinal tract.

Method

Two concentrations of STW-5 were predigested in-vitro according to the InfoGest consensus method ^[2]. After protein precipitation, all samples were analyzed by UHPLC-HRMS and data were processed with Compound Discoverer 2.1 (Thermo Fisher Scientific). In order to assess metabolization during in-vitro digestion, UHPLC chromatograms of samples taken after simulated digestion were compared to respective STW-5 dilutions. For bacterial fermentation, fecal samples of one donor were anaerobically incubated with InfoGest predigested STW-5 as well as with non-predigested STW-5 (two concentrations). Samples were taken after 30min, 4h and 24h of incubation, and analyzed by UHPLC-HRMS. After data processing, peak areas were compared to respective STW-5 dilutions incubated with PBS-buffer only.

Results

The majority of STW-5 main constituents were not significantly changed by in-vitro digestion. Microbial fermentation, however, led to fast metabolization of the major compounds detected in STW-5, such as flavonoids, caffeic acid derivatives, and triterpene glycosides.

References

- [1] Kelber O, Bauer R, Kubelka W. Phytotherapy in Functional Gastrointestinal Disorders. Dig Dis 2017; 35 Suppl 1: 36–42
- [2] Verhoeckx K, Cotter P, López-Expósito I, Kleiveland C, Lea T, Mackie A, Requena T, Swiatecka D, Wichers H. The Impact of Food Bioactives on Health. Cham: Springer International Publishing; 2015

Discovery of new GPBAR1 agonists by combined in silico - in vitro screening

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The G protein-coupled bile acid receptor (GPBAR1) is a possible new drug target for the treatment of metabolic and inflammatory diseases, including type 2 diabetes. Previously, it has been shown that a number of herbal remedies and natural small molecule metabolites were able to activate this receptor and thus may exert antidiabetic and anti-inflammatory effects. The fast-forward identification of GPBAR1 agonists is highly relevant, as metabolic diseases like type 2 diabetes have become an epidemic, and novel treatments are urgently needed ^[1].

The aim of this study was to generate reliable prediction models for the fast-forward assessment of GPBAR1 activating natural compounds. A cheminformatics workflow including ligand-based pharmacophore- and shape-based virtual screening was set up. The workflow was validated theoretically and employed for the prospective virtual screening of open-source and in-house molecular databases. From 34 chemically diverse virtual hits subjected to experimental testing, 14 were confirmed as GPBAR1 activators, including new scaffolds from natural and synthetic origin. Triterpenes previously isolated from the South African tree *Burkea africana* ^[2] and coumarins from the middle-eastern spice *Ferula assa-foetida* ^[3] showed activities comparable to the endogenous ligands chenodeoxycholic acid and lithocholic acid.

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[3] Rollinger JM, Steindl TM, Schuster D, Kirchmair J, Anrain K, Ellmerer EP, Langer T, Stuppner H, Wutzler P, Schmidtke M. *J Med Chem*. 2008 Feb 28;51(4):842-51

Bioactive polysaccharides from *Codonopsis pilosula*

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Radix *Codonopsis*, the root of *Codonopsis pilosula* (Franch) Nannf, *C. pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen, and *C. tangshen* Oliv, is a traditional herbal medicine in Asian countries, with various pharmacological effects, such as heart protection, lowering blood pressure, anti-ulcer, etc ^[1]. It was used as the replacement of ginseng because of the similar pharmacological activities. Polysaccharide is one of the major contributors to the biological activity. In our group, we aimed to find out different bioactive polysaccharides from three species of radix *Codonopsis*. *C. pilosula* from Gansu province, *C. pilosula*

Nannf. var. modesta (Nannf.) L. T. Shen from Sichuan and C. tangshen from Guizhou province, were collected and the polysaccharides were hereby obtained. Three crude polysaccharide fractions and more than 20 purified polysaccharide fractions were obtained from those three species using anion exchange chromatography and gel filtration. The structural elucidation of some of the polysaccharide fractions were finished and the results indicated both neutral fractions and acidic fractions are present in all three species. The crude polysaccharides showed activity in regulating intestinal flora and immunity. Complement fixing activity, antioxidant activity, prebiotics activity ^[2] and immunomodulating activity in intestinal epithelial cells were performed in all purified polysaccharide fractions obtained. The results from biological tests indicated that polysaccharides from different species of Codonopsis showed different activity. The further study aimed structure-activity relationship analysis would be performed.

Reference:

[1] Zou YF , Chen XF, Malterud KE, et al. Carbohydr Polym 2014; 113: 420-429.

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Nanostructured Lipid Carriers Of Silymarin: Design, Characterization and In Vitro Studies

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Silymarin is the main constituent of the extract from seeds of *Silybum marianum* L. Gaertn. and has been used for decades as hepatoprotectant. Recently it has been proposed to be beneficial in type 2 diabetes patients. However, silymarin is a poorly water soluble drug with limited oral bioavailability ^[1]. Nanoparticles-based delivery systems resulted a promising strategy to resolve these issues. In this work, nanostructured lipid carriers (NLCs) with two different lipid combinations were prepared through emulsion/evaporation/solidifying method ^[2]. Stearic acid:Capryol 90 (NLCs-SA) and cetyl palmitate:Lauroglycol 90 (NLCs-CP) were selected as lipid mixtures. Brij S20 was used as surfactant. The optimized formulations were of 210-270 nm in particle size and with zeta potential between -31 and -35 mV. Surface morphology was determined by TEM. NLCs showed good encapsulation efficiencies (80% for NLCs-SA and 93% for NLCs-CP). No degradation phenomena were observed in simulated gastrointestinal fluids. Storage stability of suspensions and lyophilized products was also investigated. Glucose and mannitol were selected as cryoprotectant agents for freeze drying and it was observed that glucose was superior to mannitol especially with regard to the physical stability. About 60% of silymarin was released in 24 h in PBS. In vitro permeation experiments

with artificial membranes and Caco-2 cells revealed that both NLCs enhanced the permeation of entrapped compound. Cellular uptake studies indicated that active processes are involved in the internalization of developed formulations.

Acknowledgments: Financial support was granted by Ente Cassa di Risparmio di Firenze 2016, financing n. 2016.0802.

References:

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Fusicoccane diterpenes from *Hypoestes forskalii* (Vahl) R.Br.

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In order to obtain bioactive secondary metabolites, we investigated the chemical constituents of *Hypoestes forskalii* Vahl. Roem. & Schult. (Acanthaceae) roots. *H. forskalii* is a perennial herb widely distributed in many African countries as well as in the Arabian peninsula^[1]. The whole plant is popularly used as natural insecticide, moreover fresh leaves are used by locals to accelerate the healing process. Various biological properties have been attributed to the plant, including antiplasmodial, antifungal, antiparasitic and cytotoxic properties^[2]. Several *Hypoestes* species have been chemically investigated before establishing that diterpenes belonging to the class of fusicoccane, isopimarane, and labdane are the main skeleton synthesized by these species^[3].

Fusicoccanes characterized by a complex 5-8-5 dicyclopenta-cyclooctane nucleus, are powerful phytotoxins in *Hypoestes* and different fungi species^[3].

Eleven new fusicoccane diterpenes were isolated from the dichloromethane extract of *H. forskalii* roots by CC, MPLC and RP-HPLC chromatography, together with two known lignans. All structures were elucidated on the basis of NMR and HR-MS spectroscopic methods. Additionally, the affinity of isolates towards Hsp90, one of the most promising targets for anti-cancer therapy, was tested by surface plasmon resonance. Results

demonstrated that 17-hydroxy-hypoestenone efficiently interacted with the protein (K_D 0.32 mM). The study of the activity of 17-hydroxy-hypoestenone by means of a panel of biochemical and cellular approaches was achieved. 17-hydroxy-hypoestenone showed an antiproliferative activity with an EC_{50} of 26 μ M on the HeLa cell line by inducing a G2/M cell cycle block through the down-regulation of pCdc2 protein levels.

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[2] Pettit G R, Goswami A, Cragg GM, Schmidt M, and Zou JC. *J Nat Prod* 1984, 47: 913-16

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Preparation of B-ring saturated nontoxic protoflavones as novel xanthine oxidase inhibitors

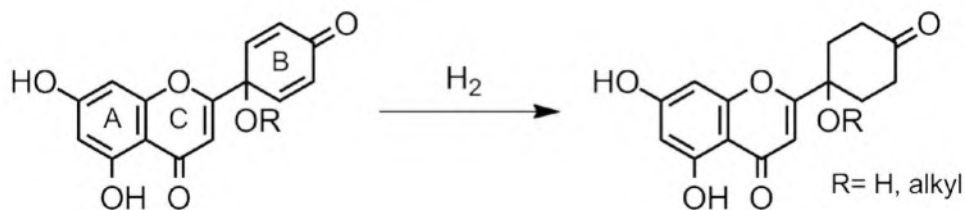
Máté Vágvolgyi ¹, Gábor Girst ^{1,2}, Zoltán P. Zomborszki ¹, Ferenc Fülöp ², Sándor B. Ötvös ², Attila Hunyadi ¹

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Protoflavones express a non-aromatic, p-quinol B ring that confers them a unique 3D structure among natural flavonoids. Anticancer activity of these compounds is intensively studied, but their cytotoxicity is a strong limitation for investigating their other possible uses ^[1].

Our group identified protoapigenone 1'-O-propargyl ether as the first non-planar flavonoid that is a strong inhibitor of xanthine oxidase (XO), however its cytotoxicity was similarly strong. XO plays a crucial role in the pathomechanism of gout, and it also significantly contributes to oxidative stress ^[2]. Recently, our group reported a selective method to saturate the protoflavone B ring of protoapigenone and its 1'-O-butyl ether through continuous flow chemical hydrogenation to obtain non-cytotoxic derivatives with the rare, naturally occurring tetrahydroprotoflavone moiety ^[3].



In our present work, we aimed to investigate the structure-activity relationships of differently substituted 1'-O-alkyl tetrahydroprotoapigenone derivatives concerning their potential to inhibit XO. We utilized both batch and continuous flow chemical approaches for the preparation. With an aim to investigate the isotope effects on the XO-inhibition protoapigenone analogues, selective deuteration of the B ring was also achieved.

Our preliminary studies on the obtained derivatives indicated that saturation of the p-quinol B ring could not only eliminate the cytotoxicity, but it could also further increase the XO-inhibition potential of protoflavones as compared to their parental compounds. Related pharmacological studies are currently ongoing, and results of these will also be presented.

[1] Hunyadi et al., *Phytochem Rev* (2014) 13 69-77.

[2] Hunyadi et al., *Tetrahedron Letters* (2013) 54 6529-6532.

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Acknowledgments

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Phytochemical analysis of antioxidant extracts obtained from the aerial parts of Greek *Cistus* species

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The majority of published research on *Cistus* species focuses on the aromatic resin labdanum, which demonstrates high antimicrobial activity. However, the aerial parts of rockrose plants have been used in Greek traditional medicine as anti-inflammatory agents for rheumatism and skin diseases^[1]. This study emphasizes on the exploration of the phenolic content and anti-oxidant potential of five species (*C. monspeliensis*, *C. salvifolius*, *C. parviflorus*, *C. creticus* spp *creticus*, *C. creticus* spp *eriocephalus*) belonging to the Greek flora. After successive extraction with *n*-hexane, ethylacetate, methanol and water by Accelerated Solvent Extraction, all extracts were tested for their phenolic content and antioxidant capacity by TPC, ABTS and DPPH assays and their chemical profile was compared with HPTLC.

The methanolic extract of *C. monspeliensis* as well as methanolic and ethylacetate extracts of *C. parviflorus* were further analysed. However, the challenge faced for the isolation of the bioactive compounds was the presence of high amount of tannins. For this purpose, two methodologies were implemented, FCPC chromatography using a three-phase solvent system (*C. monspeliensis*) and adsorption resin XAD-4 (*C. parviflorus*), resulting to removing of tannins and fractionation of the extracts. Further purification

of compounds was accomplished with MPLC, Sephadex CC and prep-TLC. Their purity and identity was confirmed by NMR and LC-MS. The abovementioned process afforded to the isolation of over 40 compounds, among them 5 novel metabolites and over 15 known compounds isolated for the first time from the genus *Cistus*.

To the best of our knowledge, this is the first thorough phytochemical investigation of the methanolic extracts of the aerial parts of Greek *C. monspeliensis* and *C. parviflorus*, demonstrating simultaneously that the enrichment of crude extracts in polyphenols increase their antioxidant properties, while wise-chosen methodologies simplify the separation of natural compounds from complex matrices.

[1] Demetzos CN, et al. *Fitoterapia* 1990; 61(5): 439-442;

Cytotoxicity of natural halimane and labdane diterpenes by mitochondrial dysfunction in human lung cancer cells

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Medicinal plants from the *Plectranthus* genus are a valuable source of natural products such as diterpenes^[1,2]. Mitochondrial dysfunctions (MD) have been associated with several pathologies such as ROS increase and uncontrolled *Mycobacterium tuberculosis* (Mtb) replication^[3,4]. The electrochemical gradient produced by mitochondria generates the mitochondrial membrane potential (MMP), which is a key parameter for evaluating MD^[4]. Previous works have reported the cytotoxicity of *Plectranthus* diterpenoids and pointed their potential against *M. smegmatis*^[2,5]. In this work, diterpenoids from *P. ornatus* Codd. (previously isolated^[1,2]) were evaluated for their cytotoxicity and for the mechanisms of cell death associated with MD in A549 cell line (humanlung adenocarcinoma). One halimane HAL: (11R*,13E)-11-acetoxyhalima-5,13-dien-15-oic acid and two labdane diterpenes PLEC: Plectornatine C and the MRC: 1,6-di-O-acetylforskolin:1,6-di-O-acetyl-9-deoxyforskolin (1:1). Our pioneer study showed that only HAL and PLEC were cytotoxic (IC₅₀=60 and 8 µg.mL⁻¹, respectively). Also, the ROS level observed after 1h was significantly higher (p < 0.01) with HAL

and this effect was maintained for up to 48h. All compounds were able to decrease mtDNA copy number, but only HAL increased MMP and exhibited DNA damage of 8.78 lesions per 10 kb (ND5 region). In conclusion, HAL has a cytotoxic effect associated with MD on lung cancer cells, that may be further evaluated on the Mtb replication mechanism. Additional studies are ongoing, aiming to unveil the coexistence of tuberculosis and lung cancer that has remained controversial, since the middle of the 19th century.

[1] Rijo P., et al. *Magn Reson Chem* MRC. 2005;43(7):595–8.

[2] Rijo P., et al. *Biochem Syst Ecol.* 2007 Apr;35(4):215–21.

[3] Duan L., et al. *J Immunol.* 2002;169(9):5181–7.

[4] Sakamuru S., et al. *Methods Mol Biol.* 2016;1473:17–22.

[5] Burmistrova O., et al. *J Nat Prod.* 2013;76(8):1413–23.

Scale up fermentation of *Streptomyces* sp. (CA-129531 strain) - a potential source of bioactive compounds with skin-whitening activity

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In the frame of MICROSMETICS EU project 56 potential candidate actinobacteria strains of global biodiversity were selected to be studied using OSMAC strategy. In total 614 extracts were produced and cell-free bioassays have been used for the evaluation of their skin-whitening bioactivity. Among the initial 614 extracts, the actinomycete strain CA-129531 of the genus *Streptomyces*, originated from Martinique and cultivated in the fermentation medium DNPM, exhibited the most promising skin-whitening activity (i.e. tyrosinase inhibition). This activity was confirmed in cell-based assays in mouse melanocytes (B16F10 cell line). Preliminary study of this strain, including the scale up fermentation in 1lt and bioguided fractionation of the active EtOAc extract, led to the isolation and identification of trichostatin A and trichostatic acid. Scale-up process optimization was performed in a bioreactor Biostat C+ (total volume 30 kg). Direct liquid/liquid extraction of the culture medium with EtOAc was performed and the yield of trichostatin A was measured in different experiments performed under modified media. The highest amount of this marker was observed when using media DNPM*3, a modified formulation of DNPM that used as carbon source dextrose in agreement with requirements of cosmetic legislation. After confirming the tyrosinase inhibition in cell-free assay of the EtOAc extract of this broth (IC₅₀=63.27µg/ml), preparative HPLC was used in order to bioguided isolate the active compounds. The full set of spectroscopic data (HRMS and NMR) was recorded for all

active isolated compounds in order to unambiguously elucidate their structure, while it was also identified the main metabolite responsible for this activity ($IC_{50}=3.07\mu\text{g/ml}$). Therefore, this extract can be considered as potential candidate for industrial development and this optimized small-scale process can then be transferred to pilot scale following established scale-up strategies in cosmeceutical industry.

Monday, 27th August, 2018 - Bibo BallroomAB - 14:00 - 16:00

Invited & Short Lecture - Session 1-1

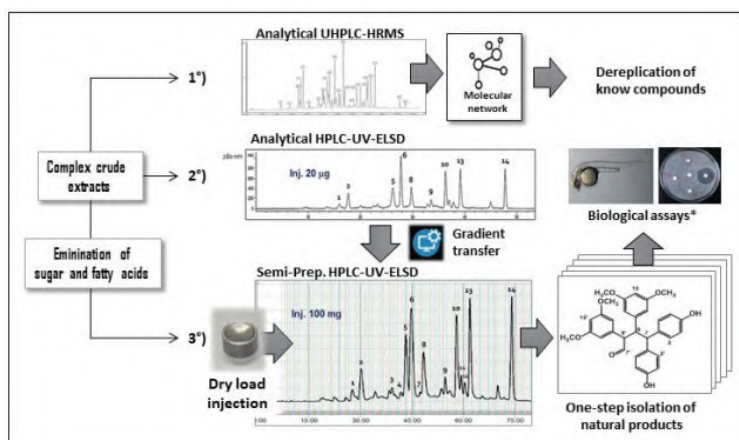
Session 1-1-IL-01:

Efficient Natural Products Isolation with Dry Load Injection of Extracts and Software Assisted Gradient Transfer at the Semi-Preparative HPLC scale

Emerson Queiroz, Abdulelah Alfattani Alfattani, Adlin Afzan, Laurence Marcourt, Davide Righi, Davy Guillaume, Jean-Luc Wolfender

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The isolation of compounds from crude extracts represent a key step common to all natural product investigations. Obtaining pure natural products at sub-milligram scale is crucial for assessment of their bioactivity profile and their full de novo structure determination when they cannot be dereplicated with LC-MS profiling methods. Ideally, this process should be performed in one-step directed from the crude extracts. For this semi-preparative chromatographic resolution should be improved and perfectly match the performance obtained by analytical HPLC . One of the limits of this method is the amount of extract that can be purified. Usually the sample is solubilized in organic solvent and injected on column through rheodyne-loop. Since the solubility of extracts is limited, high solvent volume are needed significantly compromising the resolution of separation. Moreover, when large amounts of the extract are injected there is a risk of system over pressure. A new approach was developed in order to overcome these issues. The procedure involve an initial treatment of the extract to eliminate compounds from the primary metabolism such as sugars. The separation is first optimized on an HPLC analytical column, and the conditions are transferred by software to a semi-preparative column packed with the same stationary phase to ensure similar selectivity. The sample is then injected by dry load on a dedicated cell that affords loading of hundreds of milligrams of crude extract without loss of resolution or overpressure. ELSD detection provide a semi-quantitative view of the fractions content and post-column ¹H-NMR and UHPLC-HRMS profiling of all fractions gathered in 2D matrices gave a precise view of the separation and possible co-elution issues. The approach was successfully used for the fractionation of several complex natural extracts from plant and microbial origin. In all cases, an important number of pure compounds was obtained in one single step.



Session 1-1-IL-03:

Effective Components of Chinese Medicines and Their Biocatalytic Modification

Min Ye

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Traditional Chinese medicines (TCMs) usually have complex chemical composition, and unknown effective components. Meanwhile, some of their bioactive compounds suffered from poor bioavailability. These have been major hurdles for the modernization of TCM. Herein, we integrated chemical investigation, in vivo metabolism, and bioactivity evaluation to systematically elucidate the effective components of licorice. We then used biocatalytic approaches to improve the pharmacological properties of these compounds.

Firstly, 311 compounds were detected in licorice by using comprehensive 2DLC/MS. In total 257 compounds were isolated from three medicinal *Glycyrrhiza* plants (*G. uralensis*, *G. glabra*, *G. inflata*), including 44 new compounds. Chemical difference among the three species was revealed by quantifying 151 secondary metabolites in 95 samples using LC/MS/MS. Successively, 247 compounds were screened using 11 cell- and enzyme-based bioassays. A number of isoprenylated phenols were found as bioactive compounds for the first time. Furthermore, the in vivo metabolites of licorice were analyzed, where 90 metabolites were detected and 55 were monitored by multi-component pharmacokinetic analysis. Finally, compounds with potent bioactivity and high plasma exposure were confirmed for their bioactivities using animal models. For example, glycycomarin showed hepatoprotective effect to chronic and acute alcoholic liver injuries, and isoangustone A induced apoptosis in colorectal cancer cells principally by inducing mitochondrial outer membrane permeabilization.

Biocatalytic approaches were then used to modify the structures of effective components. MhGT1, a glycosyltransferase from *Mucor hiemalis*, catalyzed the regio- and stereo- specific glycosylation of

isoprenylated flavonoids. YjiC, a glycosyltransferase from *Bacillus subtilis*, catalyzed the 3-O-glycosylation of glycyrrhetic acid. These modifications improved the bioavailability of the bioactive components.

The study established the chemical–bioactivity correlation for the popular herbal medicine licorice, and found a number of bioactive compounds related to its clinical application. It set a good example for the discovery of bioactive natural products, and for the modernization of TCM.

Session 1-1-SL-04:

Natural Products Chemistry Bloopers and Blunders in the 21st Century

Mahmut Miski

Istanbul University, Faculty of Pharmacy, Dept. of Pharmacognosy, Istanbul, Turkey

We are living in an era of the wonders of analytical instrumentation, advanced computational chemistry tools and biochemical discoveries that facilitates the isolation, identification of structure and biological activities of natural products. In particular, development of the high resolution NMR spectroscopy equipment beyond 800 MHz resolution, sophisticated NMR techniques, rapid X-ray crystallographic analyses, advanced molecular modeling and docking applications performing on high performance data workstations have revolutionized natural products chemistry and drug discovery studies. In contrast to these developments, examination of natural products papers published in respected journals, compound structures published in well known chemical reference sources, books and/or electronic structure databases reveal many mistakes in the published structures. Many of these structural mistakes suggest the lack of proper stereochemistry training of author(s) and referees. Although these mistakes may not cause any serious issues frequently, nevertheless, when a prospective scientist decides to take the published structure and extrapolate its promising biological activity by synthesis and computational chemistry studies where a misrepresented structure could result in the loss of precious resources and time. Several examples of such misrepresented structures and their possible causes will be discussed during the presentation.

Session 1-1-SL-05:

Afriplex Green Rooibos Extract and Post-Ischemic Heart Recovery in Diet-Induced, Pre-Diabetic Rats

Sybrand Smit¹, Barbara Huisamen^{1,2}, Erna Marais¹, Rabia Johnson²

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Cardiovascular diseases are the leading cause of death worldwide and exacerbated by the presence of risk factors such as obesity and insulin resistance. *Aspalathus linearis* (commonly known as rooibos) is a plant indigenous to South Africa containing bioactive phenolic compounds, including aspalathin, implicated for its strong antioxidant potential.

In this study, 60 mg/kg/day Afriplex green rooibos extract (GRT), containing 12% aspalathin, was used as an intervention in treating cardiometabolic disease risk factors induced by a 16-week high-fat, high-caloric diet (HCD) in Wistar rats. Rats received GRT for 6 weeks before the hearts were excised and mounted on a working heart perfusion apparatus and its recovery post-ischemia/reperfusion injury investigated.

HCD led to increased body weight ($368.6 \pm 6.5\text{g}$ vs $394.4 \pm 6.1\text{g}$ = $\Delta 9.6\%$) and visceral adiposity (14.5g vs 23.1g = $\Delta 59.0\%$) compared to age-matched control animals. Pre-ischemia, GRT supplementation improved the heart's total work performance (a measure of pressure power and kinetic power) compared to both untreated controls (13.5 ± 0.4 vs 11.6 ± 0.3) and untreated HCD (11.70 ± 0.3 vs 10.3 ± 0.2). Post-ischemia, HCD hearts had poorer coronary output recovery after reperfusion ($60.2 \pm 2.6\%$ vs $69.2 \pm 2.6\%$), and GRT treatment restored coronary capacity ($72.7 \pm 3.7\%$) to that of controls. Infarct size was also highest in the HCD group ($45.9 \pm 2.2\%$ vs $35.9 \pm 1.4\%$ in controls), with GRT treatment protecting against the damage incurred (HCD: $24.3 \pm 1.8\%$; controls: $26.6 \pm 1.0\%$). Pre-ischemia, HCD hearts presented with low expression of PKB while GRT treatment elevated both PKB and GSK-3 β , phospho-p38 and phospho-ERK. During ischemia, GRT treatment further elevated GSK-3 β levels in both control and HCD hearts. In early and late reperfusion, low levels of GSK-3 β , PKB and JNK in HCD hearts were reversed by GRT treatment. It furthermore increased ERK activity during late reperfusion in the HCD hearts.

This preliminary study shows that GRT supplementation improves heart recovery post-ischemia in rats with elevated risk for cardiovascular disease by improving pro-survival and anti-inflammatory signalling.

Session 1-1-SL-06:

Using cytological profiling to discover chemical probes from the traditional Chinese medicines against Parkinson's disease

Yun-jiang Feng, Chao Wang, George Mellick

Griffith Institute for Drug Discovery, Griffith University, Brisbane, Australia

Lack of understanding of the molecular mechanism underlying Parkinson's disease (PD), a neurodegenerative disorder, has hindered the development of novel therapeutics with disease-modifying effect in parkinsonian patients. The emerging field of chemical biology which utilizes diverse small molecules especially natural products to explore the biological processes offers a "probe to drug" approach to address this problem. TCMs contain a huge reservoir of bioactive small molecules that contribute to the efficacy in

thousands of years' clinical practice. Such molecules working together in similar or different modes of action may hold the key to unlocking the mystery of PD biology ^[1].

A integrated methodology combining cytological profiling platform with NMR-guided isolation has been developed to capture and characterize the bioactive components from traditional Chinese medicines (TCM) ^[2,3]. A number of natural products have been identified from selected TCMs with characteristic phenotypes against cells harvested from PD patients. We will discuss the identification and cytological profiles of the compounds, as well as their potential as chemical probes for in-depth interrogation of the signaling pathways implicated in PD, and thus support future neuroprotective or disease-modifying drug discovery.

[1] Tang C, Ye Y, Feng Y, Quinn R. *Natural Product Reports* (2016), 33, 6-25.

[2] Wang C, Yang X, Mellick G, Feng Y. *ACS Chemical Neuroscience*, (2016), DOI 10.1021/acschemneuro.6b00245

[3] Wang D, Feng Y, Murtaza M, Wood S, Mellick G, Hooper J, Quinn R. *J. Natural Products* (2016), 79(2), 353-361.

Session 1-1-SL-07:

Pushing the limits of natural products chemistry: multi-omic definition of a novel target for an anti-mycobacterial isolated from hops (*Humulus Lupulus*)

Rafael Baptista ¹, David M. Fazakerley ¹, Sumana Bhowmick ¹, Manfred Beckman ¹, Les Baillie ², Luis A.J. Mur ¹

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² *DA, Aberystwyth, United Kingdom 2 School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, Cardiff, Wales, UK, CF10 3NB, Cardiff, United Kingdom*

Tuberculosis (TB) is as a major global threat mostly due to infection with antibiotic resistant forms of *Mycobacterium tuberculosis*, the causal agent of the disease [1]. The resulting accelerated quest for new anti-mycobacterial agents, has produced targets but with significant toxicity or physical and chemical problems. Given this many are re-examining natural products for their structural, functional and stereochemical diversity that provide unique scaffolds for further drug optimisation. To aid such efforts the targets for natural products should be defined as an essential step for the development of an anti-tubercular drug [2] .

We report the isolation of a compound from hops (*Humulus lupulus*) with significant in vitro anti-mycobacterial activity (10 µg/mL). Whole-genome sequencing of compound-resistant mutants of *M. smegmatis* identified a major target. Non-targeted metabolomic by flow infusion ionisation electrospray high-resolution mass spectrometry (FIE-HRMS), lipidomic analysis and in silico studies were employed to independently validate the putative target and obtain valuable insights into the mode of action of this antibiotic. This novel use of

FIE-HRMS [3] also permitted the characterisation of metabolic differences between mycobacteria treated with several known antibiotics (first and second line against tuberculosis) and controls. Specific metabolic patterns to pathways leading to cell arrest were defined for our compound that were discrete from any known antibacterial mechanisms. This established the importance of a metabolomic strategy in drug target discovery that could be more widely applied. Beyond this, our definition of a novel target will enable the optimisation or de novo synthesis of new anti-tubercular drugs, avoiding the resistance problems linked to known targets.

[1] WHO. 2016 Global tuberculosis report.

[2] Baptista R, et al. Future Med Chem. 2018.

[3] Baptista R, et al. Sci Rep. 2018.

Monday, 27th August, 2018 - The Chapel - 14:00 - 16:00

Invited & Short Lecture - Session 2-1

Session 2-1-IL-01:

Localization and Organization of Scopolamine Biosynthesis in *Duboisia myoporoides* R.Br.

Laura Kohnen, Oliver Kayser

TU Dortmund University, Biochemical and Chemical Engineering, Dortmund, Germany

Tropane alkaloids (TAs), especially hyoscyamine and scopolamine, are important precursors for anticholinergic and antispasmodic drugs. Hyoscyamine and scopolamine are currently obtained at commercial scale from hybrid crosses of *Duboisia myoporoides* x *Duboisia leichhardtii* plants. In this study, we present a global investigation of localization and organization of TA biosynthesis in a *Duboisia myoporoides* R. Br. wildtype line. The tissue-specific spatial distribution of TAs within *D. myoporoides* is presented, including quantification of the TAs littorine, 6-hydroxy hyoscyamine, hyoscyamine, scopolamine, and additionally, hyoscyamine aldehyde as well as scopolamine glucoside. Scopolamine (14.77 ± 5.03 mg g⁻¹), and to a lesser extend hyoscyamine (3.01 ± 1.54 mg g⁻¹) as well as 6-hydroxy hyoscyamine (4.35 ± 1.18 mg g⁻¹) (Fig.1), are accumulated in leaves during plant development with the highest concentration of total TAs detected in six-month old plants. Littorine, an early precursor in TA biosynthesis, was present only in the roots (0.46 ± 0.07 mg g⁻¹). During development, the spatial distribution of all investigated alkaloids changed due to secondary growth in the roots. Gene transcripts involved in early stages of TA biosynthesis, *pmt*, *tr-I*, and *cyp80f1* were found to be most abundant in the roots. In contrast, the transcript encoding hyoscyamine 6 β - hydroxylase (*h6h*) was highest in the leaves of three-month old plants. This investigation presents the spatial distribution of biochemical components as well as gene expression profiles of genetic

factors known to participate in TA biosynthesis in *D. myoporoides*. The results of this investigation may aid in future breeding or genetic enhancement strategies aimed at increasing the yields of TAs in these medicinally valuable plant species ^[1].

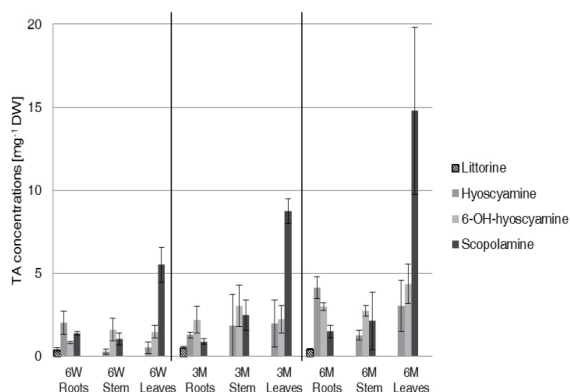


Fig. 1: Concentration of various tropane alkaloids (mg/DW) in *Duboisia myoporoides* roots, stem and leaves

[1] Kohnen, KL et al. 2017: Localization and Organization of Scopolamine Biosynthesis in *Duboisia myoporoides* R. Br. *Plant Cell Physiol*; 59:107-118

Session 2-1-IL-02:

Enantiomeric Lignanamides from the Fruits of *Cannabis sativa* and Their Neuroprotective Activity

Zhi-Hong Jiang, Guo-Yuan Zhu

Macau University of Science and Technology, Macau, China

Hemp seed, the fruits of non-drug variety of *Cannabis sativa* L., have been used as food and traditional medicine for thousands of years. Previous phytochemical studies of *C. sativa* led to the identification of cannabinoids, phenolics, fatty acids, sterols, and lignanamides. In this study, 30 lignanamides were isolated from hemp seed by a series of column chromatographic methods monitored by UPLC-TOF-MS analyses. Their structures were elucidated by NMR and HRMS spectroscopic data, calculated ECD, and CD experimental analyses. Among them, compounds 1 and 2 are novel lignanamides possessing an unique 6/5/5/5 tetracyclic benzo-angular triquinane

skeleton. Compound 3 and 4 have unusual spirolic lignan skeleton featuring with a 1-nitrospiro[4.5]deca-6,9-dien-8-one moiety. Compound 5 possesses an unprecedented neolignan skeleton fused by a caffeic acid and a coumarin group. Subsequent chiral resolution of 1–3 was performed by chiral HPLC separation to afford

three pairs of enantiomers (+)- and (–)-1, 2 and 3. Pharmacological targeting of endoplasmic reticulum (ER) stress pathway is emerging as a therapeutic strategy for several neurodegenerative diseases. Pretreatment with compounds 1 and 2 significantly reduced the ER stress-induced neuron cytotoxicity on PC12 cells and SH-SY5Y cells. Our results suggested that lignanamides from the fruits of *C. sativa* may be developed as a new type of neuroprotective agent.

Session 2-1-IL-03:

Network pharmacology to unveil the biological basis of traditional Chinese medicine

Shao Li

MOE Key Laboratory of Bioinformatics and Bioinformatics Division, BNRIST / Department of Automation, Tsinghua University, Beijing, China

Traditional Chinese Medicine (TCM) is characterized by its holistic thinking in treating a patient as a holistic system, and the use of various herbal formulae in restoring the balance of human body. The holistic philosophy and patient-tailored treatment of TCM shares a lot with the key idea of current systems biology and precision medicine. However, it is very challenging to unveil the biological basis of TCM by current reductionist "one gene, one target, one drug" approach due to the complexity of both the chemical compositions of herb formula and the biological systems of patients. To understand TCM from a systems perspective, we proposed a new concept and established a new approach of "network target, multi-component drug", which emphasizes that treating the biomolecular networks underlying diseases or TCM Syndrome as a therapeutic target to determine the systematic intervention such as herbal formula. Then, by using artificial intelligence methods, we created a set of algorithms to predict the network target underlying disease and herbal formula. The effectiveness of the network target approach has been demonstrated by the follow-up clinical and experimental investigations. Typically, we constructed for the first time the metabolism-immune imbalanced molecular network underlying Cold / Hot syndrome, a pair of thousand-year-old concepts in TCM, and validated the network in a cohort of patients with gastritis and precancerous lesions. Furthermore, we identified the novel actions of compounds, network regulation mechanisms, and modern indications of Cold / Hot herbs in fighting inflammation and cancer from a classic "Nourishing Yin" formula, Liu-wei-di-huang, a "Clearing away Hot" formula, Qing-Luo-Yin, and various herbs. Taken together, the "network target"-based network pharmacology approach is expected to offer bright prospects in understanding TCM, narrow the gap between Eastern and Western medical practices, and help achieve the TCM-based systems and precision medicine in such a Big Data and Artificial Intelligence era.

Session 2-1-SL-04:

Straightforward Process Design for the Identification and Isolation of bioactive Natural Products using Thin-Layer and Preparative Chromatography

Petra Lewits, Michaela Oberle, Michael Schulte

Merck KGaA, Darmstadt, Germany

Thin-Layer Chromatography (TLC) is still by far the most important technique to isolate and identify novel bioactive compounds from natural sources. It is easy to use, offers the possibility to detect specific groups of compounds through post-chromatographic derivatization or staining and can be combined with identification tools such as mass spectrometry and bioautography. In addition, the separation conditions, which have been optimized on the TLC plate can be transferred directly to the preparative liquid chromatography process to obtain the compound of interest in larger amounts.

We will show examples for the straightforward process design starting with the identification of bioactive compounds using TLC, optimizing the separation by appropriate choices of the solvent system and the transfer of the separation to preparative liquid chromatography. After the transfer from the TLC plate to the column we will also show the possibilities of scaling up preparative liquid chromatography to a ton-scale.

We will show the advantages of bioautographic tests on TLC plates to identify unknown compounds with specific physiological activities, e.g. antibiotic or hormone-like activity.

Session 2-1-SL-05:

Effects of Cranberry extract (*Vaccinium macrocarpon*) on bacteria in human urine: an ex vivo study

Birte Scharf ¹, Jandirk Sendker ¹, Said Rabbani ², Ulrich Dobrindt ³, Beat Ernst ², Andreas Hensel ¹

¹ *University of Muenster, Institute of Pharmaceutical Biology and Phytochemistry, Muenster, Germany*

² *University of Basel, Institute of Molecular Pharmacy, Basel, Switzerland*

³ *University of Muenster, Institute of Hygiene, Muenster, Germany*

The initial step of urinary tract infections (UTIs), frequently caused by uropathogenic *Escherichia coli* (UPEC), is the adhesion of bacteria to urothelial cells. Extracts from Cranberry fruits (*Vaccinium macrocarpon* Ait.) have long been associated with the prevention of UTIs. Within ex vivo experiments in two studies, a time-dependent, significant inhibition of bacterial adhesion of UPEC NU14 and UTI89 to human T24 bladder cells was achieved using urine from 20 volunteers after consumption of standardized cranberry extract (CE). This effect was independent of urine pH. To investigate the effect of the Cranberry metabolites on UPEC transcriptome analysis of UTI89 by Next Generation Sequencing was performed. The transcriptome of

bacteria grown in urine collected after 7 days of cranberry intake indicated no relevant differences compared to that obtained from bacteria grown in control urine. To elucidate whether the antiadhesive activity of the urine after CE ingestion is based on a direct interaction with bacterial type 1 adhesins (FimH), in vitro FimH assay was performed by recombinantly expressed FimH-lectin domain. The tests showed that the urine after CE consumption inhibits FimH more strongly than the control urine. LC-MS/MS study of the urine was performed to correlate Cranberry-related metabolites with the antiadhesive effects via multivariate data analysis. In order to be able to examine urine by LC-MS / MS, it was pretreated by solid-phase extraction (SPE). The correlation of the investigated SPE eluates with the biological data indicated substances that could not be identified as cranberry-associated metabolites. Currently, the investigation of the preliminary eluates is in progress.

Session 2-1-SL-06:

The piperine derivative LAU398 reduces cholesterol uptake in a human intestinal cell line

Verena Hiebl ¹, Laurin Wimmer ², Limei Wang ¹, Simone L. Latkolik ¹, Angela Ladurner ¹, Marko D. Mihovilovic ², Verena M. Dirsch ¹

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Atherosclerosis is a chronic disease of the arterial wall, characterized by the development of lipid-rich plaques. Cholesterol absorption and excretion pathways are therefore of great interest. Since the intestine is a major interface in cholesterol turnover, it represents a pharmacologically interesting target tissue.

To assess the influence of natural products on cholesterol uptake, a human intestinal cell model utilizing a monolayer of polarised Caco-2 cells has been established. After treating the monolayer with compounds (48 h), micelles with radioactively labelled cholesterol are added for two hours. Then, cells are lysed and intracellular cholesterol is quantified.

A major regulator of cholesterol homeostasis is the nuclear receptor LXR (liver X receptor).

Several cholesterol transporters, like ABCA1 (ATP-binding cassette transporter A1), are regulated by this nuclear receptor. A piperine derivative (LAU398, Figure 1) upregulated ABCA1 in THP-1-derived macrophages (EC₅₀ of 4.1 µM) and increased cholesterol efflux (EC₅₀ of 1.1 µM) in this cell line. Hence, LAU398 was further investigated in cholesterol uptake experiments in intestinal cells. At 10 µM, LAU398 significantly reduced the uptake of cholesterol into Caco-2 cells. Interestingly, LAU398 also significantly enhanced capsaicin-induced currents through TRPV1 channels at 100 µM in oocytes ^[1]. However, co-administration with a TRPV1 or LXR antagonist could not reverse the effects of LAU398 on cholesterol uptake.

In conclusion, LAU398 significantly reduces cholesterol uptake in Caco-2 cells and enhances cholesterol efflux from THP-1-derived macrophages. The exact implications of TRPV1 or nuclear receptors in these effects remain to be elucidated.

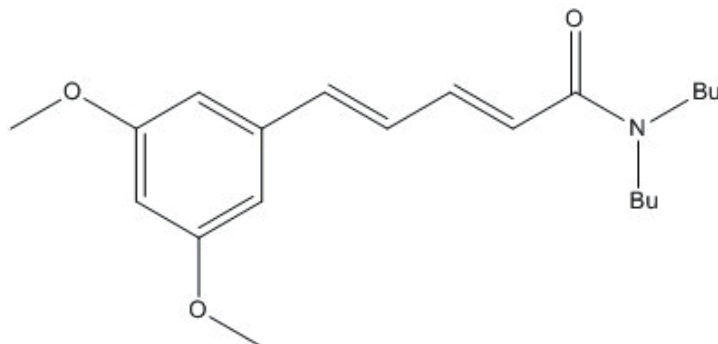


Figure 1: Structure of the piperine derivative LAU398.

[1] Wimmer, L, Schonbauer, D, Pakfeifer, P, Schoffmann, A, Khom, S, Hering, S, and Mihovilovic, M D. *Org Biomol Chem* 2015; 13: 990-994

Session 2-1-SL-07:

Archazolid, a biogenic vacuolar-type ATPase inhibitor, augments cancer cell adhesion to endothelial cells by accumulating extracellular collagen

Betty Luong ¹, Iris Bischoff ¹, Rolf Müller ², Dirk Menche ³, Robert Furst ¹

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The vacuolar-type H⁺-ATPase (v-ATPase) is a proton pump acidifying intracellular compartments, among them endosomes, lysosomes or clathrin-coated vesicles, in order to regulate various cellular functions. The v-ATPase has increasingly gained attention as a promising therapeutic target for the treatment of diseases such as osteoporosis or cancer during the last decades. In this study we focus on the in vitro effects of the natural product archazolid, a specific inhibitor of the v-ATPase first isolated from the myxobacterium *Archangium gephyra*, in the vascular endothelium. The endothelium represents an important barrier regulating cell-cell interactions. In particular with regard to pathophysiological conditions, we focused on endothelial/cancer cell interactions in vitro. We found that archazolid concentrations up to 1 nM did not impair the viability of human umbilical vein endothelial cells (HUVECs) but successfully inhibit the v-ATPase resulting in an abrogated lysosomal acidification. In in vitro experiments the adhesion of the breast cancer

cell line MDA-MB-231 to an archazolid-treated HUVEC monolayer was significantly increased. Of note, the involvement of prominent cell adhesion molecules playing a crucial role in cancer cell adhesion onto the endothelium could be excluded. Interestingly, blocking of beta1 integrins on MDA-MB-231 cells abrogated the archazolid-induced cancer cell adhesion onto HUVECs. In line with these results, we found that archazolid increased the expression of collagen on the endothelial surface, the favored ligand of beta1 integrin on MDA-MB-231 cells. This effect was accompanied by a significantly reduced expression and activity of the lysosomal proteinase cathepsin B. Over-expression of cathepsin B reversed the pro-adhesive effect in endothelial cells. This study shows for the first time that the archazolid-induced v-ATPase inhibition in endothelial cells modifies the interactions between endothelial and cancer cells by a pro-adhesive phenotype. Further approaches considering v-ATPase inhibitors as therapeutic strategy for the treatment of pathophysiological conditions should take our findings into account.

Monday, 27th August, 2018 - Bibo BallroomC+Room1 - 14:00 - 16:00

Invited & Short Lecture - Session 3-1

Session 3-1-IL-01:

Discovery of synergistic combination in herbal medicines and their regulatory mechanism

Ping Li

China Pharmaceutical University, Nanjing, China

Herbal medicines have played an important role in health maintenance and disease treatment for thousands of years. The discovery of artemisinin is one of the most brilliant examples of therapeutic research of herbal medicines (HMs). However, multi-bioactive components and their synergistic actions are the characteristics of HMs. Accompanying with hot discussions on developing multidrug therapy for multi-gene diseases, herbal medicines are receiving increasing attention worldwide. Therefore, we proposed a new theory “bioactive equivalent combinatorial components (BECCs)”, as well as a strategy to identify BECCs or synergistic combination of HMs. Furthermore, the regulatory mechanism of the synergistic combination was further investigated. The BECCs system has been applied to TCM formula such as Compound Danshen, which improved the internationalization of TCM research.

Session 3-1-IL-02:

Quality of herbal materia prima: the curious case of Saint John's wort

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² *Division of Herbal and East Asian Medicine, Department of Life Sciences, University of Westminster, London, United Kingdom*

Saint John's wort (*Hypericum perforatum* L.-HP) is well-known for its traditional medicinal use. It is currently licensed in Europe as a medicine or as a THR product, but unregulated products remain accessible through secondary means. A previous study (Booker et al. 2018), looking into the quality of HP products (including THRs and food supplements), highlighted how significantly different their chemical content could be.

The study aimed at continuing the assessment of HP products quality by looking into the variability of the starting material and using the results to pinpoint where regulations can be improved.

A combination of NMR metabolomics and Pharmacopoeia-based HPTLC fingerprinting was used to investigate the chemical profile of 86 *Hypericum* samples (77 *H. perforatum*) collected in 14 countries.

Significant variations between species' chemical fingerprints were found but limited significant differences within samples collected in distinct geographical locations. A previously reported "Chinese Saint John's wort" fingerprint, featuring a number of anomalies, when compared to the European Pharmacopoeia standard (Booker et al. 2018), has been matched to 100% of the samples coming from China. One of the characteristics has been positively identified as avicularin, which was also found in 50% of the specimens from Spain, but in no other sample. Rutin was not present in 38% of samples. Lower flavonoids concentration was detected in materials of commercial origin and were generally associated with a higher content of woody stems and possibly higher sample age.

This study reinforces the knowledge that unregulated herbal products quality is not reliable and a way to insure safety needs to be found. The importance of appropriately choosing, sourcing and handling *materia prima* is highlighted, alongside the need for a regulatory system that is informed about the natural variability of the raw material.

References:

Booker et al. *Phytomedicine* (2018);40:158-164

Session 3-1-IL-03:

Identification of bioactive diterpenoids from medicinal herbs

Yang Ye

State Key Laboratory of Drug Research and Natural Products Chemistry Department, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Natural products play an important role in new drug discovery. As one of the most important types of natural

compounds, diterpenoids have attracted considerable attentions due to their diversified structures and a spectrum of bioactivities such as anti-bacterial, anti-malarial, anti-inflammation, anti-cancer and so on. Searching for bioactive diterpenoids is still a hot topic in the field of natural products.

A systematic investigation of unique diterpenoids from a medicinal herb *Podocarpus nagi* has been carried out, resulting in the isolation of 44 nor-diterpenoid lactones with 16 of them being new, especially a novel binor-diterpenoid with dehydroxylation at C-7. Notably, most of isolated diterpenoid lactones displayed remarkable potency to increase LDL uptake in HepG2 cells at a concentration of 5 μ M. In vivo study further showed that nagilactone B, an abundant diterpene existing in this plant, dose-dependently decreased TC, TG, and LDL levels in high fat diet induced dyslipidemic hamsters. Mechanism study indicated nagilactone B exerted lipid-lowering effect by elevating LDLR mRNA and protein level. It is the first time to report anti-hyperlipidemic activity for naturally occurring diterpene lactones in vitro and in vivo. Nagilactone B represent a new type of compounds with promising lipid-lowering activity.

Session 3-1-SL-04:

Validation and implementation of an HPLC-DAD method for the simultaneous quantification of chlorogenic acid, flavonoids and flavonolignans in *Cecropia* species

Andrés Rivera Mondragón¹, Kenn Foubert¹, Sebastiaan Bijttebier¹, Tania Naessens¹, Deborah Custers¹, Catherina Caballero-George², Sandra Apers¹, Luc Pieters¹

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² Group of Pharmaceutical Research, Centre of Innovation and Technology Transfer, Institute of Scientific Research and High Technology Services (INDICASAT-AIP), Building 219, City of Knowledge, Panama, Panama

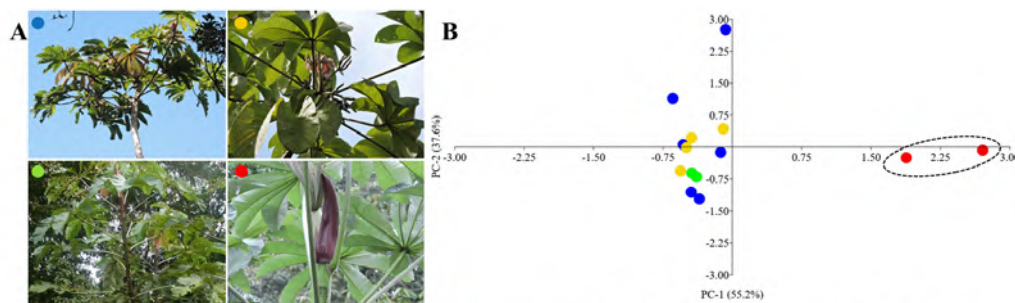


Figure 1. *Cecropia* species collected in Panama (A). Principal component analysis (PCA) on *Cecropia* species leaves: Score plot (B).
● *C. obtusifolia*, ● *C. peltata*, ● *C. insignis* and ● *C. hispidissima*. Photographs taken by Rodolfo R. Flores J.

Several plant species of the genus *Cecropia* (Urticaceae) are traditionally used in Latin America as anti-diabetic, anti-hypertensive and anti-inflammatory agents [1] and are commercially available as food supplements. However, until now there is a lack of quality control for these herbal products.

An HPLC-DAD method was validated for the quantification of chlorogenic acid (CA), total flavonoids (TF) and flavonolignans (FL) following the ICH guidelines [2]. Fourteen authentic *Cecropia* leaf samples collected in Panama and three commercial products were analyzed. The chemical composition of different species was compared by principal component analysis (PCA).

Validation parameters of the method for CA, TF and FL were satisfactory. Specificity was defined for each analyte. An adequate linear response was obtained for all analytical curves ($r^2 > 0.999$). All relative standard deviations (%RSD) for repeatability and intermediate precision were lower than 2 and 5%, respectively. Accuracy, expressed as the recovery, varied from 98 to 102% for all concentration levels. The limit of detection and limit of quantification were on the scale of nanogram per milliliter (ng/mL).

Analysis of the samples showed qualitative and quantitative differences in their chemical composition. Both analytical results and PCA revealed chemical similarities for *C. obtusifolia*, *C. peltata*, *C. insignis* and *C. hololeuca*, which contains mostly C-glycosyl flavones and C,O-glycosyl flavones, while *C. hispidissima* showed to be different due to the high content of O-glycosyl-flavonols (Fig. 1).

This study serves as a useful tool for the quality control of herbal supplements of *Cecropia* species and the subsequent interpretation of their related pharmacological effects.

References

- [1] Rivera-Mondragón A, Ortiz OO, Bijttebier S, Vlietinck A, Apers S, Pieters L, Caballero-George C. *Pharm Biol* 2017; 55:1500-1512.
- [2] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Text and Methodology Q (R1), 2005; 1-13.

Session 3-1-SL-05:

Quality Control Parameters for Jiaogulan (*Gynostemma pentaphyllum*) Production

Iftekhar Ahmed ¹, James De Voss ¹, Mark Christen ¹, Joanne Blanchfield ¹, Hans Wohlmuth ^{1,2,3}, David Leach ^{2,4}, Kerry Bone ²

¹ School of Chemistry & Molecular Biosciences, The University of Queensland, Brisbane, Australia

² Integra Healthcare, Brisbane, Australia

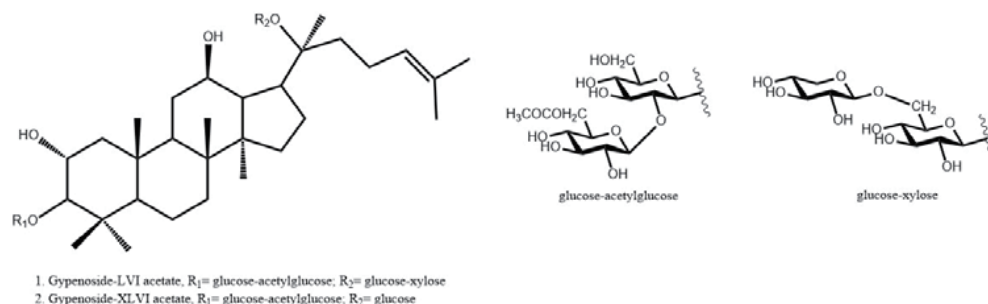
³ National Institute of Complementary Medicine, Western Sydney University, Sydney, Australia

⁴ School of Health & Science, Western Sydney University, Sydney, Australia

Gynostemma pentaphyllum (Thunb.) Makino has significant whole herb medicinal applications including its demonstrated anti-obesity effects. Patented heat and steam processing of the natural extract has increased the levels of gypenosides L & LI and damulins A & B, the latter two causing, at least in vitro, a significant increase in AMP-activated protein kinase activity. Such a patent-protected, refined extract is at odds with published monographs (1,2) of the whole herb. Insight into the identity of key marker compounds in the natural extract, especially saponins and flavonoids, are required to ensure quality and efficacy of any subsequent processed natural product.

The work undertaken and presented here encompasses identification of all major flavonoids and key saponins by UPLC-MS and NMR, in an authenticated voucher specimen (Medicinal Plant Herbarium, Southern Cross University (Accession No: PHARM160054). Plant materials surveyed include a range of retail Jiaogulan teas as well as various trade herb and extracts available in Australia. The identified saponins include the gypenosides XLVI and LVI and two novel acetylated derivatives and new damulin analogues. Eleven fully identified glycosylated flavonoids of kaempferol, quercetin and isorhamnetin provide a definitive

fingerprint profile. Previously reported constituents (3) including ginsenosides Rb1 and Rd, with the flavonoids vitexin and ombuoside are not present in the authenticated specimen. Given the wide geographic distribution of this plant species, it is likely that chemotypic variation is at play here and more detailed survey data is needed to define this variation for future quality control to be rigorously implemented.



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- [2]. Gynostemmati Herba, Hong Kong Chinese Materia Medica Standards (HKCMMS), (http://www.cmd.gov.hk/hkcmms/vol5/Vol5_pdf_English/D_Monographs/14-Gynostemmati%20Herba.pdf).
- [3]. Xie, Z, Shi, H and Yu L (2013). HPLC/MS Fingerprinting Techniques for Quality Control of Gynostemma pentaphyllum (Thunb.) Makino samples. DOI: 10.1021/bk-2013-1138.ch003.

Session 3-1-SL-06:

Integrated FIA-FT-ICR MS and LC-HRMS metabolomics as novel holistic workflow for quality control of Extra Virgin Olive Oil (EVOO)

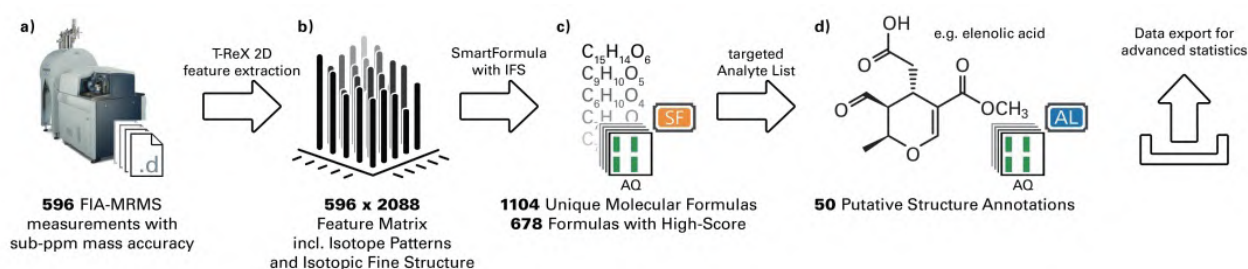
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Extra virgin olive oil (EVOO) consumption has globally increased due to its superior nutritional and sensory properties. In combination with its importance for European Union's economy [1], it has been established as a product of high economic priority and the need for its quality and authenticity control is of outmost importance. Its chemical complexity and variability enhances the hassle in investigating the most suitable methodology and consequently numerous analytical methods have been suggested [2]. However, a reliable methodology to ensure authenticity and quality of EVOO is still unavailable. In this study Fourier Transform High Resolution Mass Spectrometry (FTHRMS) techniques were integrated for metabolomics analysis of Greek EVOO and their corresponding biophenols (polar constituents). In particular, Ultra High Performance

Liquid Chromatography coupled with orbitrap analyser (UPLC-Orbitrap-MS) and Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS) using Flow Injection Analysis (FIA) method were incorporated providing novel data for EVOO chemical discrepancy and classification. More than 300 EVOO samples were collected from the main Greek olive oil producing regions, for two harvesting years. After pre-treatment, data were subjected to multivariate data analysis (MDA). Clear trends and clusters were observed correlating certain biomarkers with selected discriminants factor. To our knowledge this is the first time that two FT MS platforms combining LC and FIA methods were integrated to give solutions to quality control aspects of EVOO. Moreover, this is the first time that both lipophylic components and polyphenols are analysed together providing a holistic quality control workflow for EVOO.



The author would like to thank IKY institute for the financial support and the Greek olive oil producers for their kind offer of EVOO samples.

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2. Kalogeropoulos N, Tsimidou M. Antioxidants 2004 ; 3: 387-413.

Session 3-1-SL-07:

USP Botanical Monographs from Traditional Chinese Herbal Medicines Origin

Cui-ying Ma, Gabriel Giancaspro

U.S. Pharmacopeial Convention, Maryland, United States

USP botanical monographs contain science-based quality standards that include multiple interrelated tests to provide a full quality characterization for each article in terms of its identity, purity, and content. USP develops pharmacopeial monographs for Traditional Herbal Medicines from different countries including Traditional Chinese Medicines. USP harmonizes botanical monographs to other pharmacopeias, such as European Pharmacopeia (EP) and Chinese Pharmacopeia (ChP), but USP monographs usually have more advantages

in both methods and content specifications. In this work, we present USP monographs of Chinese skullcap root^[1] and Coptis species rhizome^[2].

USP monograph of Chinese skullcap root consists of the dried root of *Scutellaria baicalensis* Georgi. It contains multiple flavonoids including baicalin, baicalein, wogonoside and wogonin. Both HPLC and HPTLC analysis can identify and the HPLC can quantify the four flavonoids. Moreover, the monograph allows efficient differentiation between the Chinese and the American skullcap (*Scutellaria lateriflora*).

The USP monograph of Coptis species rhizome is represented by the dried rhizome of *Coptis chinensis* Franch., *Coptis deltoidea* C.Y.Cheng & P.K.Hsiao, or *Coptis teeta* Wall.. The three species included in the USP monograph are in agreement with those of EP and ChP. However, according to USP monograph six alkaloids including berberine, palmatine, coptisine, epiberberine, jatrorrhizine, and columbamine can be determined by both HPLC and HPTLC, and the sum of the six alkaloids is requested to be NLT 9.0% and NMT 20%. In contrast in EP only berberine is specified; in ChP, four alkaloids are separately specified for *C. chinensis*, and berberine is only specified for *C. deltoidea* and *C. Teeta*.

References

[1] USP, Chinese Skullcap Root. Pharmacopeial Forum, 44 (2). Available at www.usp.org

[2] USP, Coptis Species Rhizome. Pharmacopeial Forum, 44 (5). Available at www.usp.org

Monday, 27th August, 2018 - Poster Area - Poster Shed - 16:00 - 18:00

Poster Session-PO-01:

Extraction optimisation using water/glycerol for the efficient recovery of phenolic antioxidants from aerial plants of *Echinacea purpurea*

Plamen Momchev, Barbara Fumic, Petar Ciganovic, Jasna Jablan, Suzana Inic, Marijana Zovko Koncic

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A successive optimisation of the extraction process for polyphenol recovery from aerial plants of *Echinacea purpurea* (L.) Moench (Asteraceae) was performed. The ultrasonication-assisted extraction was carried out using water/glycerol as an eco-friendly solvent mixture. The first step of optimization was performed according to a two-factor interaction model intended for selection of independent variables that were critical to the process. The dependent variables were caftaric and cichoric acid, analysed using the HPLC-DAD method. Radical scavenging activity of the extracts was analyzed using the DPPH free radical. Analysis of variance (ANOVA) has shown that the most important extraction factors were glycerol concentration, temperature, ultrasonication power and extraction time. In an attempt to fine-tune the extraction procedure, subsequent Box Behnken analysis was performed by varying those factors. The results have shown that

the yield was significantly influenced by glycerol content and temperature of the extraction solvent as linear terms, as well as by all the variables as quadratic terms. In order to confirm the validity of the model, the set of the extraction conditions optimized for the extraction of the phenolic acids (70% glycerol, 60 °C, ultrasonication power 72W and 60 min) was chosen for preparation of one extract. The analysis has shown that the actual sum of concentrations of caftaric and cichoric acid in the extract was 1.542 ± 0.228 mg/mL, while the predicted value was 1.525 mg/mL. This infers that the deviation of the experimental result was only 1.1% and confirms the validity of the quadratic model. The prepared extracts were good radical scavengers with IC₅₀ values starting from 3.97 mL extract/mL. The results indicate that the ultrasonication-assisted extraction using water/glycerol mixtures is a promising tool for extraction of bioactive phenolics from *E. purpurea* suitable for inclusion into cosmetic and pharmaceutical preparations.

Poster Session-PO-02:

Antifungal activity and toxic effects of isolated compounds from the roots of *Ximenia caffra* var *natalensis* used for oral candidiasis

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Oral candidiasis is the fungal infections caused by *Candida albicans* and is mostly prevalent in immunocompromised patients. *Ximenia caffra* var. *natalensis* Sond. is used traditionally in South Africa for the treatment of oral candidiasis by the local people and traditional healers. The aim of the study was to investigate the in vitro antifungal activity of the selected plant extracts and isolate compounds from the most promising plant species. The roots extracts of *Ximenia caffra* var. *natalensis* was evaluated for antifungal activity against *Candida albicans* using serial dilution assay. Acetone extracts were active against the tested fungal pathogen with MIC values of 0.20 mg/ml and were selected for further phytochemical analysis. Bioautography assay was used to determine the number of active compounds in the plant extracts. Antifungal compounds were observed in the acetone extracts and were subjected to column chromatography. Bioassay-guided fractionation using column chromatography of acetone extract led to the isolation of four antifungal compounds. Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) were used for the identification of isolated compounds. Cytotoxicity of *Ximenia caffra* var. *natalensis* and isolated compounds was determined using the MTT (3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide) assay against Vero kidney cells. Compound 1 was relatively not toxic against the cells with LC₅₀ 32.2 mg/ml. Compound 1 was identified as epigallocatechin gallate, while compounds 3 was identified as

Kaempferol-3-O-rhamnoside and 3 and 4 were not identified due to the presence of mixtures of long chain fatty acids. The results support the traditional use of the selected plant species for the treatment of oral candidiasis by the local people and traditional healers in Aganang Local Municipality. *Ximenia caffra* var. *natalensis*, Cytotoxicity, Minimum inhibitory concentration (MIC).

Poster Session-PO-03:

The essential oil composition and antioxidant activity of *Achillea* spp growing in south west of Iran

roozbeh farhoudi

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Yarrow (*Achillea* spp) belongs to Asteraceae family and more than 100 species have been recognized in this genus. The composition of essential oil isolated from *Achillea eriophora*, *Achillea millefolium*, *Achillea biebersteinii* and *Achillea tenuifolia* growing wild in south west of Iran, was analyzed. *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oils were characterized by sabinene, 1, 8-cineole, α -bisabololoxide A, Apigenin-7-glucoside, terpinene-4-ol and α -pinene. Results indicated essential oil obtained from *A. eriophora*, *A. millefolium*, *A. tenuifolia* and *A. biebersteinii* exhibited a dose-dependent increase with a radical scavenging effect of 85.0 %, 82.0% , 82.0% and 64.0 % at 350 μ g/ml, which are close to the 1,1-diphenyl-2- picrylhydrazyl inhibition of the positive control Butylated Hydroxytoluene (88.0%) at the same concentration. It was shown that the *A. biebersteinii* essential oil exhibited the weakest antioxidant effect than Butylated Hydroxytoluene or other *Achillea* spp essential oils . In this study chamazulene, α -bisabolol, α -bisabololoxide and apigenin-7-glucoside percentage were higher in *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oil compared to *A. biebersteinii* essential oil and these compounds improved antioxidant capacity of *Achillea* spp. This study indicated that *Achillea* spp collected from South-west of Iran may be considered as a good source of natural antioxidants to be used in medicinal and food products to promote human health and prevent diseases. Results indicated some of *A. eriophora*, *A. millefolium* and *A. tenuifolia* essential oil compounds like 1,8-Cineole, Apigenin-7-glucoside, α -bisabolol, α -pinene and β -pinene were higher compared *A. biebersteinii* essential oil and it can be improve antioxidant activity of these plants ^[1,2].

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Poster Session-PO-04:

Antioxidant activity and Biocompounds of Lamiaceae Family Herbal Species

roozbeh farhodi

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The present study was designed to evaluate the antioxidants compound and activities in *Salvia officinalis*, *Mentha spicata*, *Lavandula angustifolia*, *Satureja hortensis* and *Origanum vulgare* methanol extracts. Antioxidant activities of plant extracts were assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ability to reduce Fe³⁺ to Fe²⁺ assay. Free radical scavenging activity of *S. officinalis* was recorded as high as 90.1 % followed by *L.angustifolia* (88.7 %) at 300 µg/ml. This value was found close to the activity of synthetic antioxidant, butylated hydroxytoluene (BHT) (94.0 %) at the same concentration. In this study, *S. officinalis* and *L. angustifolia* had higher total phenolic contents (21.7 ± 1.18 and 19.2 ± 1.06 mg GA/100 g DW) whereas *S. officinalis* had the largest flavonoid contents (4.8 ± 0.12 mg CE / 100 g DW). Results showed strong correlation between antioxidant activity and carotenoids content ($r=0.82$), phenolic compounds ($r=0.92$), vitamin C ($r=0.81$), vitamin E ($r=0.79$) and tannins content ($r=0.81$). It can be concluded from the current results that antioxidative potency of *L. angustifolia* and *S. officinalis* is attributed to the presence of higher contents of biocompound like carotenoids, phenolics, vitamins and flavonoids. Plant secondary metabolites play an important role in defense mechanism against free radicals. The current study shows that there are differences in the antioxidant activity of the Lamiaceae species commonly consumed in Iran. Some of the plants like *S. officinalis* and *L. angustifolia* can be considered as good sources of natural antioxidants since their extracts were found to possess high antioxidant activity ^[1,2].

[1]. Ceker S, Nardemir G, Alpsoy L, Agar G, Mete. . J. Essent. Oil Bearing Plants 2013; 15(3): 122-129.

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Poster Session-PO-05:

Comparative Study of the Biological Activities of *Jatropha pelargoniifolia* and *Jatropha gauca* Native to Saudi Arabia

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Many *Jatropha* species have found applications in folk medicine. This study aimed to evaluate the biological activities of the total alcoholic extracts from the roots and aerial parts of *Jatropha pelargonifolia* and *Jatropha glauca* that belong to Euphorbiaceae family native to Saudi Arabia. In vivo and in vitro studies were carried out to evaluate their possible activities as hepatoprotective, antinociceptive, anti-inflammatory, and antidiabetic/hypoglycemic agents in comparison to standard clinical drugs. Hepatoprotective effect was assessed via changes in serum biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), and total bilirubin content. Tissue parameters, such as presence of malonaldehyde (MDA), non-protein sulfhydryl groups (NP-SH), and total protein (TP), were also measured. Antinociceptive activity was explored by the hot plate and writhing methods. In addition, the anti-inflammatory effect was evaluated by carrageenan-induced paw edema. Finally, hypoglycemic and antidiabetic activities in alloxan-induced diabetic mice were evaluated for extracts from both the *Jatropha* plants.

Results: the roots of *J. glauca* showed higher hepatoprotective activity as well as superior antinociceptive activity (63.85%) in comparison to indomethacin (69.8%). In addition, the root extracts from *J. pelargonifolia* exhibited higher anti-inflammatory effect (50.63%) and hypoglycemic activity (37.98%) as compared to phenylbutazone (64.63%) and glibenclamide (51.57%), respectively. Finally, extracts from aerial parts of *J. glauca* showed significant antidiabetic activity in alloxan-induced diabetes (39.93%) cases as compared to glibenclamide (47.13%). Conclusion: the ethanolic extracts from *J. glauca* and *J. pelargonifolia* (root and aerial parts) induced analgesic and anti-inflammatory effects; these results emphasizes the main purpose of using these plants in folk medicine as analgesic and for the treatment of inflammatory conditions. As well, the selected plants showed significant hepatoprotective and hypoglycemic/antidiabetic activities.

Poster Session-PO-06:

Investigation of antiinflammatory properties of traditionally used East and Central African medicinal plants in the COX/PGH₂ and 15-LOX/15-Hydroperoxyeicosatetraenoic acid pathways

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The majority of plant and insect species of the tropical rainforests in western Uganda and eastern DRC have not yet been discovered; 90% have never been screened for bioactivity. Approx. 60% of the world's population relies almost entirely on plants for medication. The knowledge of East and Central African

plants and their traditional uses are mainly transferred orally from one generation to the next by traditional healers, leading to the loss of vital information due to lack of records. Our study provides documentation of 16 different African medicinal plants traditionally used to treat inflammation and related disorders such as pain, arthritis, osteoporosis, asthma, dermatitis and even cancer. One possible methodology for the discovery of novel antiinflammatory compounds is screening selected plant extracts for a broad array of pharmacological activities. Phenolic compounds are often thought to possess antiinflammatory properties. The MOAs of many phenolic compounds are most likely associated with their inhibition of proinflammatory enzymes in the arachidonic acid pathway such as lipoxygenases (LOX) and cyclooxygenases (COX) in inflammatory cascades or with their free radical scavenging activity. Due to undesirable effects of non-steroidal antiinflammatory drugs (NSAIDs) such as gastrointestinal bleeding, selective inhibition of COX-2 is preferred to the COX-1 inhibition. We present results of diverse in vitro experiments performed with 61 different plant extracts: 1. 15-LOX inhibition screening; 2. Selective COX-2 and COX-1 inhibitor screening; 3. DPPH assay for antioxidant activity; 4. Determination of the total phenolic content. Traditional use could be scientifically validated in 15 out of 16 plant species tested (in vitro). This study was performed according to the international and national rules considering the Convention on Biodiversity and the Nagoya Protocol.

Poster Session-PO-07:

Structural characterization of phenolic acids, flavonoids, saponins and flavonolignans in *Cecropia* species using an HPLC-DAD-QTOF method

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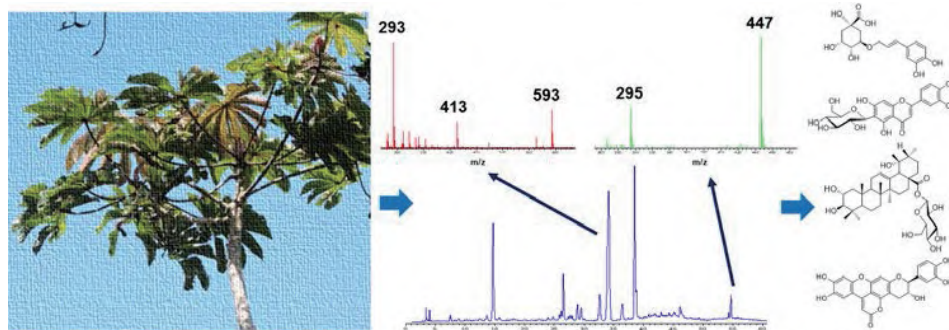


Figure 1. Characterization of *Cecropia* species by HPLC-DAD-QTOF-HRMS. Photograph taken by Rodolfo R. Flores J.

Plant species popularly known as 'guarumo', 'embauba', 'ambay' and 'trumped tree' belong to the genus

Cecropia Loebl. (Urticaceae) and are distributed from Mexico to Central and South America^[1]. Several species are traditionally used in Latin American folk medicine for the treatment of diabetes, hypertension and inflammation^[2]. These therapeutic properties have been correlated to their content of phenolic acids, flavonoids, proanthocyanidins, triterpenoids and steroids. However, there is insufficient phytochemical information for the comparison of different Cecropia species.

The aim of this study was to characterize the polyphenolic profile from the methanolic extracts of *C. obtusifolia*, *C. peltata*, *C. insignis* and *C. hispidissima* leaves collected in the Republic of Panama, using a method of high-performance liquid chromatography-diode array detection- quadrupole time of flight-tandem mass spectrometry (HPLC-DAD-QTOF) method (Fig. 1). Forty-seven compounds comprising two phenolic acids, thirty-three flavonoids, three flavonolignans and nine saponins were identified or tentatively characterized. Thirty nine of these compounds have not been previously reported in these species. A diverse composition of flavone mono-C-glycosides and di-C,O-glycosides were detected in *C. obtusifolia*, *C. peltata* and *C. insignis*. In contrast, quercetin mono- and di-O-glycosides were mainly found in *C. hispidissima* samples. Therefore, the reported results showed that it is possible to discriminate between *C. hispidissima* samples from the remaining species under study.

Considering the importance of the description of novel chemical entities and the increasing interest and use of natural products, this study may be of great help for the interpretation of medicinal properties and for the quality assessment of herbal supplements containing Cecropia species leaves.

References

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Poster Session-PO-08:

Quantitative relationships between structure and antiadhesive activity of flavonoids against uropathogenic *E. coli*

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Urinary tract infections (UTIs) are worldwide one of the most common bacterial infections, responsible for extensive medical costs and morbidity. UTIs are predominantly caused by uropathogenic *Escherichia coli* (UPEC). To date, UTIs are mainly treated with antibiotics, leading to the ubiquitous problem of increasing resistance against most of the currently available antimicrobial agents. Therefore, new treatment strategies are urgently needed. An innovative approach for prevention is the development of specific inhibitors of bacterial adhesion to bladder cells. Within a flow cytometry based adhesion assay, a variety of five plants

containing flavonoids as well as isolated flavonoids showed promising antiadhesive properties. Therefore QSAR models for predicting the antiadhesive activity were created. Molecular descriptors were calculated from 3D models of 25 flavonoids and used as independent variables. The dependent variable was expressed as Inhib (Inhibition of adhesion [%] at a concentration of 500 μ M). The variables were then analyzed by multiple linear regression (MLR) to build a linear QSAR regression model. The entire dataset consisting of 25 flavonoids was used as a training set. Regarding the obtained MLR QSAR model, $R^2=0.8764$, $XR^2=0.8343$ were achieved. The predictability of the QSAR model was evaluated with five previously untested flavonoids. The Inhib-values of these flavonoids were predicted by the QSAR model and an $R^2=0.9456$ was obtained. The excellent results for internal and external predictions indicate that this QSAR model can be used to predict the antiadhesive activity of further, yet untested, flavonoids, which can then be retrieved for testing.

Poster Session-PO-09:

Impact of herbal substances on efflux pumps in bacterial and human cells

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Since increasing bacterial resistance to antibiotics is currently one of the biggest threats to human health, there is an urgent need of new antibacterial drugs in development. Efflux pumps represent membrane transport proteins, which eliminate toxic substrates out of the cell. This mechanism contributes to the importance of efflux pumps in bacterial antibiotic resistance as well as in chemotherapy resistance. ^{[1], [2]}

As plants show an enormous compound diversity and low toxicity, they are promising substances for new efflux pump inhibitors to combat increasing bacterial resistance. Different plants of the *Artemisia* species (e.g. *A. scoparia*, *A. annua*) within the family of Asteraceae as well as some species of *Scutellaria* within the family of Lamiaceae are screened in order to examine a potential inhibition of efflux pumps. The respective plant parts are extracted with four solvents of varying polarity and screened on the non-pathogenic model strains *Mycobacterium smegmatis* mc2 155 and *Escherichia coli* K12 MG1655. The results showed that extracts of a *Scutellaria* species (MIC=8mg/l) and an *Artemisia* species (MIC=64mg/l) possess strong antimicrobial activities against *Mycobacterium smegmatis* mc2 155. Another extract of a *Scutellaria* species showed good resistance- modulating effects against *Escherichia coli* K12 (MF=8) and AG100 (MF=16). Since the human intestinal and lung epithelium are equipped with efflux pumps, another essential part of this study is to investigate the impact of potent plant inhibitors on these features. For this, standardized in-vitro models are used, which recapitulate the intestinal barrier and serve to study the permeability behaviour of drugs. Caco-2 cells are tested for the oral route and Calu-3, A549 and H441 cells for the respiratory route.

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Poster Session-PO-10:

Characterization of tyrosinase inhibitors from Lotus receptacle

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Tyrosinase is a copper-containing enzyme, which is widely distributed in microorganisms, animals and plants and is a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin and hair [1]. Consequently, tyrosinase inhibitors are probably the most non-invasive strategy for the control of skin pigmentation. However, synthetic agents often result in inflammation of the skin, insufficient penetration or undefined clinical efficiency [2]. Currently, the development of more depigmenting agent from natural products continues to arouse great interest. In this investigation, the phytochemical study, including fractionation and purification, of 95 % ethanol extract of Lotus receptacle led to the isolation of nine compounds and their structures were elucidated as (-)-epigallocatechin (1), catechin (2), procyanidin B2 (3), quercetin (4), hibifolin (5), dihydrokaempferol-7-O- β -D-glucoside (6), taxifolin (7), trans-dihydromorin (8), and oxyresveratrol (9) mainly based on their spectral and chemical clues. Among these nine compounds, eight compounds, compound 1-3 and 5-8, were isolated from Lotus receptacle for the first time. Compounds 1-4, 8 and 9 showed strong inhibitory activity against mushroom tyrosinase with IC_{50} values ranging from 5.58 to 54.36 μ M, comparing with kojic acid which was used as a positive control with IC_{50} value of 60.14 μ M. Therefore, the residues of Lotus receptacle may serve as potential candidates as remedy for hyperpigmentation and as skin-whitening agents in cosmetics industry.

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Acknowledgements:

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Cardiac glycosides from the roots of *Nerium indicum* Mill.

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Nerium indicum Mill. belongs to the Apocynaceae family. It is a large branched shrub that is found throughout China, Japan, and India, where it grows both wild and as an ornamental garden plant ^[1]. The roots, flowers, barks, and leaves of *N. indicum* have long been used in traditional medicines for treating skin diseases and wound infections, and as an antidote, antileprotic, emetic, expectorant, and sternutatory agent ^[2]. Phytochemical and pharmacological studies have confirmed that cardiac glycosides are the major bioactive constituents of *N. indicum*, and these compounds have cardiotonic, diuretic, cytotoxic, antibacterial, anticancer, antiplatelet aggregation, antiinflammatory, hepatoprotective, antihyperlipidemic, antiulcer, and central nervous system depressant properties ^[3]. In continuation of our studies on chemical constituents from the roots of *N. indicum*, five cardiac glycosides were isolated and elucidated as gitoxigenin 3-[O- β -D-glucopyranosyl-(1 \rightarrow 4)-O-glucopyranosyl(1 \rightarrow 4)- β -D-digitaloside] (1), digitoxigenin 3-[O- β -D-glucopyranosyl-(1 \rightarrow 6)-O-glucopyranosyl(1 \rightarrow 2)- β -D-digitaloside] (2), digitoxigenin 3-[[O- β -D-glucopyranosyl-(1 \rightarrow 2)]-[O- β -D-glucopyranosyl-(1 \rightarrow 3)]-rhamnoside} (3), 1-acetoxy-3-[O- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-digitaloside]-14-hydroxy-20(22)-enolide (4), and 3-O- β -D-diginoside-1,14-dihydroxycard-20(22)-enolide (5). To the best of our knowledge, all these compounds were obtained from the genus *Nerium* for the first time.

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Poster Session-PO-12:

Impact of herbal substances on adipocyte differentiation

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The role of fat tissue as an endocrine organ, central to the synthesis of various hormones is - next to its close association to several metabolic diseases particularly in those suffering from obesity- a focus for a large body of ongoing research.

Given the various reported effects of herbal substances on several organ systems, the effects of herbal substances on adipose tissue is a promising, yet insufficiently explored area of research. A major goal of this dissertation is to explore the impact of herbal substances on adipocyte differentiation.

In a first step (screening phase) the research for this dissertation will aim to investigate the impact of herbal substances on adipocyte differentiation by examining the effects of selected plants on adipocytes prior to differentiation. In a second step (model development) the research will use the insights from the screening phase to development of a cell model. In this phase effective ethanolic extracts, based on the current knowledge of adipocyte metabolism and the established interactions with other herbal compounds, will be investigated. For this, the well-known cell model, the murine 3T3-L1 preadipocytes, is used. Several extracts showed positive effects but especially the family of Rutaceae showed very good results on adipocyte differentiation in the first tests.

This phase will also cover the approach to the fractionation of these extracts, the isolation of active single compounds and the study of the underlying mechanisms. The last phase of the proposed research will focus on identification of the active substances.

Poster Session-PO-13:

Escaping Bio-Assay Guided Isolation: Nature's Tools for Chemical Biology

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ABSTRACT

The classical bio-assay guided approach is to screen extracts for biological activity and then use an iterative

cycle of fractionation/assay/fractionation until the active compound is purified. This is time consuming, often requiring many cycles of fractionation before the compound of interest is purified and requires resources to run the assay after every chromatographic (fractionation) step. Structure elucidation occurs once the pure compound is obtained and, in many cases, known compounds are re-discovered. This project aims to apply ^1H NMR in combination with multivariate statistical analysis (PCA) in order to achieve a metabolic profile for natural product fractions, that are active on TB targets. As NMR reveals all compounds containing hydrogen and is quantitative, it can be used to guarantee that all compounds within a fraction are isolated. TB is an infectious disease worldwide, causing death every 20 seconds, and a new drug is urgently needed because of the multi-drug resistance developed by *Mycobacterium tuberculosis*. The NMR approach is being used to analyse the results of a recent HTS screen against *M. tuberculosis* H37Rv and the recombinant mycobacterial lipoamide dehydrogenase (Lpd) enzyme, which is involved in *M. tuberculosis* defence against the host immune system.

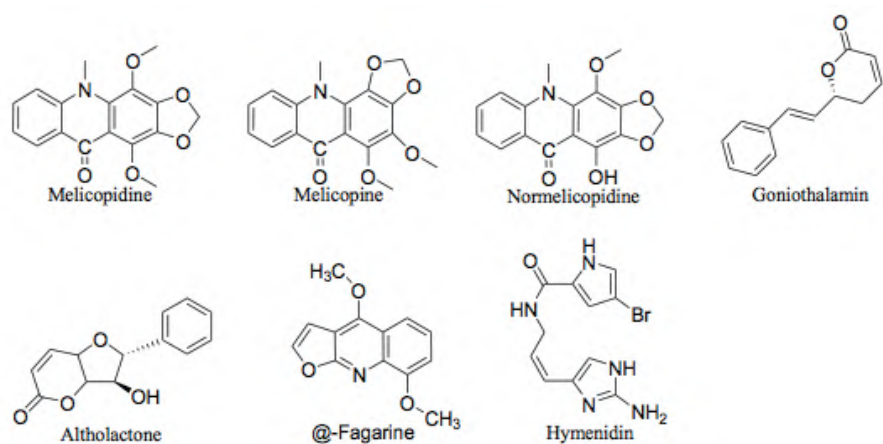


Figure 1. Isolated compounds.

Poster Session-PO-14:

Characteristics of low molecular weight extractives from the roots of *Cinnamomum camphora*

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Cinnamomum camphora (L.) Presl (family: Lauraceae), commonly known as the camphor tree, is one of the most common species in subtropical evergreen broad-leaved forest widespread in southern China [1]. All parts of *C. camphora* contain a special aroma and volatile oil that has been used as a insect repellent and as an antibacterial agent [2]. Pharmacological studies have revealed that the plant possesses a wide

range of bioactive properties. Previous phytochemical investigation of the genus *Cinnamomum* has resulted in the isolation and structural elucidation of essential oil compounds, lignans, flavones and polyphenols [3]. However, no research has focussed on the secondary metabolites of *C. camphora* roots.

In the current work, phytochemical investigations of *C. camphora* roots led to the isolation and purification of seven known compounds, including oleanolic acid (1), β -sitosterol (2), daucosterol(3), tricosanoic acid (4), dimethylmatairesinol (5), and luteolin (6), luteolin-7-O- β -D-glucoside (7), tricetin-7-methyl ether (8), and quercetin-3-O- β -D-glucoside (9). Their structures were elucidated by MS and 1D and 2D NMR spectroscopy, as well as comparison with literature data. All the nine compounds (1~9) were isolated for the first time from *C. camphora* roots, and compounds 4, 6, 7 and 8 have previously never been reported from Lauraceae.

Acknowledgements

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Poster Session-PO-15:

The Anti-inflammatory Activity of *Cinamomum camphora* Extracts and Its Active Components

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Cinamomum camphora is proved to possess a lot of biological activities, including antifungal, antioxidant, antibacterial, anti-allergic, and anti-inflammatory activities ^[1-2] . However, the bioactive compounds responsible for the anti-inflammatory effect of leaves have not been yet determined. The objective of the present study was to isolate these bioactive compounds by bioassay-guided isolation technique and to determine the mode of action of isolated compounds in LPS-induced macrophages. We demonstrated that ethyl acetate fraction significantly decreased the production of inflammation mediator nitric oxide (NO) and

inflammatory cytokines tumor necrosis factor PGE_2 and $\text{TNF-}\alpha$ in a dose-dependent manner (10, 30, 100 $\mu\text{g/ml}$). In addition, Seven compounds ursolic acid (1), β -sitosterol (2), trametenolic acid (3), (-)-sesamin (4), dimethylmatairesinol (5), oleanolic acid-3-O- β -D-glucoside (6), and quercetin-3-O- β -D-glucoside (7). were isolated from ethyl acetate fraction, among them, three compounds, ursolic acid (1), trametenolic acid (3) and oleanolic acid-3-O- β -D-glucoside (6) were reported for the first time in *Cinamomum camphora*. NO and inflammatory cytokines (PGE_2 and $\text{TNF-}\alpha$) secretion indicated that ursolic acid (1), β -sitosterol (2), trametenolic acid (3) and oleanolic acid-3-O- β -D-glucoside (6) showed a more outstanding anti-inflammation potential at non-toxic concentrations (10, 30, 100 μM) than the other four compounds.

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Acknowledgements

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Poster Session-PO-16:

Cell penetrating peptides functionalized gambogic acid-nanostructured lipid carrier for cancer treatment

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Tumor-targeted delivery is considered a crucial component of current anticancer drug development and is the best approach to increase the efficacy and reduce the toxicity. Nanomedicine, particularly ligand-based nanoparticles have shown a great potential for active targeting of tumor. Cell penetrating peptide (CPP) is one of the promising ligands in a targeted cancer therapy. In this study, the gambogic acid-loaded nanostructured lipid carrier (GA-NLC) was modified with two kinds of cell penetrating peptides (cRGD and RGERPPR). The GA-NLC was prepared by emulsification and solvent evaporation method and coupled with cRGD, RGERPPR, and combination cRGD and RGERPPR to form GA-NLC-cRGD, GA-NLC-RGE, and GA-

NLC-cRGD/RGE, respectively. The formulations were characterized by their particle size and morphology, zeta potential, encapsulation efficiency, and differential scanning calorimetry. In vitro cytotoxicity and cellular uptake study of the formulations were performed against breast cancer cell (MDA-MB-231). Furthermore, in vivo biodistribution and antitumor activity of the formulations were determined by in vivo imaging and in tumor-bearing nude mice, respectively. The result of in vitro cytotoxicity study showed that GA-NLC-RGE exhibited a significantly higher cytotoxicity on MDA-MB-231 as compared with GA-NLC and GA-Sol. Similarly, RGE-Cou-6-NLC showed remarkably higher uptake by the cells than other NLCs over the incubation period. The in vivo imaging study has demonstrated that among the formulations, the RGE-decorated DiR-NLC were more accumulated in the tumor site. The in vivo antitumor activity revealed that RGE-GA-NLC inhibits the tumor growth more efficiently than other formulations. In conclusion, RGERPPR has a potential as an effective carrier in tumor targeting drug delivery.

Poster Session-PO-17:

Easy and quick detection and purification of extracellular vesicles

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Extracellular vesicles (EVs) are a heterogeneous group of membranous vesicles comprising apoptotic bodies, microvesicles and exosomes. The distinct species are classified by their size and origin. Despite there is upcoming interest on animal derived EVs- resulting in an increasing knowledge of their function, composition, and cargo- little is known about EVs in the plant kingdom ^[1]. Agarose gels in concentrations between 0.5% - 2% are typically used for electrophoretic separation of nucleic acids ^[2]. Our investigations have shown that they are also suitable for quick and easy EV detection and purification. We isolated EVs from the apoplastic fluid (APF) of *Nicotiana tabacum* L., *Hedera helix* L., and *Viscum album* L. Even though intact plant derived EVs do not migrate into the gel matrix, they can be visualized inside the well. If 3,3-Dihexyloxacarbocyanine iodide (DiOC6) is added to the specimen in excess, membranous components can already be detected by eye, or with higher sensitivity, using a UV transilluminator. The detected subtype of EVs depends on the previous sample preparation. Moreover, EVs are purified from small charged contaminants and dye excess, passing through the gel.

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The analgesic effects of the hydro-ethanol stem-bark extract of *Burkea africana* (Hook) using animal models.

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Burkea africana (Hook) has been used traditionally for managing pain related ailments. However, there is little scientific documentation to complement its use in the management of pain. This experiment therefore evaluated the anti-nociceptive effects and the potential acute toxicological profile of the hydro-ethanol of the stem bark extract of *Burkea africana* (BAE). Acetic acid-induced abdominal writhing nociception^[1], formalin-induced nociception^[2], carrageenan-induced hyperalgesia^[3] and acidic-saline induced hyperalgesia^[4] tests were used to determine the anti-

nociceptive effects of BAE. The acute toxicological profile of the extract was also assessed after single and multiple administrations in mice and rats respectively. Oral administration of BAE (50, 500, 1000 mg/kg) showed a significant anti-nociceptive effects in all the models used.

In the toxicological study, acute administration of high doses of extract did not elicit any lethality with the lethal dose 50 (LD₅₀) of BAE estimated to be above 10000 mg/kg.

In conclusion, the hydro-ethanol extract of the stem bark of *B. africana* possess some anti-nociceptive effects justifying its use in traditional medicine as an analgesic.

Acknowledgement: Ghana National Petroleum Corporation (GNPC) for sponsorship.

Burkea, anti-nociception, acidic-saline,

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Cyclocarya paliurus leaves improve dyslipidemia in diabetic mice: a lipidomics-based network pharmacology study

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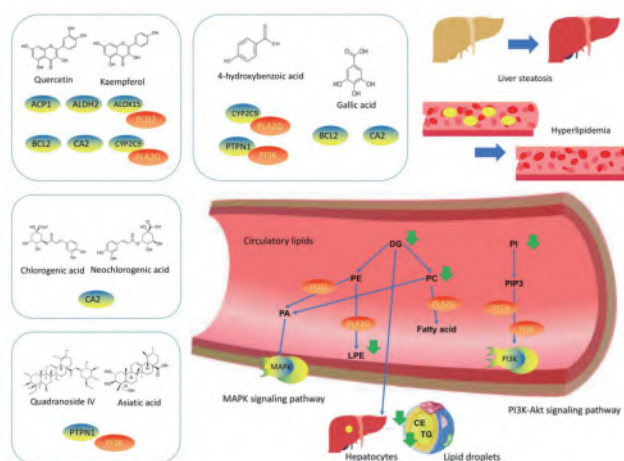
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Hyperlipidemia and hepatic steatosis affect over 75% of patients with type 2 diabetes, resulting in dyslipidemia in diabetes. *Cyclocarya paliurus* (Batal.) Ijinskaja (CP) leaf is a herbal tea which has long been consumed by the Chinese population, particularly people suffering from obesity and diabetes. CP appears to exhibit a hypolipidemic effect in rats ^[1], although the detailed mechanisms and active ingredients responsible for this hypolipidemic effect have not been elucidated yet. In this study, we investigated the beneficial effects of CP and predicted the mechanisms by utilizing lipidomics, serum-pharmacochimistry and network pharmacology approaches. Our results demonstrated serum and hepatic total triglyceride (TG), total cholesterol (T-CHO), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) levels, as well as 30 lipids including cholesterol ester (CE), diglyceride (DG), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and sphingomyelin (SM) in CP-treated mice were improved in comparison with those of diabetic mice without treatment. In parallel, 14 constituents of CP were determined in mice serum after CP administration. Mechanistically, the network pharmacological analysis revealed the predicted targets ALOX12, APP, BCL2, CYP2C9, PTPN1 and linked lipidome targets. PLD2, PLA2G(s) and PI3K(s) families are potential markers to be responsible for the CP effect on diabetic dyslipidemia, based on association analysis of the change of lipid metabolites in diabetic mice and active ingredients absorbed in blood after CP administration. In conclusion, this study verifies the beneficial effects of CP on diabetic dyslipidemia, which are associated with reducing accumulation of hepatic lipid droplets and regulating circulatory lipids in diabetic mice, possibly through PI3K signaling and MAPK signaling pathways.

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Poster Session-PO-20:

New pterocarpan and derrisisoflavones from the root of *Millettia aboensis*

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Pterocarpan and derrisisoflavones have been associated with a broad range of biological activities, including insecticidal, antimicrobial, cytotoxic, and antioxidant activities. These phytochemicals have been isolated from the genus *Derris* belonging to the Leguminosae family [1]. In the present study, some pterocarpan and derrisisoflavones were isolated from the root bark of *Millettia aboensis* using a combination of different chromatographic techniques including VLC, Sephadex LH-20 separation and semi-preparative HPLC. The structures of the compounds were elucidated by a combination of MS and NMR spectroscopy. The crude methanol root extract was screened for its cytotoxic activity on mouse lymphoma cell line (L5178Y) and the isolated compounds were tested for their antioxidant activity using 2, 2-diphenylhydrazyl (DPPH) radical scavenging model. The crude methanol root extract exhibited a growth inhibition of 87.5% on mouse lymphoma cell line (L5178Y). The ethyl acetate fraction of the methanol root extract of *Millettia aboensis* yielded three new compounds; one pterocarpan, 9-hydro-3,8-dimethoxyl pterocarpan (1); two derrisisoflavones, derrisisoflavone L (3) and derrisisoflavone M (4), which were identified on the basis of spectroscopic techniques. Two known compounds maackiain (2) and derrisisoflavone G (5) were also isolated. The isolated compounds showed promising antioxidant activity with compound 2 having the highest

activity with an IC₅₀ of 83 µg/ml. This is the first report on the isolation of pterocarpan and derrisoflavones from the root of *Millettia aboensis*. These compounds hold great potential for development into novel therapeutic molecules

Reference

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Poster Session-PO-21:

Antiproliferative and antioxidant effects of anthocyanin from *Acer palmatum* cv. 'Chishio'

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The reddish color of *Acer* genus leaves is due to anthocyanin pigments. In previous paper, these anthocyanins from *Acer* were reported as cyanidin glycosides and delphinidin glycosides. Galloyl cyanidin glycosides are distributed as a common anthocyanins from red leaves of *Acer*, but these pharmacological activities have not been investigated^[1]. In this study, phytochemical composition of anthocyanins of *Acer palmatum* cv. 'Chishio' was analyzed, and their antiproliferative and antioxidant activities were evaluated. Phytochemical profiles of anthocyanins extracted from the leaves with 1% TFA in methanol were analyzed by HPLC-MS, and 4 cyanidin glucosides (cyanidin glucoside, cyanidin rutinoside, galloyl cyanidin glucoside and galloyl cyanidin rutinoside) were found in the leaves. Antiproliferative activities of each anthocyanins were evaluated against T47D, LLC and C3H10T1/2. Antiproliferative activities of galloyl cyanidin glucoside and galloyl cyanidin rutinoside against these cells with IC₅₀ values of 100.7 and 118.2 µM (T47D), 124.0 and 303.8 µM (LLC) and 158.4 and 326.9 µM (C3H10T1/2), respectively. Antioxidant activities of each anthocyanins were evaluated using in vitro free radical scavenging assay (DPPH method). The antioxidative activities of all compounds were comparable to ascorbic acid used as the positive control.

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Poster Session-PO-22:

The diverse functions of fungal endophytes in *Artemisia annua* L.

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Artemisia annua L. is the sole natural source of antimalarial drug artemisinin. Endophytic fungi, which spend their life inside the healthy *A. annua* plants, exhibit intimate impact on the growth and physiology of their hosts and diverse bio-functions: 1) New antimicrobial and antitumor metabolites; 2) The role of plant growth promotion; 3) The regulation on artemisinin biosynthesis; 4) The biodegradation of toxic organic chemicals. The research provided novel references for the rational exploitations on the fungal endophytes in *A. annua* and for the understanding of mutualism of the plant-endophytes in *A. annua*.

Acknowledgement:

The study is supported by the NNFC (No. 81273487 and 81473183).

Artemisia annua ; endophytic fungi; antimicrobe and antitumor; growth promotion; artemisinin; biodegradation

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Poster Session-PO-23:

The role of fruiting body-associated *Pseudomonas* sp. in eliciting hypocrellin biosynthesis in *Shiraia bambusicola*

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Shiraia bambusicola is a pathogenic fungus on bamboo, whose fruiting body has been used in TCM for the treatment of vitiligo, stomachache and rheumatic in China. Hypocrellin A (HA), natural red perylenequinone pigment isolated from the fruiting body, has attracted intense interest as clinically useful photosensitizers in photodynamic therapy (PDT) for various cancers. We first analyzed the bacterial community in the fruiting body of *S. bambusicola*. *Bacillus* and *Pseudomonas* were the predominant taxa in the stroma of *S. bambusicola*. It was a surprising finding that *Pseudomonas* isolates such as *P. putida*, *P. fulva* and *P. parafulva* exhibited a general capacity to stimulate the secretion of HA in the mycelium of *S. bambusicola*. *P. fulva* SB1 showed the most significant effects on HA accumulation. When live SB1 at 100 cells /mL was applied to the submerged culture of *S. bambusicola* on day 4, the HA content was most effectively enhanced 2.47-fold (17.43 mg/g DW versus 7.06 mg/g DW in control) on day 8. The results provide a reference of interaction between fruiting-body associated bacterium and medicinal fungus *S. bambusicola*. Our study will facilitate its potential application as a novel bacteria-fungi co-culture strategy for mass production of pharmaceutically important metabolites.

Acknowledgement:

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Shiraia bambusicola ; fruiting body; hypocrellin; *Pseudomonas*; elicitation

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Poster Session-PO-24:

Antiprotozoal activity of *Hypericum afrum* and *Cytisus villosus*, two Algerian Medicinal Plants

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Leishmaniasis and trypanosomiasis are serious and neglected tropical diseases (NTDs) caused by the group parasites Leishmania and Trypanosoma, respectively. These infections remain a major public health problem and cause high morbidity and mortality especially in developing countries ^[1]. Current available drugs for the treatment of these infections suffer from high toxicities which may cause serious side effects.

The chloroform, ethyl acetate and n-butanol extracts of the aerial parts of Hypericum afrum and Cytisus villosus were screened in in vitro assays for their antiprotozoal activity against L. donovani (promastigotes, axenic amastigotes and intracellular amastigotes in THP1 cells) and T. brucei brucei using standard procedures ^[2,3]. Both plant extracts revealed significant trypanosomicidal activity with IC₅₀ values ranges of 7.99-19.48 µg/mL. The n-butanol extract of C. villosus showed good antitrypanosomal activity against T. brucei. brucei with IC₅₀ value of 7.99 and IC₉₀ value of 12.61 µg/mL. The chloroform, ethyl acetate and n-butanol extracts of H. afrum showed trypanosomicidal activity against T. brucei. brucei culture with IC₅₀ values of 12.35, 13.53, 12.93 µg/mL and with IC₉₀ values of 14.94, 19.31, 18.67 µg/mL, respectively. The extracts did not show leishmanicidal activity.

This is the first report of in vitro antitrypanosomal activity of these plants. The potent activities of the n-butanol extract of C. villosus and the chloroform extract of H. afrum make them promising candidates for the isolation of compounds that could develop into new leads for drugs against human African trypanosomiasis.

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Poster Session-PO-25:

In-vitro anti-inflammatory activity and phytochemical screening of Piliostigma thonningii leaf extracts from Benin - West Africa

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In West African countries like Benin traditional medicines continue to play a key-role in addition to state health care. *Piliostigma thonningii* is commonly used in the northern region of Benin to treat inflammatory disorders and to promote wound healing processes (1, 2). However, knowledge about the phytochemical profile and underlying pharmacological mechanisms of actions is still scarce. Therefore, a combination of antioxidant and chromatographic screening methods using HPLC, LC-MS and DPPH-TLC was performed to study the phenolic profile of the ethanolic and aqueous leaf extracts. Furthermore, the anti-inflammatory effect of *Piliostigma thonningii* leaf extracts in immortalized human keratinocytes (HaCaT) was investigated. To mimic an inflammatory process on skin, the cells were stimulated with tumor necrosis factor alpha (TNF α) to overproduce interleukins 8 and 6. Subsequently, cytokine levels in supernatants were measured by ELISA and the cell viability was investigated using the MTT assay. An aqueous leaf extract of *Piliostigma thonningii* concentration-dependently reduced the levels of IL-8 and IL-6 at nontoxic concentrations, resulting in IC₅₀ values of 74.4 μ g/ml and 89 μ g/ml, respectively. The phytochemical and antioxidant screenings could confirm quercitrin as the main phenolic compound for both extracts. The decrease of inflammatory mediators by *Piliostigma thonningii* aqueous leaf extract in TNF α -stimulated HaCaT-cells supports its ethnomedicinal usage for anti-inflammatory purposes. Pharmacokinetic studies are warranted to assess the absorption of the compounds during topical and oral application.

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Poster Session-PO-26:

Characterization of the anti-diabetic properties of the traditional decoction of *Phaleria nisidai*

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Currently, the Republic of Palau faces a prevalence of overweight and obese adults of around 80 % according to WHO data. A reverse pharmacology approach^[1] was implemented to study the traditional herbal medicines used in Palau in relation to obesity management. The decoction of the leaves of *Phaleria nissidai* Kaneh (Thymelaeaceae) (DAK^[2]) was selected by a retrospective treatment outcome method^[3]. Afterward, a pilot clinical study evaluated DAK as an adjuvant therapy for patients with insufficient diabetes control^[2].

Based on these encouraging findings, we examined the biological activity of DAK in a murine obesity model and its chemical composition. Mice fed a high-fat diet supplemented with DAK revealed greater sensitivity to insulin, improved glucose tolerance and higher insulin secretion. Furthermore, in vitro administration of DAK promoted insulin-stimulated glucose uptake into adipocytes and increased their capacity to metabolize glucose as measured by extracellular flux analysis.

Metabolites profiling of DAK obtained by UHPLC-PDA-ELSD-HRMS confirmed the high amount of the C-glycoside xanthone mangiferin and other minor related xanthenes.

We next aimed at evaluating whether mangiferin was the sole bioactive substance. Therefore, DAK was fractionated by MPLC into four fractions, which were tested in vivo. Interestingly, although the mangiferin-rich fraction was bioactive, another fraction showed overall higher bioactivity, including increased insulin sensitivity, ameliorated glucose tolerance and reduced random-fed blood glucose levels. Dereplication of this fraction revealed the presence of mangiferin-related xanthenes and flavones. Further research is on-going to elucidate the mode of action in vitro.

These investigations highlighted the potential of the reverse pharmacology method for the identification of traditional herbs with high therapeutic value.

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Poster Session-PO-27:

Cytotoxic xanthenes from *Metaxya rostrata* as promising new lead compounds for colorectal cancer therapy

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Natural products deriving from plants used in traditional medicine are an important source of anti-cancer agents. Our study focuses on *Metaxya rostrata* C.Presl (Metaxyaceae), a tree fern widespread in the rainforests of Central and South America, that is administered against intestinal ulcers and tumors in ethnic medicine. Colorectal cancer is among the most common malignant diseases in the western world and the search for effective new therapeutics is an ongoing task. An activity-guided study of *Metaxya rostrata* led to the isolation of two structurally related xanthenes: 2-deprenyl-rheediaxanthone B (XB) and 2-deprenyl-7-hydroxy-rheediaxanthone B (OH-XB). Our previous work demonstrated that XB causes cell death in colon carcinoma cell lines via the mechanism of mitotic catastrophe [1]. First results of the cytotoxic activity of OH-XB on SW480 cells by neutral red uptake assay indicated that this compound is at least as active as XB. The IC_{50} concentrations for dose-dependent cell loss were calculated to be $5.3 \pm 1.0 \mu M$ for OH-XB and $6.7 \pm 1.2 \mu M$ for XB. Cell cycle distribution was assessed by FACS analysis and demonstrated an OH-XB-induced S-phase cell cycle arrest in contrast to XB-incubated cells, which accumulated in G2-M-phase. Analysis by western blot confirmed a cell cycle blockade in S-phase by decreased levels of cyclins A and B, while cyclin E increased. Also in contrast to XB, treatment with OH-XB caused morphological modifications typical for classical apoptosis. This is substantiated by increased caspase activity and enhanced cleavage of PARP. Additional xanthenes are currently isolated in order to determine a detailed structure-activity relationship. This work resulted already in a further new natural compound from *Metaxya rostrata*, a methylated derivative of OH-XB, which is under testing.

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Poster Session-PO-28:

Discovery of Ligand Structure-activity Relationship by Mass Spectrometry: Identification of New Tuberculosis Inhibitors

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A set of tuberculosis fragment inhibitors has been discovered by mass spectrometry. The method is based on the observation of protein-ligand complexes by mass spectrometry.¹ These fragments may compete for common binding sites on the target protein or bind at different sites. Mass spectrometry enables identification of ternary complexes in which two ligands bind to different sites of a target.²⁻³

For a specific target, the result $(P+L_1) + (P+L_2)$ indicates binding to the same site (competitive), while the result $(P+L_1) + (P+L_2) + (P+L_1+L_2)$ shows that L_1 and L_2 bind to different sites (non-competitive). Compound design relies on using a number of competitive fragments linked to a non-competitive fragment. Therefore,

the structures of these fragments will be modified using synthetic methods to enhance their activities and produce novel inhibitors.

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Poster Session-PO-29:

Phytochemical Screening, Anticancer, Antibacterial and Antioxidant Activities of the Leaf Extracts of Mabolo (*Diospyros philippinensis* A.DC.)

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Drug resistance by cells has been the problem in the medical field for decades now. The use of medicinal plants as a source of creating powerful drugs has been nowadays recognized worldwide to treat such resistant diseases ^[1]. In the present study, the potential for *Diospyros philippinensis* A.DC. to inhibit growth of both bacteria and cancer cell line was conducted. The leaf crude extracts were screened for the presence of phytochemicals and examined for potential bioactivities by employing several assays like Kirby-Bauer disc diffusion method, DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium assay for the antibacterial, antioxidant and cytotoxic activities of the extract, respectively ^[2]. Phytochemical test results of the extracts revealed the presence of alkaloids, flavonoids, saponins, phenols, quinones, cardiac glycosides, phlobatannins, carbohydrate, cardenolides and proteins. The leaf extracts were found to exhibit antibacterial activity against gram-positive bacteria, high antioxidant activity (99.22% ± 0.005) but did not show any sign of cytotoxicity towards HCT116 (ATCC CCL-247). The study therefore concludes that *D. philippinensis* A.DC. leaf extract can be a source of antibacterial and chemopreventive agents.

This claim may be used as basis for future investigation.

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Poster Session-PO-30:

Study of Ethanolic Extract from Piper retrofractum to Prevent Thrombus Formation in Male Mice

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Platelets playing a relevant role in thrombosis and haemostasis. Thrombus formation in the arterial or venous circulation can causes serious cardiovascular diseases which can lead to death.¹ Piper retrofractum has been used widely as traditional medicine to treat various diseases. These plants also have some compounds, such as piperlongumine and piperine that are proven in vitro studies to reduce platelet aggregation which have important role in the pathogenesis of acute thrombosis.² This study aimed to evaluate the effect of ethanolic extract from P. retrofractum to prevent thrombosis formation in male mice. Male DDY (Deutschland, Denken, and Yoken) mice (weight 20-30 g) were given pretreatment orally for seven days divided into groups consisted of CMC (vehicle control), aspirin and dose 1, 2 and 3 as samples group of extract. The experiments used two parameters, bleeding time and survival rate. Bleeding time was observed on amputated mice tail and survival rate was observed by the calculation of dead or paralyzed mice after trombosis induction by collagen – epinephrine. P. retrofractum can prolonged the bleeding time and the results was statistically different ($P < 0.05$). P. retrofractum extract dose 1, 2, and 3 can prolonged the bleeding time to 14.41 ± 2.36 ; 17.43 ; 17.70 ± 2.10 ; ± 2.54 and increased the survival rate to 40%;60%;80%. Respectively. The effectiveness showed dose dependent manner with dosage of 11.76 mg /20 g (dose 3) gave the highest prolongation of bleeding time and highest survival rate.

From this research can be concluded the extract of P. retrofractum has a potential activity as antithrombotic agent with dose dependent manner.

antithrombotic agent, Piper retrofractum, bleeding time, survival rate

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Poster Session-PO-31:

Identifying Natural Products that Enhance Spinal Cord Regeneration

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Spinal cord injury (SCI) is the damage to the neural elements in the spinal cord that results in loss of function at the site below the point of injury. Cell-based regenerative therapy, which involves replacing lost cells at the injury site with glial cells has been extensively investigated for treating SCI. Transplantation of olfactory ensheathing cells (OECs) has been shown to enhance functional recovery in different SCI models and has successfully restored some function in paralysed patients in a recent human trial, suggesting OECs may be a promising transplant candidate for neural regeneration.¹ Due to low cell proliferation, growth factors are often used to stimulate the proliferation of the cells. However, their high molecular weight and potential side effects have limited the use of growth factors. To improve OEC transplantation therapy, the development of low molecular weight molecules, which are able to stimulate the activity of OECs is essential. Recent discovery of natural products such as curcumin² and linckosides³, which are able to stimulate proliferation and migration of OECs raised the potential of discovering new natural compounds that enhance the therapeutic properties of OECs. To date, 100 fractions obtained from reversed-phase high-pressure liquid chromatography (HPLC) fractionation of 10 dichloromethane/methanol extracts derived from Australian biota, that includes marine invertebrates and terrestrial plants, have been generated and screened against OECs. From the primary screen, 2 fractions from an Australian tropical rainforest plant were found to stimulate > 25% increase in OECs proliferation activity at 10 µg/mL. Further chemical investigations and biological testing are currently on-going to identify the active compounds.

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Poster Session-PO-32:

Antidyslipidemic Activity of Peanut Shell (*Arachis hypogaea* L) In High-Fat Diet-Fed Rat Model

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Peanut or groundnut is mostly cultivated in Indonesia and it's also the second most important kind of nut after soybean in Indonesia. Peanut shell (*Arachis hypogaea*) is a dreg which contains fibre, saponins, phenols and polyphenols with one of them is procyanidin, which has been known to reduce cholesterol levels in blood plasma.^{1,2} The aim of this research was to investigate the antidyslipidemic activity of the ethanolic extract of peanut shell (PSEE) in the dyslipidemic rat model. The dyslipidemic condition was induced by feeding high-fat diet for 28 days. The antidyslipidemic effect was evaluated at three different doses (150, 300, 450 mg/kg BW) administered for 7 days. The PSEE showed a significant decreased in the serum triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) as well as simvastatin. Moreover, the serum level of high-density lipoprotein (HDL) significantly increased accompanied by an increased HDL-C/TC ratio in a dose-dependent manner. Based on this investigation, it was concluded that PSEE possesses marked antidyslipidemic activity.

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Arachis hypogaea, antidyslipidemic activity, triglyceride, total cholesterol, low-density lipoprotein, high-density lipoprotein

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Poster Session-PO-33:

The standardized *Quisqualis Indica* extract ameliorates benign prostatic hyperplasia and lower urinary tract syndrome.

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Benign prostatic hyperplasia(BPH) is an age-related disease characterized by prostatic enlargement. ^[1] *Quisqualis indica*(QI) known as Rangoon creeper is mostly used against pyrexia, staphylococcal and helminth infection. ^[2] QI extract was standardized by reference to its quisqualic acid content using validated HPLC assay. This study aimed to investigate the protective effects of standardized QI extract against experimentally-induced BPH and lower urinary tract syndrome(LUTS). Testosterone induced BPH in rats was generated via daily subcutaneous injections of testosterone (3 mg/kg) for 4 weeks. The rats were divided into the following six groups (normal/vehicle; BPH/vehicle; BPH/finasteride; and three QI doses) and orally administrated for 4 weeks. The relative prostate weight (prostate/body weight ratio) was calculated. Histological changes and the protein levels of AR and ER were analyzed by immunohistochemistry and western blot. The present studies shown that dose-dependent and significant decreases in prostate weight/body weight ratio and prostate epithelial thickness in the BPH + QI treated rats compared with the untreated BPH rats. P rotein expression of androgen receptor (AR), estrogen receptor α (ER α) were shown in the standardized QI extract treated groups as compared to BPH alone treatment. Furthermore, standardized QI extract selectively inhibited α 1-adrenergic receptor in vitro and then reduced alpha1-adrenergic receptor induced contraction of prostate smooth muscle ex vivo. These results showed that standardized QI extracts could be useful in the clinical treatment of BPH and lower urinary tract syndrome. This work was supported by IPET through Agri-Bio Industry Technology Development Program, funded by MAFRA(116078-3).

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Poster Session-PO-34:

Click and shine – Fluorescence labelling of microcystins

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The recent development of bioorthogonal crosslinking techniques – so-called “click reactions”– offers new possibilities to study the function of compounds in cellular processes [1]. They allow for an easy conjugation

of the molecules of interest with probes and, thus, make them visible by suitable imaging techniques. However, the biorthogonal reactions require functional groups not found in nature, so they need to be introduced synthetically, which can be a challenging task for natural products.

Our molecules of interest – the cyclic heptapeptides microcystins – belong to the best-studied natural products from cyanobacteria, but their physiological role within the host remains unclear [2]. Some findings suggest that they play a crucial role in the adaptation of the cyanobacteria to high light conditions [3]. However, still little is known about the underlying mechanisms, the localization of microcystins in the producing cell, or their interaction partners.

Making microcystins amenable for bioorthogonal chemistry might result in new insights into their biological function and the physiology of cyanobacteria. After labelling with a probe, the microcystins can be localized by microscopic means, or their interaction partners can be identified by proteomics-based approaches. Thus, we present a rapid and feasible technique to introduce azide- and alkyne groups into microcystins: precursor directed biosynthesis. Azide and alkyne functionalized tyrosine derivatives were added to a microcystin-YR producing *Microcystis* sp. strain and successfully incorporated into this congener. This allowed for the conjugation of the “clickable” microcystin with e.g. a fluorescent dye and shed a new light on their role in our subsequent

physiological studies, of which first results are presented.

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Poster Session-PO-35:

In-depth metabolome investigation of a multi-herb formula used in Traditional Chinese Medicine

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In Traditional Chinese Medicine (TCM), preparations consist often in a mixture of herbs. Their quality control is challenging because every single herb already contains hundreds of constituents. The TCM selected for this study is used to treat atopic eczema, a common inflammatory skin disorder. Recently, an open-label clinical study showed the efficacy of this TCM oral formula containing ten herbs [1]. Using this TCM as a

model, we aimed at designing an innovative strategy for a rational and comprehensive standardization. The TCM formula and all single herb extracts were analyzed by UHPLC-PDA-ELSD-HRMS. The UHPLC-HRMS metabolite profiling allows the detection of several thousand m/z features. A strategy was elaborated to filter all this information and select key biomarkers. First, peak picking was performed on all single herbs extracts and on the TCM mixture. Alignment of all features in a 2D reconstructed ion map provided an efficient way to correlate the features to each herb of the mixture. Second, ELSD detection was used as a filter for a selection of the most abundant features. Third, these features were dereplicated by combining MS/MS based molecular network and taxonomy [2]. This information was used for a MS targeted isolation of selected markers by MPLC directly on the mixture of the ten herbs. The approach enabled an in-depth metabolome characterization of the TCM and allowed an efficient isolation of all markers necessary for a comprehensive standardization of the TCM through multiple targeted MRM quantification. This workflow takes into consideration the complexity of the multi-herb preparation and provides an efficient way for their quality control.

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Poster Session-PO-36:

Lupane, f riedelane, oleanane, and u rsane t riterpenes from the s tem of *Siphonodon celastrineus* Griff.

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Siphonodon celastrineus Griff. (Celastraceae) is a tree that grows up to 25 m. in height and is found in the northern and central parts of Thailand. In Thai traditional medicine, its roots are used for the treatment of inflammation, abscesses, skin diseases, and as a bone tonic ^[1]. Recently, an ethanolic extract of the leaves of *S. celastrineus* showed cytotoxic activity against the MCF-7 cancer cell line with an IC₅₀ value of 17.1 µg/mL ^[2]. Subsequent chemical investigations of the root bark of this plant revealed the presence of the oleanane triterpene and the quinone methide triterpene from the methanolic extract ^[3]. Preliminary screening of dichloromethane extract of the stem of this plant in our laboratory showed cytotoxic activity against the MOLT-3 cancer cell line (86% cytotoxicity at 10 µg/mL). The chemical and biological properties of this plant

were thus further investigated. In this presentation, the isolation and structure elucidation of forty-one new triterpenes consisting of a lupine, two friedelane, two oleanane, and thirty-six ursane - type triterpenoids, together with nine known compounds were isolated from the stem of *S. celastrineus*. The structures were characterized by various spectroscopic techniques, comparison with literature data, and chemical transformation. Several compounds were evaluated for their cytotoxic activity against six human cancer cell lines (MOLT-3, HuCCA-1, A549, HeLa, HepG2, and MDA-MB-231).

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Poster Session-PO-37:

Cytotoxic and cancer chemopreventive properties of prenylated stilbenoids from *Macaranga siamensis*

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The genus *Macaranga*, which is one of the largest genera of the Euphorbiaceae family, contains approximately 280 species and is distributed between West Africa and the islands of the South Pacific [1]. In Thailand, 22 species have been recorded [1]. Some species has been used as a traditional Thai medicine for antipyretic, antitussive and anti-inflammatory treatment [2]. *Mammea siamensis* S.J. Davies is a large-leaved plant of the tropical dry seasonal forest and is common throughout central and northern Thailand. No previous chemical investigation of this plant has been reported. In this work, twenty-five new compounds, including prenylated stilbenene, flavonol, flavanone, resorcinol, and 1,4-phenanthraquinone derivatives, together with five known compounds, were isolated from the dichloromethane extract of the leaves and twigs of *M. siamensis*. Their structures were established using spectroscopic techniques. Their cytotoxic and antioxidant properties were evaluated together with their effectiveness as aromatase inhibitors.

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Poster Session-PO-38:

The anti-inflammatory activity of ursolic acid isolated from *Plantago lanceolata* in mice

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Inflammation has been known to play a crucial role in the development of various human disorders such as atherosclerosis, cancer, asthma, hepatitis, rheumatoid arthritis, and periodontitis. Medicinal plants have been widely used for decades to treat numerous disorders including inflammatory-related diseases and provide an abundant source of natural compounds for medication. One of the medicinal plants traditionally used to cure inflammatory diseases is *Plantago lanceolata*. In the previous study, we showed that the n-hexane insoluble fraction of *Plantago lanceolata* leaves (HIFPL) demonstrated a potent anti-inflammatory activity. The purpose of this study was to investigate the active compounds responsible for the antiinflammatory activity of *Plantago lanceolata* by utilizing a mice experimental model.

The leaves of *Plantago lanceolata* were dried, powdered and macerated in dichloromethane. The liquid extract was then evaporated in a rotary evaporator instrument until dryness. Dried extract was then partitioned using n-hexane to obtain HIFPL and n-hexane soluble fraction. Both fraction was evaluated for their anti-inflammatory in mice model. The more active fraction (HIFPL) was then separated using a preparative TLC to isolate the major compound. The structure of the isolated compound was determined based on the NMR, IR and LC-MS spectra. The content of the isolated compound in the HIFPL was determined by TLC-densitometry. A thioglycollate-induced leukocytes migration in mice was used as a pharmacological assay to evaluate the inflammatory activity of the fractions and isolated compounds. The level of chemokines (MCP-1 and IL-8), the main cytokine regulating the chemotaxis activity was also evaluated.

We found that ursolic acid, the main compound isolated from HIFPL is responsible for the antiinflammatory activity. It demonstrated antiinflammatory activity by inhibiting the migration of leukocytes, at least partly, by lowering the level of IL-8 and MCP-1. This results provide a scientific evidence regarding the traditional usage of *Plantago lanceolata* for inflammatory disorders.

Poster Session-PO-39:

Polyphenol extracts from berry press residues: characterization of chemical composition and biological activity

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Berries of Northern European bogs and forests (*Vaccinium* spp.) contain significant quantities of various phenolic compounds. Most of these compounds are recovered when berry juice is produced. However, a considerable part of polyphenols remains in berry press residues and are discarded as food industry waste. The aim of the study was to compare the methods of extraction of polyphenols from press residues of American cranberry and optimize the extraction conditions. The impact of main extraction parameters (e.g., extraction time, solid/solvent ratio, solvent type) on the yield of extracted polyphenols was examined. Ultrasound-assisted extraction showed the highest potential from all studied methods, given its fast, convenient use and low cost it was possible to recover up to 5 g of polyphenolics from 100g of berry press residues (bilberry). Response Surface Methodology (RSM) was used to identify the optimal solvent composition for extraction, which was found to be 70% ethanol in combination with 1% formic acid. Antimicrobial activity of extracts was evaluated by the Agar Diffusion Method, which showed a potential use of extracts to inhibit growth of human pathogens in concentrations <0.0125 mg/L. Proliferation of human dermal fibroblasts was inhibited, and cell cytometry showed high antiradical activity in vitro, which was 2 times higher than that of Vitamin-C. The purified polyphenol extracts contained up to 15 different anthocyanins and 150 other polyphenols (identified by Orbitrap-MS) of which 37 were quantified and used for chemotaxonomic analysis. Taken together, these results put the waste product- berry press residues- as a valuable source of compounds that can be used to develop new nutraceuticals and functional foods with various health benefits.

Poster Session-PO-40:

Detection of chemical profile in *Ophiopogonis Radix* under different drying methods by LC-MS

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Ophiopogonis Radix, the root of *Ophiopogon japonicus*, it was rich in steroidal saponins, flavonoids,

polysaccharides and organic acids, with various pharmacological activities, including cardiovascular protection, anti-inflammation, anti-cancer, anti-oxidation, and anti-diabetes^[1]. Drying methods can be a limiting factor of TCM'S development, it is one of the most important factors related to its quality, as it can benefit in preserving bioactive components, decreasing toxic and side effects of Chinese medicine.

Based on our previous research, we found that there were 3 main drying methods in main production area of *Ophiopogonis Radix*: traditional sun drying (TSD) , sulfur coal drying (SCD) , sulfur -free coal drying (SFCD). So the optimized drying method was studied by evaluate the quality of *Ophiopogonis Radix* in 7 drying methods: TSD , SCD , SFCD , and hot-air drying under different temperatures (30, 40, 50, and 60°C). Moisture, total ash, water-soluble extracts contents were studied, and the constituents of 7 homoisoflavonoids were detected by LC-MS . The results showed that 60°C hot-air drying was the optimized method, for samples under 60°C hot-air drying condition had a higher concentrations of homoisoflavonoids, saponins, and water-soluble extract contents, while their total ash content was lower.

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Poster Session-PO-41:

Natural Products as Modifiers of Antibiotic Resistance

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The resistance of commensal bacteria to first and second line antibiotics has reached an alarming level in many parts of the world and endangers the effective treatment of infectious diseases. It is a complex global public health challenge that leads to prolonged illness and increased mortality, increases the costs for the health-care sector, and has an impact on animal health, which also could lead to an effect on food production¹. The development of resistance-modifying agents (RMAs) can mitigate the spread of bacterial drug resistance and possibly extend the useful life of an antibiotic, importantly in consideration of the lack of new antibiotics². We investigated the activity of nine methanolic extracts of plants, which were used traditionally to cure wounds, and some single substances as an RMA of multi-resistant clinical isolates of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* (MRSA) and *Enterococcus faecium* (VRE) which can be involved in wound infections. The extracts and single substances were combine with ampicillin, piperacillin, imipenem, vancomycin, gentamicin and aztreonam and the effects were investigate with the chequerboard method. We found 29 combinations that worked synergistically, mainly

with ampicillin and gentamicin on the gram positive strains. Five synergistic combinations were found for the first line antibiotics ampicillin (*Enterococcus faecium*) and piperacillin (*Pseudomonas aeruginosa*) and two for the last line antibiotic vancomycin (*Enterococcus faecium*). The highest diminishment of antibiotic resistance shows the extract of *Cetraria islandica* with gentamicin (MIC shift: 16 to 0.001953125 µg/mL), extract of *Salvia officinalis* with vancomycin (MIC shift: 256 to ≤0.015625 µg/mL) and glycyrrhizic acid with gentamicin (MIC shift: 131072 to between 8 and 16 µg/mL, high-level resistant isolates) on *Enterococcus faecium*.

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Poster Session-PO-42:

Proteomic analysis of the anti-obesity and anti-diabetes effects of *Platycodon grandiflorum* in mice

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Natural products are getting much attractions to prevent and cure these metabolic diseases. Investigation of therapeutic mechanisms of the natural products is necessary to use the natural products as complementary medicines. Recently, extract of *Platycodon grandiflorum* root (PGE) is known to have beneficial effects on obesity and diabetes. However, its detailed mechanism of anti-obesity and anti-diabetes effect is poorly understood. In this study, we investigated the anti-diabetic role of PGE by proteomic and bioinformatic analysis in the mice liver tissue. Mice were fed with 60% kcal of high fat diet (HFD) with or without 5% of ethanol extract of PG root. After 16 weeks of diet, mice were tested for their physiologies of obesity and diabetes. Mice fed with PGE showed dramatic reduced body weight. Weight of liver and fat tissue also decreased in PGE group. Furthermore, mice of PGE group showed lower fasting glucose level and improved glucose and insulin sensitivity. In order to investigate the underlying mechanism of anti-obesity and anti-diabetic effect of PGE, the livers were isolated and hepatic proteome was examined by LC-MS/MS. Comparison of protein expression profiles revealed that changes of protein expression in HFD mostly reverted to normal state by intaking PGE. The proteins were mainly involved in lipid metabolism, fatty acid β-oxidation, oxidative phosphorylation, energy production, and mitochondrial dysfunction. Interestingly,

proteins associated with epithelial adherents junction signaling are also regulated by PGE in the rescue process of type 2 diabetes.

Poster Session-PO-43:

Fomosamines A–D, New Alkaloids from Formosan Zoanthid *Zoanthus vietnamensis*

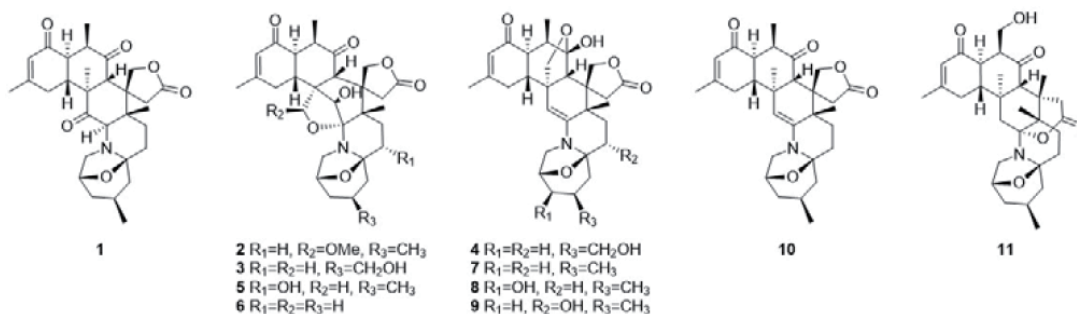
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Sea anemones of the genus *Zoanthus* (family Zoanthidae) are commonly found in subtropical or tropical coastal area. These marine invertebrates are usually cultured in aquarium because of its various colors. *Z. vietnamensis* Pax & Müller, having a pale pink oral disc with black and white tentacles, was collected in north coastal area of Taiwan and identified by its mitochondrial and nuclear sequence-based phylogenies.¹ In previous natural product studies, alkaloids, ceramides, steroids, and sphingolipids were regarded as the major components of zoanthid. In our chemical investigation of *Z. vietnamensis*, four new alkaloids named fomosamines A–D (1–4), along with seven known compounds, 7 α -hydroxykuroshine A (5), kuroshine A (6), zoanthenamine (7), 3 β -hydroxyzoanthenamine (8), 7 α -hydroxyzoanthenamine (9), 28-deoxyzoanthenamine (10), and oxyzoanthamine (11) were identified. The structures of all isolated compounds were determined by the interpretation of spectroscopic methods, especially 2D NMR analyses (COSY, HSQC, HMBC, and NOESY). Compound 1 is an oxidized analogue of 28-deoxyzoanthenamine (10) that contains a keto group at the C-11 position. Compound 2 can be characterized as a new kuroshine A type alkaloid with a hemiacetal at C-28. Compounds 3 and 4 are new hydroxy derivatives of kuroshine A (6) and zoanthenamine (7), respectively. All compounds were evaluated by MTT assays for cytotoxicity against MDA-MB-231, A549, HepG2 cancer cell lines.



alkaloids; *Zoanthus vietnamensis*; marine natural products

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Poster Session-PO-44:

Harnessing the Anti-inflammatory Potential of South African Medicinal Plants used traditionally in the Treatment of Sexually Transmitted Infections.

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Inflammatory responses and pain are the first lines of action triggered by the body's immune response to fight off infections [1]. They play a major role in the etiology of sexually transmitted infections (STIs) and could lead to more chronic inflammatory diseases when treatment is delayed [2]. The anti-inflammatory activities of twelve plants used traditionally in the treatment of STIs were investigated in this study. Acetone extracts of selected plant parts were tested for inhibitory activity against 15-lipoxygenase (15-LOX), xanthine oxidase (XO) and inducible nitric oxide production in lipopolysaccharide (LPS) stimulated RAW 264.7 murine macrophages. Cytotoxic assessment of extracts was also conducted on murine macrophages and Vero cells using the MTT assay. Extract of *Lannea schweinfurthii* was most active against 15-LOX (IC_{50} 40 ± 3 $\mu\text{g/mL}$), this was followed by the extracts of *Ficus abutilifolia*, *Cassia abbreviata* and *Pseudolachnostylis maprouneifolia*. Interestingly, activities against XO was recorded for the first time for most of the extracts and the best activity was observed with extract of *C. abbreviata* (IC_{50} 46.8 ± 1.5 $\mu\text{g/mL}$). All the extracts showed a dose-dependent suppression of inducible nitric oxide production in murine macrophages. Particularly, extracts of *F. abutilifolia*, *Faurea saligna*, and *Zanthoxylum capense* had activities comparable to quercetin used as the positive control ($IC_{50} < 30.0$ $\mu\text{g/mL}$). Most of the extracts showed mild or no toxic effect against tested cells lines. The exception was however observed with the stem bark extracts of *Z. capense* which had low (4.4%) murine macrophage viability at the highest tested concentration (100 $\mu\text{g/mL}$). Findings from this study reveal the potential of active extracts as new sources of anti-inflammatory compounds which can be further explored for the management of STI related inflammation.

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Poster Session-PO-45:

Flavonoid compounds of *Crataegus x bornmuelleri* Zabel leaves

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Turkey has a rich flora because of its geographical location. Some of these plants have the qualities to be used therapeutically due to their chemical compounds. *Crataegus* genus, known as Hawthorn, are used against a variety of diseases, mainly cardiovascular diseases, due to the flavonoids they contain. *Crataegus x bornmuelleri* Zabel, which is hybrid species of *C. orientalis* and *C. tanacetifolia*, grows as endemic in Turkey. In this study, *C. x bornmuelleri* leaves were collected from Beypazari (Ankara - Turkey) district in July 2017. The plant samples have been identified by Prof. Dr. Sukran Kultur (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University) and deposited in the Herbarium of the Faculty of Pharmacy at Istanbul University (ISTE). 8 flavonoids (2 aglycons and 6 glycosides) were isolated from the leaves of *C. x bornmuelleri* and these flavonoids were determined by using various spectroscopic techniques.

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Crataegus x bornmuelleri, Flavonoids, Medicinal Plants, Turkey.

Poster Session-PO-46:

Oil composition and Antioxidant activity of Safflower (*Carthamus tinctorius* L) Germplasm : A Comparative studies

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We have investigated the fatty acid composition and antioxidant activity in the seeds of 100 safflower accessions collected from India. The oil was recovered using in a soxhlet extraction method. The fatty acids were analyzed using gas chromatography [1]. Antioxidant activity was measured using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays. The total polyphenol content (TPC) was estimated using Folin-Ciocalteu method [2]. The oil was ranged between 15.82 and 32.23% across the entire accessions. Linolenic, palmitic, and stearic acid ranged from 0.02 to 2.00%, 4.84 to 7.57%, and 1.30 to 3.27%, respectively. Oleic and linoleic acid showed wide

variation from 10.69 to 75.73% and 15.54 to 79.87%, respectively, compared to other fatty acids. TPC, ABTS, and DPPH assay results were in the range 5.07 to 52.08 µg gallic acid equivalent (GAE) /mg dried extract (DE), 4.64 to 26.95 µg trolox equivalent (TE) /mg DE, 3.40 to 74.97 µg ascorbic acid equivalent (ASCE) / mg DE, respectively. The cluster analysis showed that accessions were segregated into three clusters. Group II, comprising 11 accessions, had high oleic acid composition. However, group III which contained 25 accessions had high antioxidant activity safflower accessions compared to other groups. TPC showed a significant positive correlation ($r = 0.875$) with ABTS. The first two principal components had accounted for the 60.75% of the total variance. Our results showed that K184660 and K184530 could be used as a source of valuable natural antioxidant materials, whereas IT300325, IT300326, and K185831 which exhibited high oleic acid composition, could be useful to develop new functional oil materials. [1] Velasco, L., Goffman, F. D., & Becker, H. C. Genetic Resources and Crop Evolution 1998; 45: 371-382. [2] Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. Journal of Agricultural and Food Chemistry 2002; 50: 1619-1624.

Poster Session-PO-47:

Identification of NO production inhibitory constituents from *Catalpa ovata* using LC-QTOF MS/MS

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The ethyl acetate fraction of *Catalpa ovata* (Bignoniaceae) exhibited a strong inhibitory effect on NO production in lipopolysaccharide-induced BV2 microglia cells. Recently, LC-QTOF MS/MS spectrometry has been used extensively to identify compounds in crude extracts without the need for separation; this hyphenated technique also provides information on the molecular weight and type of the compounds of interest [1,2]. We tried to identify the active constituents of *C. ovata* by using LC-MS coupled with a cell-based assay. A new iridoid compound, 6-O-trans-feruloyl-3 α -hydroxy-7-deoxyrehamaglutin A (4), and nine known compounds were isolated from the extracts of *C. ovata* by repeated column chromatography. Compounds 1-3, and 5-10 were identified as p-hydroxybenzoic acid (1), arillatose B (2), p-hydroxyphenylferulate (3), 6-O-(E)-feruloyl- α -glucopyranoside (5), 1-O-p-coumaroyl- β -D-glucopyranose (6), 6-O-caffeoyl- β -D-glucose (7), minecoside (8), catalposide (9), and 6-O-trans-feruloyl-5,7-bisdeoxycynanchoside (10) through comparison of the $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and MS data with reference spectra. Among them, compounds 3, 4, 5, 7, and 8 significantly attenuated lipopolysaccharide-stimulated NO production in BV2 cells. Our results indicate that time-dependent LC-MS coupled with a cell-based NO production inhibitory assay successfully predicted active compounds without a time-consuming isolation process.

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Poster Session-PO-48:

Three new monoterpene glycosides from the twigs of *Hamamelis japonica*

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Hamamelis L., the witch hazel genus, is disjunctly distributed in eastern Asia mainly China and Japan and eastern North America. Compared to other *Hamamelis* species, only few studies have been done with *H. japonica* Sieb. et Zucc. In the present investigation, the chemical constituent study of the twig of *H. japonica* has performed for the first time. Three new monoterpenoid glycosides (1–3) together with 15 known compounds were isolated from the twig of *H. japonica*. The structures of compounds were determined based on extensive spectroscopic methods including 1D and 2D NMR and CD spectra data. Planar structure of aglycone of compounds 1 and 2 were p-menthane-1,2,8-triol, while compound 3 was a camphene. The remaining known compounds identified as three flavanoids (4–6), seven phenolics (7-13), one glycoside (14) and four stilbenes (15-17) by comparing with reported data.

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Poster Session-PO-49:

Unusual Flavones from *Primula macrocalyx* as Inhibitors of OAT1 and OAT3

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Primula macrocalyx Bunge (Primulaceae) is a perennial herbaceous plant, which has been widely used as an expectorant, diuretic, sedative, spasmolytic, in folk medicine to treat vitamin deficiencies, colds, fever,

insomnia, paralysis, scurvy, heart disease, rheumatism, and kidney diseases.¹

The organic anion transporters (OATs) play key roles in the distribution and excretion of drugs. Specifically, OAT1 and OAT3, which are highly expressed in the kidney, play important parts in the renal elimination of a range of substrate molecules.² Apparently, the development of several above diseases is closely related to OATs.

As part of an ongoing program exploring the interaction of dietary phytochemicals with the OATs, a bioactivity guided fractionation was performed on the methanol extract of *P. macrocalyx* collected in Armenia, followed by structure determination of the isolated compounds based on LC-MS and NMR data, leading to the elucidation of twelve flavones, including one previously unreported compound.

To our knowledge, this study is the first to evaluate these compounds as inhibitors of the OATs, and may allow an initial elucidation of the structure activity relationships within this group. These data may also provide a rational basis for the therapeutic applications of *P. macrocalyx* in traditional medicine.

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Poster Session-PO-50:

Phytochemical Investigations of *Eremostachys molucelloides* Bunge (Lamiaceae)

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The genus *Eremostachys* Bunge (Lamiaceae) consists of 30 species distributed from Europe through Central Asia to Pakistan and Afghanistan. Of these, five are known to occur in China [1]. Reports in the literature indicate that essential oils and glycosides of iridoids, phenylethanoids, flavonoids, and diterpenes have been isolated from members of this genus. The limited nature of these reports makes this genus an attractive target for further investigation. In this study, the aerial parts of *Eremostachys molucelloides* Bunge (syn. *E. macrophylla* Montbret & Aucher ex Benth.) were collected in Kotayk Province, Armenia, between Garni and Zovashen, near Kotayk. Chromatographic fractionation of the methanol extract of this plant has afforded a variety of compounds including flavonoids, flavonoid glycosides, phenylethanoids, vanillic and p-coumaric acids. The structures of these compounds have been elucidated primarily on the basis of their 1D and 2D NMR spectroscopic data and LC-MS.

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Poster Session-PO-51:

Phytochemical Investigations of *Anchusa azurea* Mill. (Boraginaceae)

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Anchusa, comprised of approximately 35 species mainly distributed along the Mediterranean coast and Africa, Europe and western Asia, is one of the major genera of the family Boraginaceae. Some species in *Anchusa* are used as a folk medicine in different countries for a variety of ailments including wound healing, as a diuretic, and as a sedative. In previous reports, triterpenes and triterpene glycosides, flavonoids, alkaloids and phenolic acids have been isolated from species in this genus. Reported bioactivities, such as anticancer and antioxidant effects, also makes it an attractive target for further investigation. In the present work, a number of flavonoids, flavonoid glycosides, phenylpropanoids, phenylpropanoid amides and monoterpenes have been isolated from the methanol extract of a sample of the entire plant of *Anchusa azurea* Mill. from Armenia. Several of these constituents have been isolated for the first time from the genus *Anchusa* and the family Boraginaceae. The structures were elucidated by 1D and 2D homonuclear and heteronuclear NMR spectroscopy and by comparison with closely related compounds published previously. phytochemistry, Boraginaceae, *Anchusa azurea* , flavonoid glycosides, 2D NMR.

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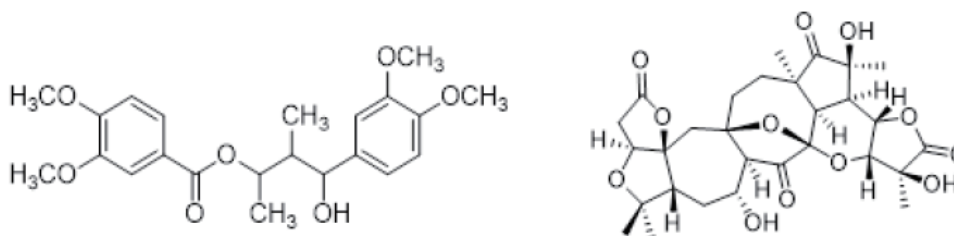
Poster Session-PO-52:

Chemical Components from the fruits of *Schisandra sphenanthera* Rehd. Et Wils and their cytotoxicity

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Schisandra sphenanthera (Schisandraceae) has been used for the traditional medicines in Asia as a sedative and anti-tussive agent and reported to have antihepatitis, antiviral and anti-HIV activities. *S.sphenanthera* is widely distributed in the East-Asia and Southeast Asia. Using various chromatographic resins and isolation techniques, a new lignan, 4-(3,4-dimethoxyphenyl)-4-hydroxy-3-methylbutan-2-yl-dimethoxybenzoate (1) along with six known lignan derivatives (2-7), one new nortriterpenoid, sphenadilactone G (8) with four known nortriterpenoids (9-12), three known phenolic compounds (13-15) and one known triterpenoid (16) were isolated from the fruits of *S.sphenanthera*. The structures of all the isolated compounds were identified by extensive spectroscopic methods including 1D and 2D NMR. All the isolated compounds were tested for their cytotoxicity against Hela, HepG2 and HCT-116 cells. Among the tested compounds, schisanlactone C (16), gomisin D (7) and lancifodilactone L (9) showed significant cytotoxicity.



Schisandra sphenanthera Rehd. Et Wils, Schisandraceae , Lignan, Nortriterpenoid, Cytotoxicity

Acknowledgements:

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Poster Session-PO-53:

Phytochemical and pharmacological screening of Chinese medicinal plants for inhibitory effects on NO production in LPS/IFN- γ stimulated RAW 264.7 macrophages

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Nitric oxide (NO) is a key messenger in the pathogenesis of inflammation, linking innate and adaptive immunity. Overproduction of nitric oxide (NO) has been associated with damage to normal cells and tissues,

leading to pathogenesis of various inflammatory diseases [1]. Due to the global relevance, the search for natural products targeting inflammatory diseases has been intensified in recent years [2].

The aim of this project is to identify Chinese medicinal plants with anti-inflammatory properties and to subsequently isolate new anti-inflammatory lead structures. Using Accelerated Solvent Extraction (ASE), we generated a high number of extracts with solvents of different polarities. The pharmacological screening of the extracts was performed at 50 µg/ml in an iNOS assay using LPS/IFN-γ stimulated RAW 264.7 mouse macrophages [3]. Out of 374 (100%) tested extracts from Chinese herbal drugs, 45 (12%) showed inhibitory effects over 20%. 21 extracts (5.6%) thereof turned out to be substantially active with inhibitory effects over 40%. Out of 45 extracts, 20 were obtained using dichloromethane, 21 were methanol extracts, and only three were water extracts. Significant inhibition showed *Curcumae longae* rhizoma, *Zingiberis rhizoma*, *Inulae flos*, *Kochiae fructus*, *Chaenomelis fructus* and *Citri reticulatae* pericarpium extracts.

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Poster Session-PO-54:

Cyclooxygenase inhibitory properties of *Osmanthus fragrans* flowers

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Chronic inflammation plays a key role in many diseases like cancer, diabetes, asthma, and arteriosclerosis.[1] Anti-inflammatory drugs are often targeting cyclooxygenase isoenzymes despite some severe side effects, like gastric ulcers, kidney damage, cardiovascular incidents, and bronchospasm, especially in long term use. Hence, there is a great interest in finding new lead compounds targeting the expression of inflammatory mediators such as COX-2 or the interleukin-group. [2]

Previous studies have shown that megastigmane derivatives have anti-inflammatory properties.[3] Therefore, five plants which are known to contain such constituents were selected as candidates for the isolation of

similar and even more potent compounds. The plant material was extracted successively with solvents of different polarity, and the extracts were tested for COX-2 mRNA expression in PMA differentiated LPS stimulated THP-1 monocytes.

Among the tested plants, extracts of sweet olive flowers (*Osmanthus fragrans* Lour.) were found to be very active. n-Hexane and dichloromethane extracts showed 41.79 ± 4.22 % and 46.15 ± 5.31 % inhibition of COX2 mRNA expression, respectively, at concentrations of 20 µg/ml, whereas methanol extracts did not show significant effects. Active extracts were further fractionated using column chromatography. Isolation, structure elucidation and pharmacological characterization of the active compounds are in progress.

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Poster Session-PO-55:

Assignment of Immunomodulatory Compounds in Huangqi Jianzhong Decoction by Combining LC-MS Metabolomics and in-vitro Cytokine Production Assays

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Within non-communicable diseases (NCD), chronic inflammatory conditions represent one of the biggest challenges for modern medicine [1]. Traditional Chinese medicine (TCM) has been practiced over centuries to treat such diseases and has gained tremendous empirical knowledge. Huangqi Jianzhong Tang (HQJZT) is a famous TCM formula and decoction composed of *Radix Astragali*, *Ramulus Cinnamomi*, *Radix et Rhizoma Glycyrrhizae Praeparata cum Melle*, *Radix Paeoniae alba*, *Rhizoma Zingiberis recens*, *Fructus Jujubae* and *Saccharum granorum*, which has been used for the treatment of various chronic inflammatory

gastrointestinal diseases [2,3]. However, there is insufficient knowledge about the active compounds and mechanisms responsible for its beneficial effects.

For the analysis of the constituents of HQJZT, UHPLC- Xevo G2-XS QTOF MS based metabolomics analyses were carried out. Data processing and compound identification was performed using UNIFI software and Traditional Medicine Library. The inhibitory effects of HQJZT on TNF- α , IL-1 β and IFN- γ production were studied in U937 cells. The data were correlated by multivariate data analysis (PCA, OPLS-DA) in order to identify constituents in HQJZT which may be relevant for activity.

The investigations resulted in the identification of 16 main compounds in HQJZT which are most likely contributing to its immunomodulatory and anti-inflammatory effects. Six of the compounds, namely calycosin, formononetin, astragaloside I, liquiritigenin, paeoniflorin and albiflorin were verified by LC-MS comparison with reference substances. Literature data confirmed that these compounds have anti-inflammatory effects.

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Poster Session-PO-56:

OCT2-mediated choline uptake protects kidney proximal tubule cells from palmitic acid-induced renal toxicity

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Astragalus propinquus (known as Mongolian milkvetch in English and as huáng qí in Chinese) is commonly used in Traditional Chinese Medicine to treat chronic kidney diseases. The dominant contents of *Astragalus propinquus* were analyzed and choline was found to be one of the effective compounds for kidney disease treatment. In this study, the kidney protective effect of choline was examined in primary mouse proximal tubule cells and HEK cells. Lipidomic analysis showed that deoxysphingolipid (deoSL) levels were increased in the plasma from patients with chronic kidney disease (CKD), indicating deoSL as toxins in CKD. In vitro study revealed that palmitic acid (PA) induced increased levels of deoSL as well as intracellular ROS. Choline treatment attenuated such increases. The regulatory effect was mediated by organic cation transporter 2

(OCT2), as OCT2 overexpressing cells were protected from PA-induced toxicity. On the other hand, TNF α , which is commonly increased in chronic kidney disease, decreased OCT2 expression levels and reduced choline uptake in kidney proximal tubule cells. In summary, these results revealed a potential therapeutical role of *Astragalus propinquus* in the treatment of chronic kidney disease.

Poster Session-PO-57:

Phytochemical characterization and anti-dementia effects of major constituents from the seeds of *Zizyphus jujuba* var. *spinosa*

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The seeds of *Zizyphus jujuba* Mill. var. *spinosa* (Bunge) H. H. Hu ex H. F. Chow (酸枣仁; family Rhamnaceae) has been used as traditional medicine to treat sedation and hypnosis in Eastern Asia. Our previous studies have indicated that the ethanolic extract of *Z. jujuba* var. *spinosa* (DHP1401) improves memory impairment, a common symptom of various neurological diseases. In this study, the chemical composition of DHP1401 has been isolated and characterized. Additionally, we investigated the anti-dementia effects of major components of DHP1401 on memory impairment by scopolamine, a muscarinic receptor antagonist. Phytochemical constituents were isolated from the dichloromethane, ethylacetate, n-butanol fractions of DHP1401 using repeated chromatography and prep-HPLC systems to yield 13 compounds. The isolated compounds were elucidated as pinostrobin (1), 6-demethoxyafmosin (2), diosmetin (3), buddlenol C (4), ficusequilignan A (5), ceanothic acid (6), alphitolic acid (7), naringenin (8), 6''-feruloylspinosin (9), nicotiflorin (10), swertisin (11), spinosin (12), magnoflorine (13) by spectroscopic analysis. Among them, swertisin (11) and spinosin (12), which are well known as the major components of *Z. jujuba* var. *spinosa*, attenuated the scopolamine-induced cognitive deficit in passive avoidance task. Further studies will focus on the profiling of components in DHP1401 and the characterization of the constituents responsible for the anti-dementia effects.

Poster Session-PO-58:

Canadine from *Corydalis turtschaninovii* Enhances Myogenesis and Protects against Myotube Atrophy

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Muscle atrophy including cachexia and sarcopenia, result in a reduction in the muscle fiber area, myo-protein content, and muscle strength, with various molecular modulators being involved. To search myogenic modulators from medicinal plants to treat muscle diseases, we isolated six alkaloids from *Corydalis turtschaninovii* (CT) and evaluated their myogenic potential in C2C12. Among them, canadine showed the strongest transactivation of MyoD and increased the number of multinucleated and cylinder-shaped myotubes during myogenesis. The activation of p38 MAPK and Akt are major mechanisms that contribute to the myogenesis by canadine.

In addition, canadine ameliorated the muscle protein degradation caused by CT26 colon carcinoma culture medium (CT26 CM), which is established to mimic cachexia, by down-regulating the muscle specific-E3 ligases, MAFbx/atrogin-1 and MuRF1.

In this study, we found that canadine from CT stimulates myogenesis and also inhibits muscle protein degradation. Therefore, we suggest canadine as a protective agent against muscle atrophy.

Poster Session-PO-59:

Asana, *Pterocarpus marsupium* as a functional material for supplemental food.

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The decoction from a tumbler made of heartwood of the Asana (Indian kino tree, *Pterocarpus marsupium*) was a remedy for diabetes mellitus in Ayurvedic medicine. In our research program for investigating novel anti-aging agent, we evaluated the anti-aging potency of Asana heartwood using disseminated intravascular coagulation syndrome (DIC), skin lightening activities and anti-oxidant activities as indicators. As results, oral administration of the extract at 200 and 500 mg/kg to DIC model rats reduced blood passing times on MC-FAN with 23 and 56%, respectively. In addition, the extract demonstrated showed suppressive effects

on collagen-induced platelet aggregation with 23 and 73% at 200 and 500 µg/ml, respectively. Furthermore, the active principle was determined to be pterostilbene with activity-guided purification and the compound showed 31, 56 and 91% of inhibition at 5, 20 and 50 µM, respectively. Moreover, the extract showed inhibition against both cyclooxygenase-1 and -2.

Inhibitory effects on tyrosinase and melanogenesis were tested for skin lightening activities and the extract showed 45 and 68% inhibition against tyrosinase at 50 and 200 µg/ml, respectively. In addition, the extract showed 45, 53 and 69% of anti-melanogenesis activity at 5, 20 and 50 µg/ml, respectively. Oxyresveratrol was determined as an active principle by activity-guided purification and showed 73, 81 and 85% of tyrosinase inhibition at 2, 5 and 10 µM, respectively.

In the anti-oxidative activities, the extract showed DPPH radical scavenging activity with 16, 36 and 67% at 5, 10 and 20 µg/ml, respectively. An active principle was determined as (+)-dihydrorobinetin by activity-guided purification. The extract also suppressed the production of AGEs with 48, 54 and 60% and 0.78, 3.13 and 12.5 µg/ml, respectively. Liquiritigenin was identified as the one of the active principles.

These results demonstrate that the extract of Asana heartwood may be a promising candidate as a multifunctional material for anti-aging.

Poster Session-PO-60:

Inhibitory effects of essential oil extracts from Panax plants against β -secretase and cholinesterases.

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The genus Panax (Araliaceae) plant is one of the most important crude drugs used in ancient Chinese medicines (Kampo in Japan) prescriptions. Triterpenoid saponins were identified as active components of the genus Panax. The previous reports were mainly on pharmacological effects derived from triterpenoid saponins. However, few reports on other compounds were published. In the previous study, we investigated pharmacological effects of aromatic components of *P. ginseng* (Pg). We reported preventive and mitigation effects against Alzheimer's disease (AD). The purpose of this study is to perform comparative study on essential oil obtained from various Panax plants for preventive and mitigation effects against AD. Inhibitory activities against β -secretase (BACE), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) of essential oil of Pg, *P. japonicus* (Pj), *P. notoginseng* (Pn), and *P. quinquefolius* (Pq) were evaluated. As results, Pn (50 µg/mL) showed most potent inhibitory activity with 27.7% of inhibition against BACE (Table). In addition, Pg (50 µg/mL) showed most potent activity with 35.3% and 80.2% against AChE and BChE, respectively. We performed GC-MS analysis about essential oil of Pg and determined the main components

as β -elemene and α -humulene from database search (Wiley7th and NIST08). These two components showed a significantly inhibitory effect on AChE and BChE, especially β -elemene (250 μ M) showed 99.8% and 80.7% inhibition against AChE and BChE, respectively. From these results of this study, the inhibitory effect of essential oil obtained from genus *Panax* against BACE, AChE and BChE was clarified. Furthermore, essential oil of Pn would be effective for prevention of AD while essential oil of Pg would be effective for mitigation of cognitive dysfunction.

Table. Inhibitory activities against BACE, AChE and BChE of essential oil obtained from genus *Panax*.

Enzymes	Samples (50 μ g/mL)			
	<i>Pg</i>	<i>Pj</i>	<i>Pn</i>	<i>Pq</i>
BACE	2.9%	16.4%*	27.7%**	9.3%
AChE	35.3%**	12.8%	13.8%*	9.4%
BChE	80.2%**	71.3%**	41.5%**	47.3%**

Data are indicated as inhibition rates (%).

Significantly different from the control at *: $P < 0.05$, **: $P < 0.01$.

Poster Session-PO-61:

Inhibitory effects of *Anemarrhena asphodeloides* and major constituent in acute lung injury model

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The rhizomes of *Anemarrhena asphodeloides* have a long history of use against lung inflammatory disorders in traditional herbal medicine. However, the therapeutic potential of this plant material in animal models of lung inflammation has yet to be evaluated. In the present study, we prepared the alcoholic extract and derived the saponin-enriched fraction from the rhizomes of *A. asphodeloides* and isolated timosaponin A-III, a major constituent. Lung inflammation was induced by intranasal administration of lipopolysaccharide (LPS) to mice, representing an animal model of acute lung injury (ALI). The alcoholic extract (50 – 200 mg/kg) inhibited the development of ALI. Especially, the oral administration of the saponin-enriched fraction (10 – 50 mg/kg) potently inhibited the lung inflammatory index. It reduced the total number of inflammatory cells in the bronchoalveolar lavage fluid (BALF). Histological changes in alveolar wall thickness and the number of infiltrated cells of the lung tissue also indicated that the saponin-enriched fraction strongly inhibited lung inflammation. Most importantly, the oral administration of timosaponin A-III at 25 – 50 mg/kg significantly inhibited the inflammatory markers observed in LPS-induced ALI mice. All these findings, for the first time, provide evidence supporting the effectiveness of *A. asphodeloides* and its major constituent, timosaponin A-III, in alleviating lung inflammation.

Generation of Natural Product-Based Screening Libraries for Drug Discovery

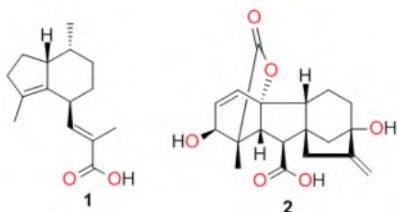
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Natural products (NPs) represent a unique source of chemical diversity that exhibit a wide variety of biological activities. Exploring and designing diverse compound collections using unique and under-utilized NPs as scaffolds for medicinal chemistry studies increases the chances of discovering new chemical probes and/or future leads that could be developed into new drugs.¹

This project is significant as it applies knowledge from synthetic, medicinal and combinatorial chemistry to novel NP-scaffolds that results in the creation of small (10–20 analogues) but unique discovery libraries. This study will allow for the exploration of unique chemical space and provide distinct screening libraries containing potential drug, lead or probe molecules.

Two NP scaffolds, valerenic acid (1) and gibberellic acid (2), have recently been modified to generate libraries of analogues via parallel solution-phase synthesis.^{2,3} The library based on 1 was screened for anti-inflammatory activity. This library was tested in two separate anti-inflammatory in vitro assays based on IL-8 and TNF- α inhibition. Six analogues showed moderate inhibitory activity in the IL-8 assay with IC₅₀ values of 2.8–8.3 μ M, while none of the tested compounds showed any significant effect on inhibiting TNF- α release.² The gibberellic acid library was evaluated for in vitro cytotoxicity and deregulation of lipid metabolism in human prostate cancer cells (LNCaP). While no cytotoxic activity was identified at the concentrations tested, five semi-synthetic analogues substantially reduced cellular uptake of free cholesterol in LNCaP cells, suggesting a novel role of gibberellic acid derivatives in deregulating cholesterol metabolism.³



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Poster Session-PO-63:

Assessment of antiproliferative activity of oligosaccharides obtained as acid-hydrolysis products of (1-3)- α -D-glucan from *Laetiporus sulphureus*

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Edible mushrooms are valuable source of biologically active compounds with beneficial impact on health. Their anticancer and immunomodulatory properties are mainly attributed to polysaccharides, especially their derivatives. Polysaccharide-derived oligosaccharides have been widely used as food additives, prebiotic supplements, drug delivery, immunoenhancers and animal feed. The biological activity of (1-3)- α -D-glucooligosaccharides obtained as acid-hydrolysis products of (1-3)- α -D-glucan from *Laetiporus sulphureus* were analyzed. Their influence on normal human colon epithelial cells (CCD 841 CoN) viability was analyzed by means of LDH assay. Anticancer properties of oligosaccharides were screened on human colon adenocarcinoma cell lines (Caco-2, LS180 and HT-29) by means of MTT and BrdU assays. Furthermore, flow cytometry was applied to cell cycle analysis. Performed studies revealed that tested compounds were not toxic to normal colon epithelial cells in whole range of investigated concentrations (0-1000 μ g/mL), at the same time antiproliferative effect of oligosaccharides was observed in colon cancer cells. The strongest inhibition of cells proliferation was observed on HT-29 cells (IC₅₀ 310.6 μ g/mL) (MTT test). Nevertheless more specific BrdU assay revealed significant decrease of DNA synthesis only in case of LS180 cells. In order to determine mechanism by which tested compound influence cancer cell proliferation flow cytometry was applied. Performed studies demonstrated alterations in cell cycle progression only in case of HT-29 cells, where (1-3)- α -D-glucooligosaccharides at concentration 1 mg/mL induced cells accumulation in S phase. Our results indicate that oligosaccharides from (1 \rightarrow 3)- α -D-glucan possess an anticancer potential and may provide a new chemopreventive option against colon cancer.

Poster Session-PO-64:

Anti-inflammatory activity of the fungus growing termite's strain *Macrotermes bellicosus* (Macrotermitinae) used in traditional medicine in Benin

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Background:

The fungus growing termites' species *Macrotermes bellicosus* are used in traditional medicine in Benin for the treatment of infectious and inflammatory diseases such as digestive disorders, mumps, snake bites, cough, diarrhea, dysentery, and pulmonary infection. In vitro studies revealed that *Macrotermes bellicosus* is efficient against various pathogenic microorganisms. However, the occurrence of anti-inflammatory effects remains unexplored.

Aim:

Aim of the present study was to investigate the anti-inflammatory properties of *Macrotermes bellicosus* in activated human monocyte-derived macrophages (THP-1) and intestinal epithelial cells (Caco2) on which inflammation was induced in vitro to model the pathophysiology of inflammatory diseases.

Methods:

The effect of termite extract was evaluated on LPS-induced TNF α release from differentiated human macrophages (THP-1) and, IL-8 release from human intestinal epithelial cells (Caco-2) induced with a cytokine-mix (IL-1 β , TNF α and IFN γ). Production of the pro-inflammatory mediators (TNF α and IL-8) was measured by enzyme-linked immunosorbent assay (ELISA) and IC₅₀ values were determined. Budesonide and dexamethasone served correspondingly as positive control. Concomitant cytotoxicity controls were performed using MTT assay.

Results:

It was found that the production of the induced pro-inflammatory cytokines TNF α (IC₅₀: 41.99 μ g/mL) and IL-8 (IC₅₀: 56.93 μ g/mL) was significantly inhibited after treatment with the termite extract. These effects were concentration-dependent and comparable to those of budesonide and dexamethasone. Moreover, no alteration of cell viability was observed in the applied concentrations of termite extract throughout the assays. Conclusion: *Macrotermes bellicosus* used as a traditional medicine showed anti-inflammatory properties in the tested human in vitro cell culture models by inhibiting pro-inflammatory cytokine release. This termite species could be an interesting natural source contributing to the treatment of inflammatory diseases including digestive diseases.

Keywords :

Termite, *Macrotermes bellicosus* , anti-inflammatory activity, Macrotermitinae, Benin.

Poster Session-PO-65:

Evaluation of osteogenic potential of Taiwanese endemic plants using in vitro model

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Oxidative stress has been reported to influence osteoporosis occurring at the older age. Osteoblasts play an important role in bone remodeling. The secondary metabolites of plants including phenols and flavonoids show the abilities to scavenge free radicals. We evaluated eight extracts obtained from different parts of Taiwanese endemic plants by using chemical analysis and antioxidant assays and establishing two in vitro models for osteogenic activities. Chemical analysis was evaluated by determining total phenolic and flavonoid contents. The antioxidant activities were determined by scavenging DPPH and ABTS radicals. Osteogenic activities were evaluated in osteosarcoma cell lines (MG-63) and primary human osteoblasts (HOb) by alkaline phosphatase (ALP) activity, an early-stage biomarker, and mineralization, a late-stage biomarker. Results exhibited that four extracts, ESS, BBW, TFL, and TFR, demonstrated higher total phenolic contents (more than 100 mg gallic acid equivalent/g of extract). ESS, BBW, TUW, and AKR contained higher total flavonoid contents (more than 100 mg rutin equivalent/g of extract). Six extracts, ESS, BBW, TFL, TFS, TFR, and TUW, significantly scavenged DPPH and ABTS radicals by more than 50%. None of the extracts showed cytotoxicity in MG-63 and HOb cells. Four extracts, ESS, BBW, TFL, and TFR, significantly enhanced ALP activity, and five extracts, ESS, BBW, TFL, AKR, and WTL, increased mineralization in MG-63 cells. In addition, ESS, TFL, TFS, and WTL significantly enhanced ALP activity in HOb cells. From these results, we deduce that ESS and TFL contained higher total phenolic and flavonoid contents, antioxidant and osteogenic activities were also higher in these two plants. Our in vitro models are well established for screening the agents with the osteogenic activity. This will provide us with the potent Taiwanese endemic plants that can serve as the sources of preventive agent for oxidative stress and osteoporosis.

Poster Session-PO-66:

HPLC determination of sugars and organic acids in twenty cultivars of *Sorbus L.* fruits

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Sorbus L. fruits are rich in phenolics, carotenoids, sugars and organic acids [1]. In this study the sugar and organic acid profiles were determined in the fruit samples of *S. esezianii*, 'Alaja Krupnaja', 'Burka', 'Businka', 'Dodong', 'Fructo Lutea', 'Granatnaja', 'Kirsten Pink', 'Konzentra', 'Krasnaja Krupnoplodnaja', 'Likernaja', 'Miciurinskaja Desertnaja', 'Nevezinskaja', 'Pendula Variegata', 'Red Tip', 'Rosina Variegata', 'Rubinovaja', 'Sorbinka', 'Titan', 'White Swan'. HPLC-HILIC-ELSD method was applied for the analysis of xylose, fructose, glucose, sucrose, and sorbitol. HPLC-UV method was applied for the analysis of ascorbic and malic acid. Fructose, glucose and sorbitol were found in the profiles all Sorbus fruits. The greatest amount of sorbitol was determined in the fruit samples from 'Pendula Variegata' and 'Konzentra' (265.31 ± 3.80 and 261.78 ± 2.87 mg/g). The contents of malic acid ranged from 38.35 to 102.37 mg/g. The greatest amount of malic acid was determined in the fruit samples of 'Rosina Variegata' cultivar. Not all fruit samples of the tested Sorbus cultivars contained ascorbic acid. Ascorbic acid was absent in the fruit samples of 'Burka', 'Businka' and 'Likernaja' cultivars. The detected ascorbic acid content in rowanberry powders varied from 0.11 mg/g ('Miciurinskaja Desertnaja') to 2.20 mg/g ('Fructo Lutea'). In conclusion, the sugars and organic acids profiles are dependent on the cultivars.

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Sorbus L., HPLC-HILIC-ELSD, ascorbic acid, sugars.

Poster Session-PO-67:

Phytochemical composition and antioxidant evaluation of *Pelargonium sidoides* DC. root extract and proanthocyanidins

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Pelargonium sidoides roots have been used for medicinal purposes in various traditional medicine systems. Proanthocyanidins (PACN) are bioactive constituents of *Pelargonium sidoides* root extracts (PSRE) and possess antioxidant, anti-inflammatory, antibacterial properties [1]. PACN enriched extracts inhibit biofilm formation and adhesion of periodontopathogenic bacteria, inhibit proteolytic activities of bacteria [2]. PACNs could be perspective bioactive compounds beneficial in development and progression of periodontal

diseases. The aim of this study was to determine proanthocyanidin composition in PSRE and collected PACN fraction. UPLC-MS analysis was used for the determination of proanthocyanidin profile and the estimation of the mean degree of polymerization (mDP) for the oligomers and polymers in PCAN. Reducing and radical scavenging activities of the extracts were determined using FRAP and ABTS assays, respectively. Five prodelphinidin derivatives were determined in the PSRE and PACN fraction (from dimers to hexamers). The calculated mean degree of polymerization for analysed PCANs sample was 6. The determined radical scavenging and reducing activities were $3747.75 \pm 534.56 \mu\text{mol/g}$ (PSRE) and $4472.75 \pm 707.71 \mu\text{mol/g}$ of TE (PACN) and 3210.17 ± 421.21 (PSRE) and 4006.13 ± 685.75 (PACN), respectively. The PACN fraction consisting of prodelphinidin oligomers showed significantly ($p < 0.05$) higher Trolox equivalent antioxidant activity compared to PSRE. PACNs could be perspective for functionalization drug delivery systems of local action with natural active substance for treatment of periodontal diseases.

Acknowledgement: The PELARGODONT Project ("Engineering and functionalization of delivery system with Pelargonium sidoides biologically active substance on inflamed periodontal surface area") is financed under the M-ERA.NET 2 Programme, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 685451.

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- Pelargonium sidoides, proanthocyanidins, antioxidant, UPLC-MS.

Poster Session-PO-68:

Four new 2-(2-phenylethyl)-4H-chromen-4-one derivatives from the resinous wood of Aquilaria sinensis and their inhibitory activities on neutrophil pro-inflammatory responses

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Aquilaria sinensis (Lour.) Gilg. (Thymelaeaceae) is an evergreen tree distributed in Guangdong, Hainan, Guangxi, and Fujian provinces of China. The aromatic resinous heartwood derived from the wounded trees of Aquilaria species is called 'agarwood'. It is a highly prized product which can be used in fragrances, incense, and traditional medicine [1]. According to a review, agarwood possesses aphrodisiac, cardiogenic, and carminative properties. Previous phytochemical investigations on agarwood revealed sesquiterpenes and

2-(2-phenylethyl)-4H-chromen-4-one derivatives to be the major constituents. These derivatives have been reported to exhibit several biological activities, such as acetylcholinesterase inhibitory, cytotoxic, antibacterial, and neuroprotective activities. In our studies on the anti-inflammatory constituents of Chinese herbal medicines, many species have been screened for in vitro inhibitory activity on neutrophil pro-inflammatory responses. *A. sinensis* was found to be an active species. Phytochemical investigation of the resinous wood of *A. sinensis* led to the isolation of four new 2-(2-phenylethyl)-4H-chromen-4-one derivatives, 6-hydroxy-5-methoxy-2-[2-(4'-methoxyphenyl)ethyl]chromone (1), 6,7-dimethoxy-2-[2-(2'-hydroxyphenyl)ethyl]chromone (2), 5-hydroxy-6-methoxy-2-[2-(3'-methoxyphenyl)ethyl]chromone (3), and 7-chloro-8-hydroxy-2-[2-(4'-methoxyphenyl)ethyl]chromone (4), and 16 known compounds (5–20) including further congeners and sesquiterpenes. The structures of the new compounds were established by spectroscopic analyses (1D and 2D NMR, HRESIMS, IR, and UV). Nine compounds including 1, 2, and 7-methoxy-2-[2-(4'-methoxyphenyl)ethyl]chromone (5) inhibited superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) with IC_{50} values $\leq 36.28 \mu\text{M}$.

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Poster Session-PO-69:

Antioxidant, anti-inflammatory and anthelmintic activities of crude extracts, fractions and an isolated compound from southern African medicinal plants

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For decades, new compounds from plants have been used to treat inflammation and helminthiasis. Eleven plant species were selected based on their use in traditional medicine against inflammation in southern Africa. The crude extracts, fractions and compounds were tested for their antioxidant, anti-inflammatory and anthelmintic activities. The antioxidant activity was determined using radical scavenging DPPH and electron reducing ABTS assays. The anti-inflammatory activity of crude extracts was evaluated via the 15-lipoxygenase inhibitory assay and the nitric oxide (NO) inhibition assay using lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophages. The anthelmintic activity was determined against the nematode helminth *Haemonchus contortus* using the egg hatch assay (EHA) and larval development assay (LDA).

Fractions and a compound (isorhamnetin-3-O- β -D-glucoside) isolated from *Typha capensis* were also tested. The acetone extract of *T. capensis* exhibited the highest antioxidant (ABTS) activity with an IC_{50} value of 0.6 μ g/mL. The hexane extract had good 15-lipoxygenase inhibitory effect with an IC_{50} value of 4.62 μ g/mL which was significantly ($p < 0.05$) higher than that of quercetin (IC_{50} of 26.60 μ g/mL). It also had strong NO inhibition (77.45%) and cell viability (97.49%) at 100 μ g/mL. The butanol fraction had very good antioxidant activity (IC_{50} = 0.26 μ g/mL), NO inhibition (94.60%) and cell viability (94.75%) at 100 μ g/mL. However the isolated compound had poor antioxidant and anti-inflammatory activity. In addition, the acetone crude extract (IC_{50} = 13.87 μ g/mL), and butanol fractions (IC_{50} = 24.01 μ g/mL) from *T. capensis* were the most efficient against *Haemonchus contortus*. This study revealed that *T. capensis* had good antioxidant, anti-inflammatory and anthelmintic activity against *Haemonchus contortus*. This plant is therefore a potential source of compounds that could be used against inflammation and diseases where inflammation is commonly implicated, such as helminthiasis.

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Poster Session-PO-70:

The phytochemical analysis of lime flowers infusions in search of chemotaxonomic markers of pharmacopoeial *Tilia* species

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Linden flower is a widely used plant material among patients in the treatment of common cold symptoms and mucosa inflammations. The pharmacopoeial monograph refers to three *Tilia* species as a valid source of a medicinal plant material – *Tiliae flos*. Those species are *T. platyphyllos*, *T. cordata* and *T. x vulgaris* (*T. europea*). Up to date there are no reports on differences in the chemical composition of the extracts prepared from flowers of those three species.

The aim of the study was to investigate the differences in a polyphenolic composition of infusions prepared from flowers obtained from three pharmacopoeial species and two non-pharmacopoeial species (*T. tomentosa* and *T. americana*).

Almost two hundred samples of flowers of *Tilia* collected between 2013-2016 in different regions of Poland and Europe were investigated. The UHPLC-DAD-MS method using Kinetex XB-C₁₈ column was developed.

Chromatograms were recorded at 254, 280 and 350 nm. The MS spectra were analyzed in negative ion mode. Over 40 compounds were detected and characterized comprising flavan-3-ols, flavonoids and phenolic acids. The identification was based on the comparison with available chemical standards and on the literature data. Major compounds from groups of flavan-3-ols and flavonoids were quantified using a validated method with epicatechin and isoquercitrin as reference standards.

It was shown that linarin (acacetin 7-O-rutinoside) is a valid chemotaxonomic marker for *T. cordata* and *T. x vulgaris*. Two quercetin O-rhamnohexosides were identified as markers for *T. platyphyllos* and *T. x vulgaris*. No differences in the chemical composition was observed as far as flavan-3-ol derivatives are considered. The study for the first time provided simple solution for the differentiation of extracts from three pharmacopoeial *Tilia* species using a single UPLC-DAD-MS analysis.

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Poster Session-PO-71:

Defining antimicrobial compounds in traditional Chinese medicinal herbs

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In the past few years, natural products have already shown to be fundamental, when combined with other fields of knowledge, in anti-infectious drug research. Innovation in omics' technologies, chemical biology and genetics have recently made possible the discovery of new drugs and their targets [1] . Natural products assume a privileged role in the drug discovery due to their, intrinsic cell permeability, structural diversity, rich functionality, and stereochemistry. They also provide unique scaffolds for further drug optimisation towards increased potency and selectivity. China has been using herbs in medicine for centuries and these are now attracting global interests. In response to the challenge of increased antimicrobial resistance, we developed a China-UK collaborative team to explore the potential of Chinese Medicines to reveal new drug leads.

Currently, our project focuses on 19 traditional Chinese herbs where bio-actives are screened using a well-established analysis pipeline. Thus, bioactivity screening of the extracts, isolation, purification using various

chromatography techniques and identification using high-resolution Mass Spectrometry and NMR. We have performed an assay-based evaluation of isolated compounds from *Artemisia annua* against mycobacteria [2] and evaluated the antimicrobial properties of extracts from the other 18 herbs using high-throughput assays. These have revealed varying levels of anti-microbial activity, further purified. Each was tested for cytotoxicity, anti-parasite (*Schistosoma mansoni*) and anti-cancer properties. Our studies can help find different naturally available compound and help in the identification of targets to fight different diseases including the drug-resistant disease.

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Poster Session-PO-72:

Chemical profile of wild and cultivated *Salsola soda* aerial parts

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The genus *Salsola* (Amaranthaceae) includes about 120 species of herbaceous or shrubby plants, widespread especially in the brackish grounds of the moderate and subtropical regions of Europe, Asia, Africa, and North America. Previous phytochemical investigations on this genus reported the isolation of flavonoids, alkaloids, acetophenones, coumarins and sterols. *Salsola* species are well-known in folk medicine as anti-hypertensive, diuretic, anti-cancer, antioxidant, emollient, purgative, anti-ulcer, and anti-inflammatory remedies [1]. Wild *S.soda* L. is an erect glabrous annual plant widespread in South Europe, particularly in marginal areas near the coast [2]; the plant buds, called "agretti", are edible and cultivated as a food plant and in the past also as a source of impure sodium carbonate [1]. To date, in the literature there isn't any complete phytochemical characterization of the wild and cultivated plants. For this reason, the aim of the present study was to evaluate and compare the chemical content of wild and cultivated *S.soda* aerial parts. The dried and powdered plant materials were sequentially extracted with n-hexane and MeOH. The MeOH extracts were partitioned between n-BuOH and H₂O to remove sugar and other inorganic salt and the n-BuOH fraction was firstly submitted to LC-MS analysis. On the same time, the n-BuOH extract of the wild plant was chromatographed on Sephadex LH-20 column, and then, subsequently, fractioned with CPC and RP-HPLC, to obtain pure compounds. Flavonoids and saponins were finally isolated and characterized by NMR and MS analyses. The chemical profile of both plants exhibited that wild *S.soda* is richer than the cultivated one in

flavonoids and saponins content.

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Poster Session-PO-73:

Modulation of iNOS Expression in LPS-Stimulated BV-2 Microglia by Prenylated Chalcones from *Cullen corylifolium* (L.) through Inhibition of I- κ B α Degradation

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The overproduction of nitric oxide (NO) and prostaglandin E2 (PGE2) by microglia may cause neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. From the activity-guided purification of *Cullen corylifolium* (L.) Medik. (syn. *Psoralea corylifolia* L.), three prenylated chalcones were identified: isobavachalcone (1), bavachromene (2), and kanzonol B (3). These prenylated chalcones showed concentration-dependent inhibitory effects on NO and PGE2 production in lipopolysaccharide (LPS)-activated microglia. Western blotting and RT-PCR analysis demonstrated that these prenylchalcones reduced the expression of protein and mRNA of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in LPS-activated microglia. Furthermore, three prenylated chalcones blocked the inhibitory- κ B α (I- κ B α) degradation and down-regulated nuclear factor κ B (NF- κ B) level of nucleus in LPS-stimulated BV-2 microglia. Therefore, these prenylated chalcones from *Psoralea corylifolia* may be beneficial for the treatment of neuro-inflammatory diseases by modulating iNOS and COX-2 expressions in activated microglial cells.

Poster Session-PO-74:

Prenylated Polyphenols from *Broussonetia kazinoki* as Inhibitors of Nitric Oxide Production

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Excessive nitric oxide(NO) production by macrophages has been involved in inflammatory diseases. Seven polyphenols (1–7) were isolated from *Broussonetia kazinoki* (*B. kazinoki*) and investigated as potential inhibitors of NO overproduction in lipopolysaccharide (LPS)-activated RAW 264.7 cells. Among them, four prenylated polyphenols (2–4 and 6) with a catechol moiety efficiently suppressed the LPS-induced high level of NO with IC₅₀ values of less than 6 μM. The compounds 2–4 and 6 also attenuated protein and mRNA levels of inducible nitric oxide synthase (iNOS). Moreover, they suppressed the nuclear factor κB (NF-κB) activity by inhibiting the degradation of inhibitory-κB-α (I-κB-α) and the translocation of NF-κB into the nucleus in LPS-activated macrophages. Taken together, these findings suggest that polyphenols from *B. kazinoki* might be beneficial for treatment of inflammatory diseases.

Poster Session-PO-75:

A lignan induces lysosomal dependent degradation of FoxM1 protein to suppress β-catenin nuclear translocation

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In this study, we isolated a lignan [(–)-(2R,3R)-1,4-O-diferuloyl]secoisolariciresinol, DFS] from *Alnus japonica* (Betulaceae) and investigated its biological activity and mechanism of action on colon cancer. DFS reduced the viability of colon cancer cells and induced cell cycle arrest. DFS also suppressed β-catenin nuclear translocation and β-catenin target gene expression through a reduction in FoxM1 protein. To assess the mechanism of the action of DFS, we investigated the effect of DFS on endogenous and exogenous FoxM1 protein degradation in colon cancer cells. DFS-induced FoxM1 protein degradation was suppressed by lysosomal inhibitors, chloroquine and bafilomycin A1, but not by knock-down of proteasomal proteins. The mechanism of DFS for FoxM1 degradation is lysosomal dependent, which was not reported before. Furthermore, we found that FoxM1 degradation was partially lysosomal-dependent under normal conditions. These observations indicate that DFS from *A. japonica* suppresses colon cancer cell proliferation by reducing β-catenin nuclear translocation. DFS induces lysosomal-dependent FoxM1 protein degradation. This is the first report on the lysosomal degradation of FoxM1 by a small molecule. DFS may be useful in treating cancers that feature the elevated expression of FoxM1.

Poster Session-PO-76:

Profiling of xanthine oxidase inhibitory polyphenols from the leaves of *Cedrela sinensis*

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Cedrela sinensis leaf is used as a seasonal vegetable in Korea. The 70% ethanol extract of these leaves exhibited potent xanthine oxidase (XO) inhibition. To investigate the compounds responsible for this effect, bioassay-guided purification of the butanol fraction led to the isolation of 5 metabolites which were identified as rutin (1), isoquercitrin (2), pentagalloyl glucose (3), quercetrin (4), and afzelin (5) by detailed spectroscopic analyses, including UV, MS, and 2D NMR techniques. Importantly, compound 3 was not only the most potent component with $IC_{50} = 2.8 \mu M$, but also similar inhibitory activity to the allopurinol ($IC_{50} = 2.3 \mu M$) used as XO inhibitor for clinical management of Gout. In kinetic mechanism study, XO inhibitor 3 was reversible noncompetitive-type inhibition characteristics. Furthermore, the most potent XO inhibitor 3 was proven to be present in high quantities in the leaves by a UPLC-qTof MS analysis. This result contributes to our understanding of *C. sinensis* leaves utility as functional food stuff and widely used herbal medicine.

Poster Session-PO-77:

Differentiation of *Zicao* species based on DNA analysis and chemical markers

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Because of wrongly labelled plant material and different local names, the exact identification of plant material stays a challenge in herbal medicine. Mix-ups can trigger serious health problems and undesired side effects due the fact that plants have different chemical compositions [1]. In China, *Zicao* is a commonly used herbal medicine, which represents the dried roots of plants from the genera *Onosma*, *Arnebia*, and *Lithospermum*, all belonging to the *Boraginaceae* family. All these roots contain red colored shikonin derivatives with various pharmacological properties [2]. Moreover, they contain pyrrolizidine alkaloids in low (*Arnebia*) or high (*Onosma* and *Lithospermum*) concentrations, which are well known for their hepatotoxic properties [3]. That is why there is an instant need for unambiguous identification of these roots. Despite of local names, which are used

on medicinal markets (Ying Zicao for *Lithospermum erythrorhizon*, Dian Zicao for *Onosma paniculata*, and Ruan Zicao *Arnebia euchroma*), confusions of these species are common.

In this study, we developed a simple HPTLC method to distinguish the different Zicao species unambiguously. Various Zicao samples from different places in China were purchased and identified by DNA analysis (ITS1, trnL-F). By HPTLC, we identified a blue fluorescent zone as a marker compound by which the species can be exactly identified and a safe application in TCM can be ensured.

Acknowledgements:

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Poster Session-PO-78:

Influence of urolithins and their glucuronides on pro-inflammatory response of THP-1 macrophages.

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Ellagitannins present in various medicinal plants are metabolized by human gut microbiota to bioavailable small molecule compounds- urolithins [1]. Urolithins after absorption in the gut rapidly undergo metabolism by II phase enzymes, what results in their presence in serum, tissues and urine mainly in forms conjugated with glucuronic acid. To fully evaluate biological activity of urolithins, the in vitro studies should be also conducted for their II phase metabolites, especially for dominating glucuronides.

The aim of the study was to evaluate influence of isourolithin A, urolithin A and urolithin B (iUA, UA, UB) as well as their respective glucuronides (GiUA, GUA, GUB) on TNF- α and IL-10 production after LPS stimulation of THP-1-derived macrophages.

THP-1 monocytes were differentiated into macrophages using PMA. UA at the concentration of 40 μ M was

capable of reducing LPS-induced TNF- α production by 59%, while GUA occurred to be inactive. The 24-hour incubation of THP-1 macrophages led to a significant increase in IL-10 production for all of the tested compounds, except for GiUA, whilst the strongest activity was observed for corresponding aglycone- iUA, which increased the production of this anti-inflammatory cytokine by 30%.

The above results confirm, that UA is the most active urolithin in terms of inhibiting the inflammation response after TLR4 receptor stimulation. iUA was for the first time indicated to trigger resolution of inflammation by increasing IL-10 production. II phase metabolism was shown to result in the loss of compounds' pharmacological properties, however elevated β -glucuronidase activity at inflammation site is potent to cause their re-activation.

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Poster Session-PO-79:

New prenylated flavanones from the fruits of *Amomum tsao-ko*

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The fruits of *Amomum tsao-ko* have been used as traditional medicine for nausea, abdominal distension, diarrhea, and malaria.^[1] In the course of our research program for the isolation of bioactive constituents from medicinal plants, the fruits of *A. tsao-ko* were extracted with MeOH, and sequentially partitioned with n-hexane, CH₂Cl₂, EtOAc, and n-BuOH. Four new prenylated flavonone compounds (1 - 4), along with nine known compounds, alpinetin (5), 5-methoxynaringenin (6), naringenin (7), hesperetin (8), 2',4',6'-trihydroxy-4-methoxychalcone (9), boesenbergin B (10), 4''-hydroxyboesenbergin B (11), tsaokoin (12), and tsaokoarylone (13) were isolated from the CH₂Cl₂ and ethyl acetate fraction of the fruits of *A. tsao-ko* by using various chromatographic methods.^[2] The structures of these compounds were elucidated on the basis of spectroscopic data interpretation, especially 2D NMR spectra such as HSQC, HMBC, and HR-ESI-MS. In addition, CD spectroscopy was used to determine the absolute configuration of four new compounds. All compounds were evaluated for their inhibitory effects on the NO production against LPS- induced RAW 264.7 macrophages.

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Poster Session-PO-80:

Potential immunomodulatory compounds isolated from red betel leaves (*Piper crocatum*, Ruiz & Pav.)

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Red betel leaves (*Piper crocatum*, Ruiz. & Pav.) have been traditionally used as an ingredient in a Jamu preparation intended to maintain health. The red betel leaves are thought to boost the immune system, keeping the body stay healthy. The study is aimed to isolate and identify compounds that stimulate the immune system, and determine their potential as immunomodulators in vivo.

Isolation of the active compounds was proceeded by bioassay - guided isolation. Activation of macrophage in vitro (phagocytic assay) was used to guide the isolation of active compounds. The isolated active compounds were tested for in vivo immunomodulatory effect on balb/c mice infected with *Lysteria monocytogenes*. In these assays, several parameters such as phagocytic index and capacity, concentration of nitric oxide and proliferation of lymphocyte were measured.

Two new neolignans, 2-allyl-4-(1'-hydroxy-1'-(3",4",5"-trimethoxyphenyl)propan-2'-yl)-3,5-dimethoxycyclohexa-2,5-dienone and its naturally occurring acetyl derivative were isolated and identified as active compounds. The two compounds separately (5 µg/mL) displayed immunomodulatory activity equal to Imboost (Echinaceae product), as positive control. In the in vivo immunomodulatory assay, the two compounds separately (5.0 and 10.0 mg/kgBW) increased the activity and capacity of macrophages. In addition, increases of nitric oxide and IL-12 productions were also observed in response to the two compounds (2.5, 5.0 and 10.0 mg/kgBW). However, there is no lymphocyte proliferation was observed, which might be due to inhibition on the binding between IL-12 and its receptor.

Poster Session-PO-81:

In vitro cytotoxic effects of a traditionally used extract from the bark of *Kalanchoe integra*

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An ethnopharmacological field study was performed among traditional healers in the Ashanti region of Ghana regarding their treatment of different kinds of cancer. A total of 151 plant species were mentioned as remedies used by the healers and these species were classified according to the frequency they were named in the survey, followed by intensive literature review regarding data on the cytotoxic potential of these plants [1].

13 plant species were chosen for an in vitro screening against different cancer cell lines, either because they were frequently mentioned for this indication or because they have not been assessed for cytotoxicity up to now. 22 hydro-ethanolic extracts (50:50) were prepared from different plant parts of the 13 selected species according to their traditional use and the cell viability was assessed in vitro in an MTT assay against MCF-7 (breast cancer), HepG2 (liver cancer) or A431 (skin cancer) cells.

Extracts from *Alstonia boonei* leaves and *Paullinia pinnata* climbers showed moderate cytotoxicity against MCF-7 cells at concentrations ≥ 50 $\mu\text{g/mL}$, whereas the bark extract from *Kalanchoe integra* strongly decreased the cell viability (IC_{50} 4.8 $\mu\text{g/mL}$). Doxorubicin was used as positive control (IC_{50} 1.4 $\mu\text{g/mL}$). The three extracts were also active against HepG2 and A431 cells, but at higher concentrations.

A preliminary phytochemical screening by LC-qTOF-MS revealed the presence of substances tentatively identified as bufadienolides. As this class of compounds is widely distributed among the genus *Kalanchoe* and known for its cytotoxic properties against various cell lines [2], bufadienolides could be responsible for the in vitro activity of the *K. integra* extract.

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Poster Session-PO-82:

Nitric oxide inhibitory constituents from *Lysimachia foenum-graecum*

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The plant of *Lysimachia foenum-graecum* (primulaceae) has been used as traditional medicine to treat cold,

headache, inflammation, and diseases of digestive system.^[1] Previous phytochemical investigations of this plant have led to the isolation of bioactive compounds such as flavonoids and triterpene saponins.^[2] The aim of the present research is to isolate and identify anti-inflammatory agents from medicinal plants. Bioassay-guided fractionation of the n-BuOH soluble fraction of the aerial parts of *L. foenum-graceum* led to the isolation of seven new oleanane-type triterpene saponins (1-7), which contains angeloyl and acetyl groups in the aglycone. Their structure were elucidated on the basis of spectroscopic methods such as 1D-, 2D-NMR, and HR-ESI-MS. Isolated compounds were evaluated for their inhibitory effects on the NO production against LPS-induced RAW 264.7 macrophages, and showed significant inhibitory effects with IC₅₀ values ranging from 1.6 to 47.9 μ M.

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Poster Session-PO-83:

Polysaccharides from *Codonopsis pilosula* modulate the immunity and intestinal microbiota on cyclophosphamide-treated immunosuppressed mice

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Polysaccharides are present in a wide range of plants, with abundant bioactivities, including immune-enhancement activity and colon health benefit effects. The intestinal microbiota is essential in the bioactivities of polysaccharides, with restored microbiota composition and the fermented product from these non-digestible polysaccharides after oral administration [1].

The polysaccharides from *Codonopsis pilosula* Nannf. var. *modesta* L. T. Shen (WCP) could restore the broken humoral immune on immunosuppression mice treated by cyclophosphamide (CP), for instance, the levels of interleukin-2 (IL-2), IL-10, interferon- γ (IFN- γ) and immunoglobulin G (IgG) in serum (Fig.1). In addition, the WCP elevated the ileum secretory immunoglobulin A (sIgA) in a dose-dependent manner, showing a potential mucosal immunological protective effect. At the same time, the amount of lactobacilli was increased in cecum (not shown), as well as the acetic acid content in a dose-dependent manner (Fig.1). It is probable that the WCP were fermented to short chain fatty acids (SCFAs) by beneficial bacteria, like *Lactobacillus*, thus stimulating the mucosal epithelium or receptors, promoting the secretion of sIgA [1-2]. Consequently, it could be used as a potential therapeutic natural product for inflammatory bowel disease (IBD)

by adjusting the imbalanced homeostasis of gut microbiota and the damaged intestine mucosa [3].

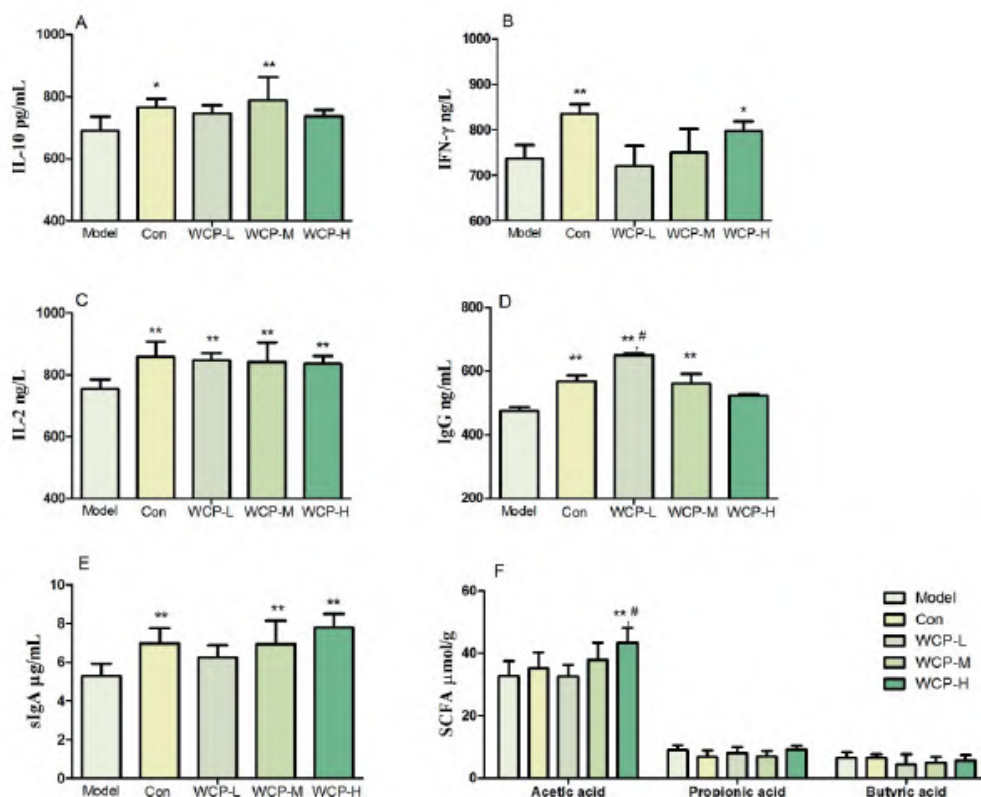


Figure 1. Effects of WCP on CP-treated mice. Model, model control; Con, normal control; WCP-L, 50 mg/kg bodyweight WCP treated group; WCP-M, 100 mg/kg bodyweight WCP treated group; WCP-H, 200 mg/kg bodyweight WCP treated group. The values were presented as mean ± SD by Duncan's test in one-way ANOVA of SPSS. 20.0. * $p < 0.05$, compared with model group; ** $p < 0.01$, compared with model group; #, $p < 0.05$, compared with normal group.

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Poster Session-PO-84:

Modulating activity of latex from *Euphorbia Mauritanica* L. on human skin models' immune response

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In recent years, literature shows that proteases have the potential to induce inflammation through activation of protease-activated receptors (PAR) [1]. Besides secondary plant constituents like phenols, di-, and polyterpenes, proteases are abundant in latices from plants of Euphorbiaceae Juss. [2,3]. It is known, that these latices induce strong skin inflammation. Therefore, we investigated the plant latex from *Euphorbia mauritanica* L. (Euphorbiaceae). Human skin is the first barrier of the body in defense from environmental influences and the inflammatory response is an important tool against pathogens. In the current study, we focused on the inflammatory potential of the combination of diterpenes with proteases on a human skin model.

Mauritanicain, a serine protease from *E. mauritanica*, was isolated by ion exchange and size exclusion chromatography. The human skin model was composed by primary keratinocytes and fibroblasts. Mauritanicain (10 µg/mL) and PMA (10 ng/mL) were incubated for one hour on the skin model. 48 hours after removing the stimulating substances, medium was removed and investigated by FACS and ELISA. When the experiment was finished, HE-staining of the skin model was performed. The occurrence of PAR2 was visualized by immunofluorescence staining.

The results showed an increased IL-1 β release by single treatment with Mauritanicain (2-fold) and by the combination of Mauritanicain and PMA (1.5-fold). The results indicate that there is no synergism between Mauritanicain and PMA.

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Poster Session-PO-85:

Chemical constituents from the heartwood of *Pterocarpus santalinus*

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Pterocarpus santalinus L. (Leguminosae) has been used for the treatment of inflammations, mental aberrations, ulcers, and diabetes. Previous reports showed that the main constituents are lignan, terpenoids,

flavonoids and sterols. As part of our ongoing research for the discovery of plant-derived inhibitors of nitric oxide (NO) production, we found that the methanolic extract of the heartwood of *P. santalinus* inhibited the LPS-induced NO production in RAW 264.7 cells. In this study, the heartwood of *P. santalinus* were extracted with MeOH, and sequentially partitioned with n-hexane, CH₂Cl₂, EtOAc, and n-BuOH. Three lignans and a flavonoid were isolated from the methylene chloride fraction of the heartwood of *P. santalinus*. Their chemical structures were elucidated on the basis of spectroscopic data interpretation, especially 1D-NMR, ESI-MS. On the basis of their spectroscopic data, these compounds were identified as savinin (1) and calocedrin (2), hinokinin (3), and pinocembrin (4).^{[1],[2]} All isolates were investigated for their inhibitory effects on the NO production in LPS-induced RAW 264.7 macrophage cells.

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Poster Session-PO-86:

The Bronchipret Story – building up evidence from bedside to bench

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The Bronchipret product family has been long and successfully used for the treatment of acute bronchitis which is commonly characterized by inflammation, cough and viscous mucus hypersecretion. Treatment efficacy has been proven for the main products Bronchipret Syrup (thyme-ivy extract combination) and Bronchipret film-coated tablets (thyme-primula root extract combination) in state-of-the-art clinical trials. However, until recently, understanding of the mechanisms of action underlying the clinical efficacy has been sparse. Over the last decade a comprehensive preclinical program - comprising several in vitro and in vivo models relevant for the indication – has been carried out, the outcome providing exciting insights into the pharmacodynamic effects of these herbal medicinal products. Not surprisingly, these studies suggest multi-modal therapeutic activity for Bronchipret: primarily anti-inflammatory, cough-soothing, and mucus-regulatory activities have been demonstrated. By applying tiered approaches combining in vivo and in vitro studies effects on pivotal aspects of mucus production as well as on key steps in inflammatory mediator production have been identified as reasonable molecular mechanisms of action. This review will provide an overview of a strategy to elucidate the pharmacological activity of a longtime established herbal medicinal product based on its known clinical therapeutic effects and presents the experimental findings on the pharmacological activities. It will also address a prospect on future tasks and challenges to help raise herbal medicinal

products from an obscure mass of traditional herbal medicines to an eye-to-eye level with chemical synthetic products further advancing the road towards an evidence-based phytomedicine.

Poster Session-PO-87:

Isoquinoline alkaloids and amides from *Chelidonium majus*

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Chelidonium majus is the only species in the genus *Chelidonium*, belongs to family Papaveraceae. The aerial parts of this plant are used as a traditional medicine for anti-bacterial, anti-inflammatory, anti-cancer activities to treat gastric ulcer, gastric cancer, many types of skin diseases and liver disorders. In the previous reports, the main constituents of *C. majus* were confirmed as isoquinoline alkaloids, flavonoids and phenolic acids. On continuing research of the bioactive compounds from natural sources, we isolated two new isoquinoline alkaloids: cis-tetrahydrocoptisine N-oxide (1), trans-tetrahydrocoptisine N-oxide (2); three known isoquinoline alkaloids: impatien B (3)^[1] spallidamine (4), oxychelerythrine (5); a phenolic acid: 3,4-methylenedioxy phthalic acid (6) and three known amides: noroxyhydrastinine (7), trans-N-coumaroyltyramine (8)^[2], cis-N-coumaroyltyramine (9)^[3] from the dried aerial parts of *C. majus*. Their structures were elucidated on the spectroscopic methods including 1D-NMR (¹H, ¹³C-NMR), 2D-NMR (HSQC, HMBC, NOESY), mass (HR-ESI, ESI). Compounds 3, 5, 6, 8 and 9 were isolated for first time from *C. majus*. Additionally, all of the isolates were tested for their inhibitory effect on LPS-induced NO production in RAW 264.7 cells. Among them, compounds 2 and 5 showed inhibitory effect with IC₅₀ values of 26.3 and 70.1 μM, while aminoguanidine, a positive control, showed an IC₅₀ value of 15.9 μM.

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Poster Session-PO-88:

Clerodanes from *Salvia pseudorosmarinus* and their activity as inhibitors of monoacylglycerol lipase (MAGL)

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As a part of our research program for characterization of the biological activity of Lamiaceae diterpenes, the aerial part extract of *Salvia pseudorosmarinus* Epling, a perennial shrub up to 150 cm high with purple flowers growing in Peruvian Ande at 3500-4000 m above sea level, were investigated [1]. Over 900 *Salvia* species are widely distributed in different regions around the world such as the Mediterranean area, Central Asia, Africa, and America, and secondary metabolites produced by these plants include mainly diterpenoids, having an abietane or clerodane skeleton, sesquiterpenoids, triterpenoids, flavonoids, and polyphenols [2]. Diterpenoids and phenolic derivatives isolated from different species showed antioxidant, anticoagulant, cytoprotective, antihypertensive, anti-fibrotic, anti-ischemia-reperfusion injury, antiviral and antitumor activities [3]. Three new and one known clerodane diterpenes were isolated from the chloroform extract of the plant, by means of flash Silica gel column chromatography and RP-HPLC. The structural characterization of all compounds was performed by spectroscopic analyses, including 1D and 2D NMR, and HRESIMS experiments. The isolated compounds were assayed for their inhibitory activity on two enzymes involved in the peculiar glycolytic or lipidic metabolism of cancer cells, human lactate dehydrogenase (LDH) and monoacylglycerol lipase (MAGL), respectively. All the compounds showed negligible activity on LDH, whereas the known clerodane jeneverol A displayed a certain inhibition activity on MAGL, showing an IC₅₀ value of 75.8 µM.

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Poster Session-PO-89:

Antileukemia effect of Xestoquinone, induced apoptosis via oxidative stress combined with inhibition of Hsp90 and topoisomerase II activities

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Xestoquinone (XQ), a polycyclic quinone-type metabolite, was isolated from the marine sponge *Petrosia* sp. which were found to inhibit a variety of cancer cell proliferation[1]. The marine polycyclic quinone-type metabolite, xestoquinone (XQ) was found to inhibit the proliferation of Molt 4, K562, SupT1, U937, T47D and DLD-1 cancer cell lines, with IC₅₀ of 1.69, 4.71, 5.87, 11.65, 14.41 and 12.5 μ M, respectively. It exhibited the most potent activity against leukemia Molt 4 cells. To fully understand the mechanism of XQ, we further explored the precise molecular targets in leukemia Molt 4 cells. We found that the use of HQ increased apoptosis by 20.8%-63.0% and caused disruption of mitochondrial membrane potential (MMP) by 30.3%-88.7% in a dose-dependent manner, as demonstrated by annexin-V/PI and JC-1 staining assays, respectively. Moreover, our findings indicated that the pretreatment of Molt 4 cells with N-acetyl-L-cysteine (NAC), a reactive oxygen species (ROS) scavenger, diminished MMP disruption and apoptosis induced by XQ, suggesting that ROS overproduction plays a crucial role in the cytotoxic activity of XQ. The results of a cell-free system assay indicated that XQ could act as HDAC and topoisomerase inhibitor through the inhibition of pan-HDAC and topoisomerase II α expression, respectively. On the protein level, the expression of the anti-apoptotic proteins p-Akt, HDAC, XIAP and Bcl-2 were inhibited by the use of XQ. However, the expression of the pro-apoptotic protein Bax, PARP cleavage and caspase activation, as well as Hsp70 and acetylated tubulin were increased after XQ treatment. Taken together, our results suggested that the antileukemic effect of XQ is ROS-mediated mitochondrial apoptosis combined with the inhibitory effect on Hsp90 and topoisomerase activities.

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Poster Session-PO-90:

13-Acetoxysarcocrassolide, a Cytotoxic Cembranolide Derivative, Exhibited Apoptotic Activity on Oral Cancer Cells through the Inhibition of Heat Shock Protein 90

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In this study, we found that the marine cytotoxic product 13-acetoxysarcocrassolide (13-AC), recently isolated from the alcyonacean coral *Lobophytum crassum* [1], exhibited potent inhibitory activity on HSP90. 13-AC induced apoptosis in oral cancer cells Ca9-22 through the disruption of mitochondrial membrane potential and the stimulation of reactive oxygen species generation. However, the pretreatment of Ca9-

22 cells with N-acetylcysteine, an antioxidant, inhibited ROS production resulting in the attenuation of the cytotoxic activity of 13-AC. Under stressful conditions, Ca9-22 cells treated with 13-AC showed a rapid induction of Keap1-Nrf2 pathway and an increase in the expression of p62/SQSTM1, but a suppression in antioxidative function of Nrf2 with immunoprecipitation, immunocytofluorescent and western blotting analysis. Inhibition of p62 expression by siRNA considerably attenuated the growth-inhibited by 13-AC treatment. Moreover, 13-AC exerted antitumor effect against oral cancer cells as demonstrated by the in vivo xenograft animal model. It significantly reduced tumor volume (55.29%) and tumor weight (90.33%). The molecular docking analysis demonstrated that 13-AC binds to N-terminal domain of HSP90 protein showing binding affinity more than 17-allylaminogeldanamycin (17-AAG), a HSP90 inhibitor of N-terminal ATP binding site and suppressed HSP90 client proteins including p-Akt, CDK4, HIF-1 α , and MMP-2. On the proteins level, 13-AC increased the expression of apoptosis related proteins such as cleaved caspases-3 and -9 as well as cleaved PARP in a dose- and time-dependent manner. Moreover, the results suggested that 13-AC exerted its cytotoxic activity through the promotion of ROS generation and the suppression of antioxidant enzyme activities. Altogether, the apoptotic effect of 13-AC was found to be mediated through the inhibition of HSP90 suggesting its potential future application as an anticancer agent.

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Poster Session-PO-91:

A Clinical study of ALM16 in subjects with mild degenerative arthritis for the development of a health functional food

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This clinical study was carried out to evaluate the efficacy and safety of ALM16 as a new functional raw material for joint health improvement. The clinical study was designed as a single institution, randomized, double-blind, placebo-controlled, and comparative study [1]. In this study, 88 patients with mild and moderate degenerative arthritis were treated with ALM16, the test product, for 12 weeks, three times a day (for a total of 9 tablets per day). In the animal studies, ALM16 exhibited excellent cartilage protection efficacy. The primary endpoint was the change of 100mm Pain VAS on walking (VAS) at the evaluation point (visit 4) after 12 weeks compared to baseline (visit 2). In the secondary endpoint, the change rate of VAS and WOMAC Scale (WOMAC), Korean Knee Score (KKS), and cytokines were evaluated. In the secondary endpoint, the

test group showed a statistically significant improvement of 44.73% (p-value < 0.0001) at the score change rate of VAS before and after the test food intake, while there was no statistically significant difference in the control group. In the total score change rate of WOMAC, the test group and control group showed statistically significant improvements of 38.18% (p-value < 0.0001) and 18.95%, respectively, before and after the test food intake. The total score change rate of KKS in test group showed a statistically significant improvement of 35.76% (p-value < 0.0001); whereas the change in the TNF-alpha, Interleukin-6, and CRP (C-reactive protein) showed some improvement in the test group, although there was no statistical significance. The results suggest that ALM16 has statistically and clinically significant improvement effects in moderate degenerative arthritis patients diagnosed with knee pain of Kellgren-Lawrence grades I-III [2] in radiological examinations.

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Poster Session-PO-92:

Anti-inflammatory effect of a Traditional Chinese Formulation tested on macrophages

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The Traditional Chinese Formulation Fang Feng Tong Sheng San (FFTSS) consists of 17 different components.[1] A previous paper reports about antioxidant, anti-diabetic, anti-tyrosinase, anti-neuro-degenerative and antimicrobial effects.[2] The water extract as well as the major ingredients of its component herbs were tested for their anti-inflammatory potential by the release of nitrite oxide (NO) in macrophages.

FFTSS was extracted by ultrasonication. The release of nitric oxide in RAW 264.7 macrophages was evaluated. The viability of the cells was determined using MTT colorimetric method after 48 h incubation with various concentrations of test samples. The yellow MTT is reduced to a blue formazan derivate by the mitochondria of the living cells. The measurement was performed spectrophotometrically at 570 nm. [3]

The water extract possessed a higher anti-inflammatory potential through nitric oxide inhibition compared to the reference indomethacin. None of the tested pure main ingredients of the formulation came close to this result which leads to the conclusion that synergistic effects are responsible for the anti-inflammatory potential of FFTSS. As 79.35 ± 0.98 % of the RAW 264.7 cells were able to survive the water extract is considered to be none toxic. The investigation of the anti-inflammatory activity of FFTSS supports its use for the treatment

of inflammatory diseases.

Acknowledgements

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Poster Session-PO-93:

Heteronemin, Induced Mitochondrial Superoxide Production and Cytoskeleton protein Talin Dysfunction Mediated Leukemia Molt 4 cells Apoptosis.

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A sesterterpene derivative, heteronemin[1] exhibited potent cytotoxic activity against several cancer cell lines. To evaluate its cytotoxic mechanism of action, we first determined of heteronemin against several cancer cell lines for 24 and 48 h with MTT assay. The most sensitive cancer cell line was Molt4 . Thus, Molt4 cells were subjected to further investigation and the apoptotic inductive effect of heteronemin on these cells was evaluated using annexin V FITC assay. The use of increasing doses of heteronemin increased the percentage of apoptotic cells from 3.7% to 97.3%. Moreover, the apoptotic effect of heteronemin was further supported with DNA ladder formation, morphology change, caspases -3, -8, and -9 activation as well as PARP cleavage. Heteronemin induced accumulation of reactive oxygen species and superoxide ions in Molt 4 cells. The use of ROS scavenger, N-acetyl cysteine, suppressed the generation of ROS production from mitochondria which was induced by heteronemin treatment. Heteronemin also decreased talin expression but activated p-talin expression, an integrin regulatory protein. The pretreatment of Molt4 cells with talin siRNA enhanced the expression of talin regulated protein FAK and FAK-related signaling pathways p-AKT (ser473), NF-kB (p65), p-ERK and anti-apoptosis protein XIAP as well as it decreased the ability of heteronemin to induce cell death. We further expanded our investigation to evaluate the antitumor effect of heteronemin in vivo xenograft animal model. The administration of heteronemin (0.3125 µg/g) reduced tumor volume 60%

compared with the control group. Taken together, these findings suggest the antitumor effect of heteronemin is associated with oxidative stress that the modulated talin and p-talin expression and mitochondrial dysfunction. Therefore, heteronemin represents an interesting anticancer drug lead against leukemia.

[1] Chang, Y.-C.; Tseng, S.-W.; Liu, L.-L.; Chou, Y.; Ho, Y.-S.; Lu, M.-C.; Su, J.-H. *Mar. Drugs* 2012, 10, 987-997.

Poster Session-PO-94:

Heteronemin, Marine Sesterpenoids-Type Metabolite, Induces Prostate LNCap Cells Apoptosis via Oxidative and ER Stress.

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Heteronemin, a marine sesterterpenoid-type natural product, possesses diverse bioactivities, especially antitumor effect[1]. To fully understand the antitumor mechanism of heteronemin, we further explored the precise molecular targets in prostate cancer cells. Initially, the growth inhibition effect of heteronemin was determined using MTT and colony formation assays. It exhibited the most potent activity against prostate cancer LNCap. With the xenograft animal model, the tumor size was significantly suppressed in the heteronemin-treated group about 51.88% as compared to the control group with no significant difference in the mice body weights. In addition, the results of a cell-free system assay demonstrated the inhibitory activity of heteronemin on Topoisomerase II (topo II). We found that the use of heteronemin triggered apoptosis by 20.13%-68.27%, caused disruption of mitochondrial membrane potential (MMP) by 66.92%-99.12% and elevated the calcium release by 1.80, 1.97 and 2.06 folds compared with the control group in a dose-dependent manner, as demonstrated by annexin-V/PI, Rhodamin 123 and Fluo-3 staining assays, respectively. Moreover, our findings indicated that the pretreatment of LNCap cells with an inhibitor of protein tyrosine phosphatase (PTPi) diminished ROS generation and apoptosis induced by heteronemin, suggesting that PTP activation plays a crucial rule in the cytotoxic activity of heteronemin. The expression of Hsp90 client proteins, phosphorylation of Akt (Ser473), STAT 3 (Ser 727 and Tyr705), PCNA, Rb2, as well as XIAP were suppressed by the use of heteronemin. However, the expression of p-HSF1, Hsp70 and acetylated tubulin were induced after heteronemin treatment. Thus, heteronemin significantly induced apoptotic and autophagic death of LNCap cells by modulating ER and oxidative stress combined with the inhibition of topo II catalytic activity and Hsp 90 function.

[1] Chang, Y.-C.; Tseng, S.-W.; Liu, L.-L.; Chou, Y.; Ho, Y.-S.; Lu, M.-C.; Su, J.-H. Mar. Drugs 2012, 10, 987-997.

Poster Session-PO-95:

Biological activities of the main compounds of the Chinese formulation Fang Feng Tong Sheng San

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The Chinese formulation Fang Feng Tong Sheng San (FFTSS) is a mixture consisting of 17 different components ^[1]. Previous investigations proved the activities of the formulation and of preparations ^[2].

12 main compounds of FFTSS's component herbs have been tested by in vitro bioassays for different activities.

Oxidative stress plays a significant role in aging processes and in the formation of cancer. The most potent antioxidants were gallic acid (IC₅₀ 4.50±0.05 µ g/ml), luteolin (IC₅₀ 4.67±0.50 µ g/ml) and baicalein (IC₅₀ 10.02±0.25 µ g/ml) compared to the reference rutin (IC₅₀ 12.66±0.84 µ g/ml). The most potent inhibitors of the lipidperoxidase were baicalein (IC₅₀ 0.37±0.03 µ g/ml), baicalin (IC₅₀ 2.50±0.05 µ g/ml) and luteolin (IC₅₀ 4.07±0.79 µ g/ml) (reference quercetin IC₅₀ 1.17±0.05 µ g/ml). Acetylcholinesterase and butyrylcholinesterase are important in the treatment of the Alzheimer's disease. The most effective substances for inhibiting acetylcholinesterase were gallic acid (IC₅₀ 2.46±4.50 µ g/ml) and wogonin (IC₅₀ 15.32±3.10 µ g/ml) (reference eserine (IC₅₀ 0.35±0.06 µ g/ml). The most potent inhibitor for butyrylcholinesterase was luteolin (IC₅₀ 24.82±0.96 µ g/ml). Baicalin showed a similar inhibitory activity (IC₅₀ 288.38±18.46 µ g/ml) as the α -glucosidase inhibitor acarbose (IC₅₀ 346.68±4.36 µ g/ml) which is used for the treatment of diabetes mellitus type II. Baicalein (IC₅₀ 44.27±2.31 µ g/ml) and baicalin (IC₅₀ 54.90±1.46 µ g/ml) turned out to be also potent inhibitors of tyrosinase (reference ascorbic acid IC₅₀ 36.73±0.67 µ g/ml) which may play a role in the pathogenesis of the Parkinson's disease.

Acknowledgements:

Financial support from the BMWF (GZ 402.000/00013-WFN/6/2016) and the CACMS is gratefully acknowledged.

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Poster Session-PO-96:

A new method for the quantification of two diterpenes, carboxyatractyloside and atractyloside, via HPLC-DAD in *Xanthii fructus*

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Xanthii fructus (*Xanthium sibiricum* PATR., Compositae) is used in Traditional Chinese Medicine for afflictions like allergic rhinitis, sinusitis and itching caused by rubella. [1] Besides fixed oil (e.g. stearic acid), volatile oil (e.g. trans-caryophyllene), also sesquiterpenes (e.g. xanthumin) and phenolic acids (e.g. 3,5-di-O-caffeoylquinic acid) are among the documented chemical constituents. [2]

Due to the fruits' apparent hepatotoxicity, content of the two diterpenes carboxyatractylosid (CATR) and atractyloside (ATR) should be quantified and limited, which is mandatory in the latest edition of the Pharmacopoeia of the Peoples Republic of China (ChP) 2015. [1–3] So far, the ChP demands a critical value for CATR for the raw herbal material and for ATR for the processed herbal material, respectively. [1]

Thus, a new HPLC-method should be developed to quantify both substances in one single run.

Method development and quantification of CATR and ATR was performed via HPLC using a C18 column and diode array detector ($\lambda=203$ nm). Mobile phase was composed of a 1 mM ammonium formate buffer (formic acid, pH 4.5) and acetonitrile using a gradient elution. HPLC analyses were performed at 40°C oven temperature with a flow rate of 1 mL/min.

The validation parameters accuracy, precision, including repeatability and intermediate precision, specificity, detection limit, quantification limit, linearity and robustness were investigated for CATR and showed adequate results.

In conclusion, a new, simple and fast HPLC method, based on the monograph of *Xanthii fructus* [1] was developed to quantify the two toxic diterpenes simultaneously.

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Poster Session-PO-97:

The herbal combination BNO2103 exerts anti-inflammatory effects by reducing immune cell recruitment

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The most noticeable and bothersome symptoms of urinary tract infections are pain, urge and frequency, which are a consequence of the ensuing inflammation, the body's innate response to infection that is triggered by release of cytokines and eicosanoids as well as recruitment of immune cells.

Here we investigated the effects of BNO2103, the active pharmaceutical ingredient in Canephron® N, on recruitment of immune cells using various in vitro and in vivo assays. To assess effects on chemotaxis, polymorphonuclear leukocytes (PMNs) were stimulated with the bacterial peptide N-formyl-methionyl-leucyl-phenylalanine (fMLP) and migration into a 150 µm membrane was measured. Adhesion of PMNs to a monolayer of hTNF (human tumor necrosis factor)- or LPS (lipopolysaccharide)-stimulated umbilical vein endothelial cells (EAhy926) and transmigration of PMNs through a layer of hTNF- or LPS-stimulated EAhy926 or kidney epithelial cells (HK-2) in Boyden chambers were also measured. Finally, effects of BNO2103 on recruitment of immune cells were investigated in a mouse model of zymosan-induced peritonitis by counting immune cells in the peritoneal exudate 4 h after injection of zymosan.

While pretreatment with BNO2103 had little effect on chemotaxis and transmigration of PMNs through endothelial cells, it reduced adhesion of PMNs to endothelial cells (max. inhibition approx. 60% at 300 µg/ml) as well as transmigration of PMNs through epithelial cells (max. inhibition approx. 100% at 300 µg/ml). In the in vivo peritonitis model, oral application of BNO2103 reduced the number of immune cells in the exudate ($2.95 \pm 0.716 \times 10^6$ cells with BNO2103 (twice the human dose) vs. $5.5 \pm 0.872 \times 10^6$ cells in control).

These data indicate that BNO2103 displays anti-inflammatory actions by interfering with the recruitment of immune cells. If pain, urge and frequency are a consequence of inflammation, then BNO2103 should also have a beneficial effect on these typical symptoms of urinary tract infection.

Poster Session-PO-98:

Modulation of sow microbiota or where demedication starts: case study of a standardized citrus extract supplementation during sow peripartum

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Farrowing is a critical period in sow's production cycle in which they are more susceptible to some troubles like constipation. These troubles result in a decrease of sow's welfare, an appetite alteration and a decrease of milk quality and/or quantity. Constipation has also a negative impact on sow's microbiota by promoting *Escherichia coli* proliferation. To avoid these situations, it's important to manage sow's microbiota during peripartum.

Citrus extract molecules effect on microbiota is documented in many research. However, the extracts available on market vary in term of composition and concentration of active compounds, which can lead to variation in the obtained results. This study was set up with a standardized natural citrus extract (SNCE) in order to evaluate its effect on sow's microbiota and productivity. In a commercial farm (west of France), 100 sows on peripartum were divided in 2 groups: a trial group (27 sows) received diet supplemented with SNCE (2500 ppm, 10 days before farrowing and 5 days after) and a control group (23 sows) received diet without supplementation. Zootechnical performances (feed intake, litter weight gain), first defection after farrowing, as a marker of intestinal transit, were monitored and microbiota composition of the 2 groups were assessed. Results showed that the SNCE supplementation significantly reduced the "first defection- farrowing" interval, which indicate a better and faster transit after farrowing. Supplemented sows had a higher feed intake and a higher litter weight gain compared to control group. Moreover, PCR analysis of faecal microbiota showed that the bacterial composition of supplemented group had more *Lactobacillus* (*L. reuteri*, *L. amylovorus*) compared to control group. These results suggest that feed supplementation with SNCE, which allow to modulate microbiota, have beneficial effects on sow's productivity and piglet in pre-weaning period. Monitoring microbiota could be the first step of a global demedication strategy.

Poster Session-PO-99:

Domestication Study of *Senecio Nutans* Sch.Bip. (Asteraceae): Its Economic Potential and Sustainable Management

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Introduction: Chachacoma, *Senecio nutans* Sch.Bip. (Asteraceae), is a herbal remedy used traditionally in Chile to prevent altitude sickness. The main compound identified is acetophenone (1-[4-hydroxy-3-(3-methylbut-2-enyl)phenyl]ethanone), which has been found to have vasodilator effects [1]. Consequently, it has acquired a reputation to be used as a sports supplement and has been used as a value-adding ingredient for new innovative health products.

Chachacoma mainly grows in the wild between 3,200-5,000 metres and has been collected by the Aymara minority group as a cash crop, overexploiting this native natural resource. The interinstitutional research collaboration between CIHDE, Chile and UCL, UK, has established an evidence base for its characterization, plausibility of action and optimization of the main ingredient, acetophenone, through in vitro tissue culture.

Methods:

We analyzed samples of Chachacoma cultivated and collected at different altitudes by NMR spectroscopy and HPTLC to assess the levels of acetophenone and establish fingerprints for the other metabolites extracted.

Results:

Acetophenone content appears to vary at different altitudes and growing conditions, the best collected samples, with relation to acetophenone content, grew in the wild, above 4500 meters, at Chungara lake. Similar levels of acetophenone and metabolite fingerprint were obtained using in vitro tissue culture as the starting material.

Conclusions:

Tissue culture cultivation may be a way to expand and develop the Chachacoma industry for the benefit of the Aymara and other minority groups living in the high Andes, and at the same time help to ensure its sustainability for future generations.

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We are grateful for a grant from the regional program of CONICYT, Grant R15F100

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Poster Session-PO-100:

Total flavonoid and tannin content variation during the vegetation period and extracts antimicrobial activity of *Filipendula ulmaria* L. herbal raw materials

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Meadowsweet (*Filipendula ulmaria* L.) is traditionally used to treat diarrhea and urinary infections. Herbal raw material characterized by astringent and antibacterial properties because of tannins [1]. Medicinal plant accumulates flavonol glycosides. Due to the flavonoids meadowsweet herbal extracts presents an anti-cancer, anti-inflammatory and immunomodulatory effects [2].

Research object is naturally growing meadowsweet herb collected in Lithuania. The aim of this study was to determine the quantitative composition dynamics of flavonoids and tannins during the vegetation period of this plant and assay the antimicrobial effects of herbal extracts against nine microbial cultures.

The highest total flavonoids content of *F. ulmaria* herb was determined during a massive flowering period (3.49 %). The total flavonoid content in herbal raw material significantly depends on the plant vegetation stage, but statistically significant changes take place from the beginning of flowering until the end of flowering. The maximum total tannin content was determined during meadowsweet massive flowering stage (16.80%). Statistically significant changes of tannin content was evaluated at the beginning of flowering, including massive flowering, to the end of flowering, ($p < 0.05$). This tendency may be due to the fact that tannins are needed in order to protect themselves from the medicinal plants threatening infections, insect or animal damage. [3]. Aqueous and ethanolic meadowsweet extracts were tested for their antibacterial activity respectively. It was found that ethanolic extracts possess a slightly higher antimicrobial effect, especially against *E. faecalis* and *B. subtilis*. It was noted that *C. albicans* was characterized by the highest resistance. The growth was inhibited only by the highest concentration of aqueous extracts (10mg/ml).

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- meadowsweet, tannins, flavonoids, antimicrobial

Poster Session-PO-101:

Piperidine alkaloid enriched fractions from young shoots of Norway spruce (*Picea abies*) are antibacterial against *Streptococcus equi* ssp. *equi*

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Streptococcus equi ssp. *equi* is a haemolytic bacterium which causes an upper respiratory infection in horses, called strangles. Strangles is the most frequently diagnosed infectious disease in horses worldwide [1]. Strangles is highly contagious and is characterized by swollen lymph nodes and fever, eventually leading to death of young horses through suffocation and is thus of substantial economic concern in horse business [1]. Resistance to drugs has been reported to be low, with the exception of gentamicin [2]. Complication rate is, however high, with 20 % of the cases leading to severe infection and fatality [3]. To the best of our knowledge, there is a limited number of investigations concerning the use of plants and their extracts or individual compounds for prophylaxis and treatment of strangles.

Piperidine alkaloid enriched fractions were extracted from young shoots of *Picea abies*, using acid water and solid phase extraction [4]. Some of the fractions were separated further using RP18-TLC. The fractions were investigated for their growth inhibitory effects against *S. equi* ssp. *equi* ATCC 9528 using a microdilution method [5]. At 265-487 µg/ml, the fractions inhibited 45-100 % of the growth. A fraction enriched with trans-pinidinol gave especially good effects resulting in a MIC at 335 µg/ml. Our results indicate that young shoots of spruce and their alkaloid enriched extracts could be used both as prophylaxis and for treatment of strangles.

Acknowledgements:

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Poster Session-PO-102:

Eupatoriopicrin inhibit the pro-inflammatory functions of human neutrophils via suppression p38 and ERK 1/2 MAP kinases pathways

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The p38 and ERK MAPK pathways are activated in human neutrophils by chemo attractants, pro-inflammatory cytokines, lipopolysaccharide (LPS), and Fcγ receptor ligation. Pharmacologic inhibition of p38 MAPK activation attenuates neutrophil respiratory burst activity, exocytosis, chemotaxis, adhesion, IL-8 synthesis and stress-induced apoptosis. Pharmacologic inhibition of ERK activity enhances neutrophil apoptosis, while the role of ERK in respiratory burst activity remains controversial [1]. Inhibition of both kinases is a promising therapeutic strategy to treat chronic inflammatory diseases.

The present study tested the hypothesis that eupatoriopicrin (sesquiterpene lactone, isolated from *E. cannabinum* L.) mediates multiple p38 and ERK1/2 MAPK-dependent responses in human neutrophils.

To verify the hypothesis we tested activity of eupatoriopicrin and positive controls (quercetin and clarithromycin) on LPS and f-MLP-stimulated neutrophils. We examined: (I) phosphorylation level of p38, ERK1/2 and JNK MAP kinases, (II) degranulation; respiratory burst activity and elastase release, (III) pro-inflammatory cytokine release (IL-8, IL-1β and TNFα) and (IV) apoptosis.

Phosphorylation of p38, ERK1/2 and JNK MAPK was determined by immunoblotting analysis. The inhibition of ROS production was determined using luminol dependent chemiluminescence method. Neutrophil elastase release was established spectrophotometrically. The effect on chemokines production was measured by enzyme-linked immunosorbent assay (ELISA). The apoptosis of neutrophils was analyzed with flow cytometry.

Inhibition of p38 kinase activity by eupatoriopicrin significantly attenuated degranulation and pro-inflammatory cytokine release. Respiratory burst and elastase activity were significantly inhibited by eupatoriopicrin at 2.5μM, compared with quercetin and clarithromycin tested at 50 μM . Lipopolysaccharide-induced IL-8 and TNFα production was significantly inhibited by eupatoriopicrin at 0.25 μM (IC₅₀ < 1 μM). Inhibition of p38 and ERK1/2 activity by eupatoriopicrin at 0.25 μM resulted in an abolition of LPS-delay of neutrophils apoptosis. These data suggest that eupatoriopicrin mediates both p38- and ERK MAPK-dependent neutrophil responses leading to potential beneficial health effects.

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Poster Session-PO-103:

An innovative Approach to Sustainable Marine Invertebrate Chemistry and a Scale-Up Technology for Open Marine Ecosystems

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The biodiversity of marine environment is a unique resource that can provide a huge diversity of natural products. So far some thousands small molecules have been isolated mainly, from invertebrates such as sponges, tunicates, and molluscs, or their associated microorganisms such as marine bacteria or fungi.

The major limiting factor in marine invertebrate chemistry is the sustainable access to the bioresource. In addition to the difficulties involved in harvesting the invertebrates, these sessile animals face various environmental and anthropogenic threats. Many are endangered species and protected through national, regional and international conventions. Attempts to cultivate marine invertebrates or their cells have not, to date, provided a satisfactory solution to the problem of sourcing. This is not only due to the low yield and slow growth rate but it is also a result of taking the invertebrates out of their natural environment, which implies also their removal from the natural physico-chemical constraints and the presence of symbionts. As a consequence the cultivated invertebrates are not able to produce the desired target compounds. Thus the future challenge is to ensure the protection of marine invertebrate biodiversity while meeting the need to provide the drug pipeline with marine natural scaffolds and bioactive compounds.

In order to face this challenge we have developed an innovative strategy for trapping molecules from invertebrates maintained alive in an aquarium. This approach allowed to capture and then isolate five small molecules without applying any stress or injury neither to the animal nor to its environment. LC-HRMS and NMR analyses were implemented for the unambiguous characterization of the isolated molecules revealing all to be guanidine alkaloids while 3 of them were new natural products.

To anticipate the validation of the approach in open marine ecosystems, a specific technology named SomarteX (Self Operating MARine Trapping EXpert) was patented and build in order to be tested in different marine locations and depths.

Poster Session-PO-104:

Ellagitannins in antimycobacterial extracts of *Combretum hartmannianum*, a savannah woodland tree

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Combretum hartmannianum Schweinf. (Combretaceae) is native to East Africa, growing naturally in woodland areas in Eritrea, Ethiopia, South Sudan and Sudan [1]. Various parts of this plant, including roots and stem are used traditionally in Africa against bacterial infections and their symptoms such as cough [2].

We have assessed the antimycobacterial activity of extracts of *C. hartmannianum* against the TB model bacterium *Mycobacterium smegmatis* ATCC 14468. The most promising growth inhibitory activity was observed for root extracts, such as a methanol soxhlet extract (IZ 31.5mm, MIC 312.5 µg/ml) and an ethyl acetate extract (MIC 1250 µg/ml). Cold methanol extracts of the stem bark and wood gave a MIC of 5000 µg/ml. Hexane and dichloromethane extracts of the stem wood and stem bark were devoid of activity. HPLC-DAD and UHPLC/QTOF-MS analysis of the ethyl acetate extract of the root resulted in the characterization of fifteen ellagitannins, among them punicalagin, castalagin, corilagin, tellimagrandin, terflavin B and S-flavogallonic acid, none of which have been identified before in *C. hartmannianum*. Six of the identified ellagitannins are unknown and NMR is needed to elucidate their molecular structures. Previously, flavogallonic acid dilactone and terchebulin have been identified in *C. hartmannianum* stem bark [3]. Our results indicate that roots of *C. hartmannianum* contain a high diversity and concentration of ellagitannins which could be potential agents against tuberculosis.

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Poster Session-PO-105:

In vitro inhibitory effect of seriniquinone on human cytochrome P450

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Background:

Seriniquinone (SQ – Fig 1) is a secondary metabolite isolated from a rare marine bacterium of the genus *Serinicoccus*, which has raised attention of the scientific community due to its specific action on skin protective antimicrobial peptide, dermcidin [1]. SQ is the only known natural product to bind and modulate such protein, which is over expressed in some cancer types and linked to a poor prognosis of such diseases [2]. The advance of this new bioactive molecule to drug development requires metabolism studies for the assessment of the potential of SQ to interact clinically with other drugs. In this context, this study aimed to evaluate the in vitro inhibitory effect of SQ on human CYP450.

Methods:

Specific reactions catalyzed by CYP450 isoforms were monitored in the presence and absence of SQ (15 μ M) using human liver microsomes and the inhibitory potential against the enzymes activities were determined. Furthermore, IC₅₀ determinations were performed for the inhibited CYP450 isoforms.

Results:

The main CYP450 isoforms inhibited by SQ and its respective inhibition percentage were CYP1A2 (93%), CYP2E1 (65%), CYP3A4/5 (55% and 35% for midazolam 1'-hydroxylation and nifedipine oxidation reactions, respectively) and CYP2C19 (47%). Weak inhibition was observed for CYP2C9 (8%) and CYP2D6 was not inhibited.

Conclusion :

These results provide useful information about the safety of SQ as a drug candidate and suggest that its concomitant intake with drugs metabolized by CYP1A2, CYP2E1, CYP3A4/5 and CYP2C19 should be carefully monitored because it may lead to adverse SQ-drug interactions.

Acknowledgments:

We are grateful to FAPESP, CNPq and CAPES for financial support.

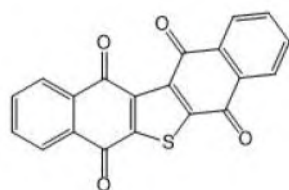


Fig 1. Chemical structure of seriniquinone (SQ)

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Poster Session-PO-106:

Studies on secondary metabolite profiling, in vitro antioxidant potential and enzyme inhibitory properties of *Rubus caesius*

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The genus *Rubus* has a number of economically important members which can be considered as natural agents in several traditional folk remedies. Taking this into consideration, the present study was designed to investigate antioxidant activities and enzyme inhibitory effects of the extracts and fractions (RC1, H₂O; RC2, 50% MeOH; RC3, MeOH; RC4, Et₂O; RC5, EtOAc; RC6, n-BuOH) obtained from the aerial parts of *Rubus caesius*. Different assays to detect antioxidant capacity, especially free radical scavenging (ABTS and DPPH assays), reducing power (CUPRAC and FRAP), phosphomolybdenum and metal chelating were performed. Enzyme inhibitory effects were also tested towards acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), tyrosinase, and glycolytic enzymes. Additionally, total phenolic and flavonoid content using colorimetric assays was examined. Strongest antioxidant potential among all samples RC1-RC6 was observed for the RC5 which ranging from 1.58 ± 0.01 to 4.98 ± 0.05 mmol TE/g extract. Also, RC5 contained highest concentration of total polyphenolics (315.02 ± 9.11 mg GAE/g) and flavonoids (38.13 ± 0.37 mg RE/g). Highest metal chelating capabilities was observed for RC2, being equal to 16.62 ± 1.71 mg EDTAE/g. All tested samples were active against AChE, however only three fractions RC1, RC2 and RC3 were active against BChE. Activity was observed in range of 1.99 ± 0.04 - 2.46 ± 0.01 mg GALAE/g and 1.00 ± 0.09 -

1.10 ± 0.01 mg GALAE/g for AChE and BChE, respectively. Tested extracts and fractions inhibited α-amylase, with RC5 showing highest activity equal to 0.70 ± 0.07 mmol ACAE/g, while the same fraction (RC5) was most effective inhibitor (2.03 ± 0.01 mmol ACAE/g) of α-glucosidase. The RC6 fraction had the strongest anti-tyrosinase inhibitor ability 63.12 ± 0.26 mg KAE/g. The present study demonstrated that *R. caesius* may be considered as a source of biologically active phytoconstituents to develop novel functional products or natural drugs in pharmaceutical field.

Poster Session-PO-107:

Cornus mas and Cornus alba fruits - the comparative study of α -amylase and pancreatic lipase inhibitory effects

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Fruits from *Cornus* spp. (Cornaceae) species have shown antidiabetic, antibacterial and anti-allergic properties and are thus considered a source of phytochemicals that are beneficial to human health [1].

The aim of the study was a comparison of the chemical compositions of the aqueous and ethanolic extracts from fresh-picked fruits of *Cornus mas* L.(Cm) and *Cornus alba* L. (Ca) as well as their α-amylase and pancreatic lipase (PL) inhibitory activities. In order to identify the compounds potentially responsible for the inhibition of pancreatic enzymes the bio-assay guided isolation was conducted.

The chemical compositions of the dogwood extracts and the fractions of the extracts were analyzed by HPLC-DAD-MS/MS. Their effects on digestive enzyme activity were evaluated using an in vitro fluorescence method [2].

Iridoids (loganic acid, cornuside) and anthocyanins (pelargonidin 3-O-galactoside) were identified in the Cm fruit extracts. Flavonoids, such as quercetin and kaempferol derivatives, and phenolic acids were detected in the Ca fruit extracts. The ethanolic extracts of both species inhibited PL activity more significantly than α-amylase activity. The IC₅₀ of ethanolic extracts of Cm and Ca fruits were 15.2 ± 3.9 µg/mL and 25.3 ± 2.0 µg/mL, respectively, in the PL assay. The chromatographic separation of the constituents of Ca fruit provided a fraction containing phenolic acids derivatives, which inhibited PL activity by 69.9 ± 4.5% at a concentration of 7.5 µg/mL.

The phytochemical constituents of Cm, and particularly of Ca fruit extracts, can inhibit pancreatic enzymes and thus should be considered effective preparations in the prevention and control of hyperlipidemia related diseases.

Acknowledgements :

This project was financially supported by the National Science Centre, Poland (grant No 2016/23/D/NZ7/00958).

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Poster Session-PO-108:

Antioxidant and Anti-inflammatory Activities of *Taraxacum mirabile* Wagenitz Roots

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The genus *Taraxacum* is a member of the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae. *Taraxacum* species are wild plants and have long been used traditionally in folk medicine for its curative properties such as dyspepsia, heartburn, spleen and liver complaints, anorexia, diabetes, cancer and gastric, renal and hepatic ailments [1, 2]. *Taraxacum mirabile* Wagenitz is an endemic species to Turkey and growing in the central part of the country. In this study, the antioxidant and anti-inflammatory activities of ethyl acetate, butanol, dichloromethane and petroleum ether fractions of the ethanol extract from the roots of *Taraxacum mirabile* Wagenitz were investigated. It was found that the extracts are able to scavenge DPPH, ABTS radicals, and reduce Fe³⁺ to Fe²⁺ in the ferric reducing antioxidant power (FRAP) assay. Ethyl acetate and dichloromethane extracts showed the highest antioxidant activity due to their richest phenolic contents, followed by butanol extracts, whereas, petroleum ether extracts containing the least phenolics, were weakest in activity. Anti-inflammatory activities of the extracts were evaluated against cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), key enzymes relating to inflammation. The ability of the extracts to inhibit COX-1 and COX-2 was determined by calculating percent inhibition of prostaglandin production measured by enzyme immunoassay. The results showed a considerable inhibitory activities up to 96 % at 10 mg/ml (final concentration of 500 µg/ml) against both enzymes, suggesting that this species might be a potential source of effective plant-derived anti-inflammatory substances.

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Poster Session-PO-109:

Isolation and purification of guaiane sesquiterpene lactones from *Ferula penninervis* Regel et Schmalh. by high-performance counter-current chromatography

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Ferula penninervis Regel et Schmalh. (feather-veined giant fennel) is a perennial herbaceous plant of Apiaceae family, widespread in the dry regions of Central Asia. Extract of various *Ferula* species have shown antiviral, antibacterial, anti-inflammatory, antitumor and antidiabetic activities [1]. Sesquiterpenes, sesquiterpene coumarins and dimeric coumarins are considered the main biologically active constituents [2]. The aim of the study was to optimize the high-performance counter-current chromatography technique (HPCCC), which has been already successfully used for separating numerous natural products, to obtain pure secondary metabolites from *F. penninervis* roots. In order to find the suitable coefficient partition (K) values of target compounds, different mixtures of n-hexane-ethyl acetate-methanol-water (HEMWat) were tested; of these, HEMWat 3:2:3:2 (v/v/v/v) was further selected for the HPCCC separation of the crude methanolic extract of *F. penninervis* roots, performed at a flow rate of 6 mL/min, 1600 rpm and 254 nm. Five guaiane sesquiterpene lactones, namely: olgin (5.9 mg, 95.7% purity), laferin (9.7 mg, 95.7%), olgoferin (4.2 mg, 95.4%), oferin (3.1 mg, 98.0%) and talassin B (4.9 mg, 98.4%), were obtained from a total amount of 800 mg of crude extract. The compounds were identified by the use of spectrometric methods and comparison with literature data [3]. Since all these compounds were isolated for the first time by HPCCC, this technique could thus open the perspectives of large scale isolation of biologically active compounds from *F. penninervis* or other plant species abundant in sesquiterpene derivatives.

Acknowledgments:

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Determination of antiseizure activity of bergamottin and lucidafuranocoumarin A, constituents of *Peucedanum alsaticum* L. using a zebrafish epilepsy model

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Epilepsies – encompassing a variety of neurological disorders of diverse etiology that have spontaneous, recurrent seizures as a common symptom – affect approximately 1% of the population. While numerous antiseizure drugs (ASDs) have been developed, these are only effective in 70% of epilepsy patients, leaving at least 20 million epilepsy patients worldwide without effective medication. In an ongoing effort to explore the potential of medicinal plants to provide novel drug leads and/or novel botanical drugs for the treatment of epilepsies, we and others have recently established the utility of zebrafish epilepsy models for the rapid and microscale in vivo screening and bioassay-guided fractionation of secondary metabolite extracts of medicinal plants [1]. Several phytochemicals with antiseizure activity have been identified to date, including coumarins [2].

Here, we describe the in vivo characterization of the antiseizure activity of main furanocoumarin constituents, isolated from the methanolic extract of *Peucedanum alsaticum* L. (Apiaceae) using high-performance countercurrent chromatography (HPLCCC). Immiscible mixture of solvents heptane, ethyl acetate, methanol and water (v/v 3:1:3:1) was successfully used in reversed phase mode to achieve 12,79 mg of bergamottin and 24,22 mg of lucidafuranocoumarin A. Both compounds exhibited antiseizure activity in zebrafish epilepsy model based on the GABAA antagonist pentylenetetrazol (PTZ), which causes zebrafish larvae to exhibit increased locomotor activity, seizure-like behavior, and epileptiform electrographic activity [3]. Lucidafuranocoumarin A exhibited more potent antiseizure activity, inhibiting 69% of PTZ-induced seizures at its maximum tolerated concentration (MTC) of 16 μ M. Further experiments will seek to validate these findings through electrophysiological analysis in zebrafish and equivalent experiments in mouse epilepsy models.

Acknowledgments: This work was supported by Preludium 11 grant 2016/21/N/NZ4/03658 from the National Science Center (NCN) of Poland.

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Liposomes for Escin and Berberine Chloride Dermal Delivery

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Berberine (BRB) is a natural isoquinoline alkaloid, used for many therapeutic activities by the Traditional Chinese Medicine, from the ancient times. Escin (ESN) is a mixture of triterpenic saponins that exhibits anti-inflammatory properties [1] and it was selected for both structural and functional activity [2]. Different liposomes, loaded with BRB HCl, were prepared according to the lipid film hydration method. All liposomes showed optimal sizes, low polydispersity and spherical shape. The dialysis bag method, followed by HPLC-DAD analysis, was used to define the Encapsulation Efficiency (EE%) and to investigate the in vitro release of BRB HCl and ESN. EE% were about 67% for the first one and 94% for the second one. Concerning the kinetic release, it was found a maximum delivery around 75% and 25% respectively, within 24 h. Liposomes had chemical-physical stability, until a month storage period, at 4°C. Deformation capability of the vesicles was evaluated by extrusion. Then, the formulations were tested by a system of parallel artificial membranes (PAMPA), miming the stratum corneum and the ex vivo permeation assay was made on rabbit ear skin, using vertical diffusion Franz cells. Passive transport through PAMPA and skin permeability were higher for BRB HCl loaded in ESN-based liposomes than BRB HCl loaded in the conventional liposomes formulated with phosphatidylcholine and cholesterol, or respect to the free molecule. In conclusion, ESN-based liposomes loaded with BRB HCl are deformable vesicles, able to cross the skin until the deepest layers of epidermis and suitable for dermatological applications to achieve a therapeutic synergy.

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Development and Characterization on Nanocochleates for Oral Delivery of Andrographolide

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Andrographolide (AG) is the main diterpenoid of *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae) and it has been reported to exhibit a wide spectrum of biological activities including anti-inflammatory, anticancer, antihyperlipidemic and so on. However it is insoluble in water, it is unstable under alkaline conditions and it is rapidly metabolized in the intestine [1]. In this work nanocochleates (NCs) based on phosphatidylcholine, cholesterol and calcium ions were prepared by trapping method. The multi-layered cylindrical structures of NCs protect the incorporated drug from degradation in hazardous environmental conditions, resulting in enhanced oral bioavailability [2]. Oral administration is characterized by good patient compliance, lower medical expenses and fewer side effects. AG-NCs exhibit an average size of 140 nm with good homogeneity and zeta potential of -22 mV. TEM observations provided details on the morphology: AG-NCs had a cigar-like shape with a regular size distribution which was comparable with Light Scattering data. AG-NCs were freeze dried without cryoprotectant and were stored at room temperature. After 60 days no variations of size, homogeneity and zeta potential were observed. The encapsulation efficiency determined by HPLC-DAD analyses was 71%. In vitro release studies in PBS at pH 7.4 indicated that after 24h approximately 95% of AG was released and Hixson model was shown to be the best-fit model to describe the kinetic of release. In vitro gastrointestinal stability studies were also performed. Finally J77A.1 macrophage cells were cultured to investigate the potential cytotoxicity and uptake of NCs.

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Poster Session-PO-113:

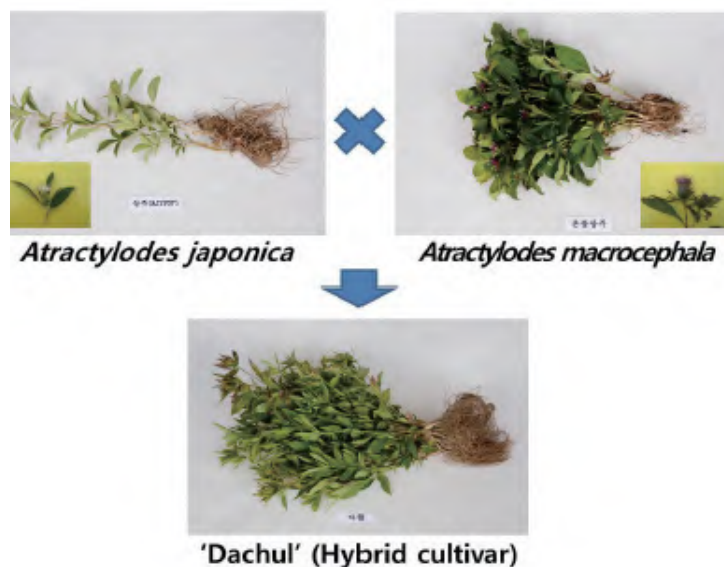
Comparison of agronomic characteristics and active ingredients among *Atractylodes japonica*, *Atractylodes macrocephala*, and their hybrid cultivars

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Atractylodes japonica koidz.(AJ) and *Atractylodes macrocephala* koidz.(AM) belong to *Atractylodes* genus (Asteraceae) and their rhizomes are used as traditional medicine 'White Atractylodes Rhizome' in Korea, China and Japan. 'White Atractylodes Rhizome' has anti-inflammatory and antinociceptive effects and atractylon is the major active ingredient. Previously, we developed 8 hybrid cultivars with disease resistance, high yielding ability and high active ingredients by interspecific hybridization between AJ and AM. In this study, agronomic characteristics of 8 hybrid cultivars were investigated in RDA experimental field. Among these cultivars, 'Sang-won' had the highest dry weight of rhizome (53.8g/plant), followed by 'Da-chul'(50.0g/plant). Most of hybrid cultivars showed higher dry weight than those of AJ (7.2g/plant) and AM (36.4g/plant). In addition, active ingredients were investigated using HPLC. As a result, Atractylon content was the highest in 'Dachul' (19.090 mg/g) that was 4 times higher than AM (4.923 mg/g). Based on these results, hybrid cultivars showed higher agronomic performance than AJ and AM. Particularly, 'Dachul' could be the superior cultivar with high active ingredient as well as high yield ability.



Poster Session-PO-114:

Studies on the chemical constituents and anti-inflammatory effect of *Pogostemon cablin*

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Pogostemon cablin (Blanco) Benth., also known as patchouli (廣藿香) is an important herb which possesses several biological activities such as antioxidant, analgesic, anti-inflammatory, and cytotoxic activities. Till now

more than 140 compounds have been isolated and identified from patchouli [1]. However, the mechanisms of some traditional uses of patchouli have not been well verified by standardizing and authenticating the bioactivity of purified compounds. In this meeting, the anti-inflammatory effect of pure compounds from patchouli will be investigated. In our search of compounds with anti-inflammatory activities, 13 compounds (1-13), including seven flavonoids, ternatin (1), 5-hydroxy-3,6,7,8,3',4'-hexamethoxyflavone (2), 3,5-dihydroxy-7,3',4'-trimethoxyflavone (3), rhamnazin (4), pachypodol (5), 5-hydroxy-3,7,4'-trimethoxyflavone (6), and retusin (7), a pyranone, pogostone (8), four benzenoids, veratric acid (9), isovanillic acid (10), α -curcumene (11), and (E)-2-methyl-6-(p-tolyl)hept-3-en-2-ol (12), and a sesquiterpene, α -guaiene (13) were isolated and identified from the aerial parts of *P. cablin*. The structures of all isolates were determined through spectral analyses and comparison of their physical and spectral data with literatures. To mimic the pathology of the inflammation, LPS treatment was performed on the RAW 264.7. Rhamnazin (4), pachypodol (5), and (E)-2-methyl-6-(p-tolyl)hept-3-en-2-ol (12) significantly decreased nitrite accumulation in LPS-stimulated RAW 264.7 cells. Western blot assay showed that pachypodol (5) ameliorated increases in inducible nitric oxide synthase (iNOS) via the inhibition of phosphorylation of JNK and elevation of HO-1 in LPS-treated macrophages. The results suggest that the above compounds effectively inhibit the NO production and may be useful in preventing inflammatory diseases mediated by excessive production of NO.

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Poster Session-PO-115:

Peach blossom extract mixture HT077 attenuates obesity and fatty liver in mice fed a high-fat diet

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Obesity, an important public health problem, is associated with the development of nonalcoholic fatty liver disease characterized by an accumulation of intrahepatic triglyceride (i.e., steatosis). Peach blossom extract mixture HT077 is the combined extracts of peach blossom (*Prunus persica*) and lotus leaf (*Nelumbo*

nucifera). The objective of this study was to assess the activity of HT077 in reducing high-fat diet (HFD)-induced obesity and obesity-associated hepatic steatosis. Male C57BL/6 mice were fed either a normal diet, HFD, or HFD containing 0.2% HT077 for 10 weeks and their body weights were measured weekly. The weights of adipose tissue and liver, the hepatic levels of triglyceride and total cholesterol, and the serum levels of alanine aminotransferase (ALT) and aspartate transaminase (AST) were measured at the end of the study. The body weight of the HT077-treated HFD group was significantly lower compared with that of the HFD control group from the third week until the end of the treatment. After 10 weeks, the body weight gain and the weights of abdominal, mesenteric, and perirenal adipose tissue significantly decreased in the HT077-treated HFD group compared with those in the HFD control group. Furthermore, HT077 treatment significantly decreased the liver weight, the hepatic levels of triglyceride and total cholesterol, and the serum levels of ALT and AST compared with the HFD control group. These findings suggest that HT077 has anti-obesity effects and prevents the development of obesity-induced hepatic steatosis. HT077 may be of therapeutic value in treating obesity and nonalcoholic fatty liver disease.

Poster Session-PO-116:

Secondary metabolites and their bioactivities from the root of *Cryptocarya concinna*

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Over 1,400 species of Formosan plants have been screened for cytotoxicity and *Cryptocarya concinna* Hance (Lauraceae) was found to be one of the most bioactive species. *C. concinna* is a medium-sized evergreen tree and distributed in altitude 500-1,500 m broad-leaved forests in Taiwan and southern China. Previously, we reported three new chalcones concichalcones A-C (1-3), two new flavanoid cryptoflavanones E-F (7-8), and 19 known compounds from the active ethyl acetate soluble layer of the root of *C. concinna*. Continuing investigation of the active layer, additional three new chalcones, concichalcone D (4), concichalcon E (5) and concitocaryone (6), together with five known compounds, including two flavonoids, two amide alkaloids, and one lignan have been isolated. The structures of these new compounds were elucidated by NMR, UV, IR, ESIMS, and HRESIMS analyses. Different human cell lines, including cervical cancer SiHa cells, osteosarcoma U2OS cells, and normal keratinocyte HaCaT cells, were used to examine the cytotoxic profiles of these compounds. A new compound concichalcone D (4) has a significant cytotoxic

activity on U2OS and SiHa cells, with IC₅₀ values less than 15 µM. The known compounds cryptocaryone, infectocaryone, cryptocaryanones A, and cryptocaryanone B exhibited potent cytotoxic activities in all the cell lines examined, with the IC₅₀ values less than 10 µM. Two compounds, cryptocaryanones A and concichalcone D (4), exhibited a mild selectivity between cancer and normal cells, as their IC₅₀ values were significantly higher in the normal keratinocyte HaCaT cells. Interestingly, a mixture of cryptoflavonones A & B showed a differential cytotoxic activity in SiHa cells (IC₅₀ value less than 20 µM) over the U2OS and HaCaT cells (IC₅₀ value higher than 20 µM).

Poster Session-PO-117:

Synergistic effects of onion peel flavonoids exert rescue of auditory function in animal model

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In recent years the main goal in hearing loss treatment has focused in prevention, since noise exposure or ototoxic agents damages cochlear sensory hair cells which lack the capacity to regenerate, leading to permanent hearing impairment [1]. Thus, natural products have become important as preventive agents due to their protective activity in the inner ear, activity attributed mainly to bioactive compounds such as flavonoids [2]. Onion peel extract (OPE) is well known for its high content of flavonoids, among these quercetin, isoquercetin, quercitrin, rutin and others. Quercetin has shown protective effect against noise-induced hearing loss and aminoglycosides-induced ototoxicity in animal models, however OPE activity has not yet been studied in hearing loss [3]. Therefore, in this study, we demonstrated that OPE effectively increased recovery of auditory function and protection of sensory hair cells in vitro (using mouse auditory cell line: HEI-OC1 cells) and in vivo (zebrafish model of neomycin-induced ototoxicity and noise-induced hearing loss mice model). Moreover, OPE protection, in HEI-OC1 cells against neomycin-induced ototoxicity, might be mediated by anti-apoptosis mechanism. Furthermore, since it is well known that the use of extracts has advantages over the use of isolated compounds, we demonstrated the synergistic effects of the flavonoids contained in OPE by using isobologram. [1] Furness DN. Cell Tissue Res 2015; 361(1): 387-99. [2] Sanz-Fernández R, Sánchez-Rodríguez C, Granizo JJ, Durio-Calero E, Martín-Sanz E. Eur Arch Otorhinolaryngol 2016; 273(2): 341-7. [3] Hirose Y, Sugahara K, Kanagawa E, Takemoto Y, Hashimoto M, Yamashita H. Quercetin protects against hair cell loss in the zebrafish lateral line and guinea pig cochlea. Hear Res. 342 (2016) 80-85.

Chemical constituents from an alga-derived fungal strain *Acremonium* sp. NTU492

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Marine natural products were the secondary metabolites of marine organisms with highly diversified structural features and varied bioactivities. It has been shown that marine-derived microorganisms were a rich source of unique chemical entities, and the algae-derived fungi remained to be less investigated so far. In this study, a number of fungal strains were isolated from marine algae collected from northeastern coast of Taiwan. In the preliminary antimicrobial screening against bacteria and fungi, including *Escherichia coli* DH5 α , *Staphylococcus aureus* YC981, *Candida albicans* SC5314, and *Cryptococcus neoformans* H99, the ethyl acetate extracts of liquid (potato dextrose broth) and solid (brown rice) fermented products of *Acremonium* sp. NTU492 isolated from the red alga *Mastophora rosea* were found to exhibit significant growth inhibitory activity against *C. albicans* and *C. neoformans*. Thus, a series of bioassay-guided fractionation and separation was undertaken, which has led to the isolation and identification of 13 compounds including 7 new compounds, namely acrepeptins A-E (1-5) and acremonisins A and B (6 and 7), along with previously reported 8-deoxy-trichothecin (8), cyclo[L-alanyl-L-threonyl-(2S)-2-hydroxy-3-methylbutanoyl-L-isoleucyl-(2S)-2-hydroxy-3-methylbutanoyl] (9), β -alanine, N-[N-[N-[1-(2-hydroxy-4-methyl-1-oxopentyl)-L-prolyl]-L-isoleucyl]-N-methyl-L-valyl]-N-methyl-L-alanyl](10), guangomide A (11), guangomide B (12), and brefeldin A (13). Their structures were elucidated by spectral analysis and compared with literatures [1, 2]. Of these, 8 exhibited significant antimicrobial activities against *C. albicans* and *C. neoformans* with MIC values of 2 and 0.5 μ g/mL, respectively.

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Metabolic Profiling of Gegen Qinlian Decoction by UPLC-DAD-ESI-MS/MS and HPTLC

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Gegen Qinlian Decoction (GQD) is a TCM formulation first documented by Zhang Zhongjing in "Shanghanlun" at the end of Han-Dynasty (ca. 220 AD). It consists of four herbs, namely Radix Puerariae lobatae, Radix Scutellariae, Rhizoma Coptidis and Radix et Rhizoma Glycyrrhizae praeparatae cum melle. The formula is known to influence bacteriostasis and is clinically used to treat diarrhea, acute enteritis, bacterial dysentery, and Helicobacter pylori infection gastritis, but also type 2 diabetes ^[1-3].

In order to gain better understanding on the chemical composition and on possible active constituents, we have characterized GQD by metabolic profiling using UPLC-MS/MS and HPTLC fingerprints. The aim of the study was to assign the constituents of the formulation to the individual herbs and to identify as many components as possible.

HPTLC and UPLC-HRMS/MS methods were developed to separate the various constituents of the single herbs and of the mixture. Compounds detected in the formula were assigned to their originating plants using Compound Discoverer 2.1 software. UPLC-HRMS/MS identification was carried out by comparing retention times and MS/MS fragmentation patterns with reference compounds and existing data from literature. HPTLC identification was done by comparison of the R_f values with reference substances. In total, 76 compounds were assigned, 17 from Radix Puerariae lobatae, 12 from Radix Scutellariae, 26 from Rhizoma Coptidis, and 10 from Radix et Rhizoma Glycyrrhizae.

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Echinacoside as active compounds from *Cistanche tubulosa* protects BPA-induced reproductive damage by targeting hypothalamic androgen receptor

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Abstract:

Cistanche tubulosa is an official plant grows in arid or semi-arid areas. *Cistanche* extracts protect against sperm damage in mice under BPA induced reproductive damage [1, 2]; Echinacoside (ECH) is one of the major active compounds of *Cistanche*. This study investigated protective effects of ECH against oligoasthenospermia in rat and identified the interaction between ECH and androgen receptor (AR). METHODS Pharmacodynamics experiment was studied with six groups of mice with different ECH dosages and positive reference drug. In ECH distribution assay, mice were divided into ten groups, ECH (20 mg/kg) was administered orally and the mice in each group were anesthetized for sample collection. ECH distribution in brain, testis, and serum was assayed by HPLC, quantity, and quality of sperms was evaluated and hormone levels were determined by radio immunosorbent assay. The AR levels and key steroidogenic-related genes were determined by Western blot and qPCR. The ECH-AR interactions were evaluated by fluorescence localization assay, indirect ELISA, and molecular docking. RESULTS ECH significantly alleviated the BPA-induced reproductive damage by increasing testicular superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and glutathione (GSH); as well as by reducing serum malondialdehyde (MDA). ECH also play role in the sperm count and motility compared to the BPA-treated group. ECH combined with hypothalamic AR in the pocket of Met-894 and Val-713 to inhibit the transfer of AR from the cytoplasm to nuclei in the hypothalamus. While negative feedback of sex hormone regulation was inhibited, positive feedback was stimulated to increase the secretion of luteinizing hormone and testosterone subsequently enhancing sperm quantity. [3].

Echinacoside; *Cistanche tubulosa*; oligoasthenospermia; Androgen Receptor; Bisphenol A

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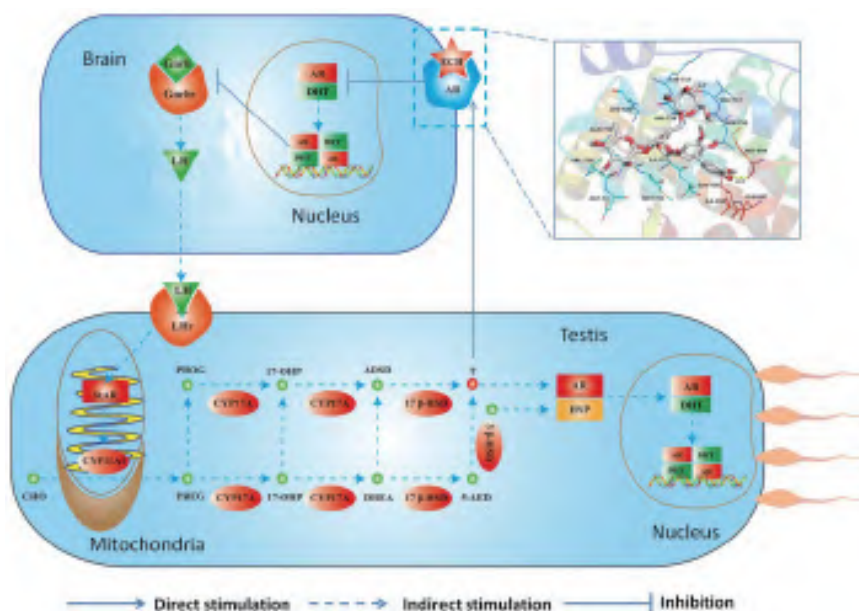


Figure 1 Mechanism by which ECH enhances the sperm counts.

Poster Session-PO-121:

Study of Bioactive Phytosterols from *Acanthus ilicifolius* Against Hepatocellular Carcinoma (Hcc)

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Hepatocellular carcinoma is one of the most commonly occurring cancer in the world. *Acanthus ilicifolius* is a mangrove plant (TCM) known for its medicinal properties. Two kilograms of plant extract yielded 0.121mg of sterols. Ethyl acetate and hexane mixture was found to be the suitable solvent for extraction in column chromatography. Subsequent HPLC and NMR analysis revealed the purity of the separated sterols. The Phytochemical compounds were analyzed by GC-MS and the structure was retrieved from PubChem. Totally, seven HCC target proteins were collected from literature, ligand and proteins were prepared for in silico molecular docking. HepG2 cell lines were used for in vitro (MTT assay). The phytochemical Cholest-5-en-3-ol (3, Beta.-), carbonochloridate, exhibited maximum docking score against the HCC target protein C-Jun N-terminal kinase 1 (JNK 1) (-6.934). The purified sterols were tested for in vitro antioxidant assay and cytotoxic activity. DPPH scavenging activities of the sterols increased with the increase in concentration when it was compared with the reference antioxidant (vitamin C). The total antioxidant capacity was

increased with increasing concentration of the plant extract at 500µg/mL. The hydrogen peroxide scavenging activity of the sample was less when compared to that of vitamin C but there was an increase with increase in concentration of the samples. Cytotoxic activity against HepG2 cell line, cell survival percentage was found to decrease with increasing concentration of the sample. The sterols exhibited potent activity in par with the standard drug doxorubicin. Therefore, an alternate drug from natural sources with potent activity and less side effects is need of the hour. Both in silico and in vitro studies indicated that the *A. ilicifolius* bioactive phytochemicals had anticarcereous activity against Hepatocellular carcinoma. There can be a possibility of synergistic activity of phytochemicals together against HCC.

Key words:

Acanthus ilicifolius; Sterol; Molecular docking; Hepatocellular carcinoma; GC-MS.

Poster Session-PO-122:

The downregulation of PCSK9 by sauchinone controls LDL-C

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is mainly expressed in the liver and contributes to cholesterol homeostasis. PCSK9 binds to the epidermal growth factor (EGF), a domain of the LDL receptor (LDLR). PCSK9 promotes degradation of these cell surface LDL receptors. Albeit the efficacy of statin therapy, some patients with familial hypercholesterolemia, an inherited autosomal dominant disorder characterized by extremely high levels of LDL-C face substantial residual risk associated with high levels of LDL-C since statin therapy is not completely successful in lowering LDL-C levels, particularly in heterozygous or homozygous familial hypercholesterolemia patients. Hence, inhibition of PCSK9 has emerged as an attractive target to control LDL-C levels. Two antibody-based drugs that directly target PCSK9 were approved by United State Food and Drug Administration (USFDA) in 2015 and several more candidates are undergoing clinical trials. So far, there are only a few reports regarding natural products with PCSK9 inhibitory activity. During our search for PCSK9 modulatory compounds from medicinal plants, we found that sauchinone from *Saururus chinensis* downregulated PCSK9 expression. In the present study, it was found that the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9) was downregulated, and the expression of LDLR was upregulated in sauchinone-treated HepG2 cells. Consequently, LDL-C uptake was increased. As a transcriptional factor of PCSK9 expression, SREBP-2 was proposed by transcriptome analysis and western blotting. In obese mice administered orally, sauchinone increased hepatic LDLR through PCSK9 inhibition and showed the reduced serum LDL-C levels and downstream targets of SREBP-2.

Secondary metabolites from the fungus, *Ophiocordyceps sobolifera*

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A fungus *Ophiocordyceps sobolifera* (Hill ex Watson) G.H.Sung, J.M.Sung, Hywel-Jones & Spatafora (Ophiocordycipitaceae) called ChanHua in Chinese. It is a parasitic fungus growing on wing-less cicada nymphs and has been used as Traditional Chinese Medicine for improving the renal function. In previous pharmacological studies, the extract of *O. sobolifera* ameliorates nephrotoxicity-induced renal dysfunction in the rat [1] and can improve the anti-tumor capacity of mice [2]. However, the chemical constituents of this fungus have never been studied. In the current research, *O. sobolifera* was processed through liquid-state fermentation, and its liquid fermentate showed cholesterol inhibitory and hypoglycemic activities based on the preliminary screening.

The liquid fermentate was partitioned and afforded ethyl acetate, n-butanol and water soluble layers. The n-butanol soluble layer of the liquid fermentate of *O. sobolifera* led to the isolation of one new diketopiperazine compound, 6-hydroxy-cyclo-(Pro-Thr) (1), along with nineteen known compounds, cyclo-(Ile-Ser) (2), cyclo-(Gly-Leu) (3), cyclo-(Gly-Pro) (4), cyclo-(Gly-Val) (5), cyclo-(Ile-Gly) (6), cyclo-(Pro-Ser) (7), butyl-2-pyrrolidone-5-carboxylate (8), 5-(hydroxymethyl)-1H-pyrrole-2-carbaldehyde (9), indole-3-carbaldehyde (10), 4-methoxybenzoic acid (11), β -carboline (12), butyl β -D-glucopyranoside (13), 2-deoxy-ribose-1,4-lactone (14), cordysin B (15), cordycepin (16), dihydrouracil (17), thymidine (18), uracil (19), and uridine (20). The structures of these compounds were elucidated by spectral analysis. The isolates are further evaluated with regard to cholesterol inhibitory and hypoglycemic activities.

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Artichoke leaf extract decreases sorbitol level in the rat lenses under high glucose

conditions

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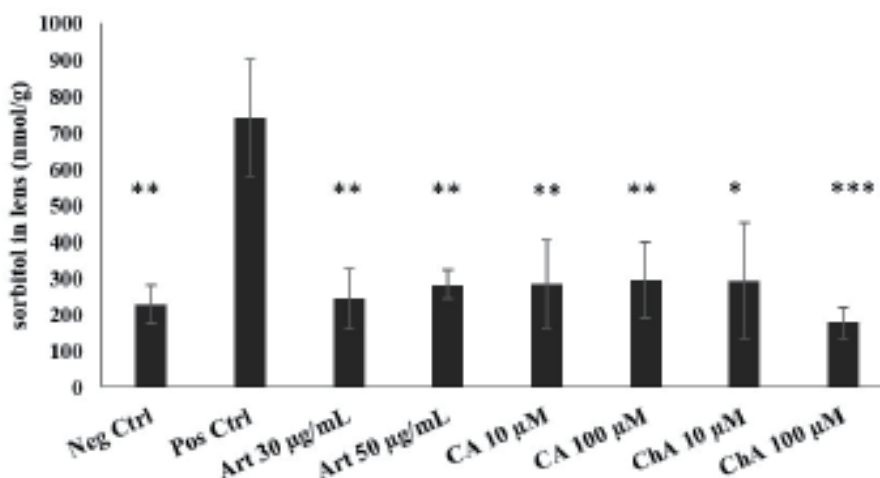
Artichokes are well known for their beneficial therapeutic effects: enhancement of lipid metabolism, choleresis, and hepatic functions. In our previous study artichoke leaves extract showed effective inhibition of aldo-keto reductases - human AKR1B1 and rat lens aldose reductase as well as effective inhibition of NF- κ B activity in cell culture during LPS incubation in human leukemic monocytes (1). Several in vitro and in vivo studies presented inhibitors of AKR1B1 and antioxidants as a potential therapeutic agents against development of chronic diabetic complications such as cataract, diabetic nephropathy, neuropathy and the others (2, 3).

The aim of this work was to elucidate the potential protective activity of artichoke leaves extract and some of its constituents (caffeic and chlorogenic acid) against increasing sorbitol level in the lens tissue induced by high glucose concentration.

Figure 1: Effects of artichoke extract (Art), caffeic acid (CA) and chlorogenic acid (ChA) on sorbitol accumulation in isolated rat lenses cultivated with high glucose concentration (except for negative control Neg Ctrl).

* indicates $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus positive control (Pos Ctrl).

As it is shown in Figure 1, significantly increased sorbitol levels were recorded in the isolated rat lenses incubated with high glucose concentration (50 mM - positive control) in comparison with a sample incubated without glucose (negative control). Sorbitol production was significantly inhibited by all tested samples.



Acknowledgements:

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Poster Session-PO-125:

Secondary metabolites from the fungus, *Diaporthe phaseolorum* var. *caulivora*

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Endophytes are microorganisms that live in the internal tissues of their host without causing any apparent disease symptoms. Instead, endophytes affect their hosts in a positive way including growth enhancement and protection against pathogens and feeding damage. *Diaporthe phaseolorum* var. *caulivora*, which was isolated from *Neolitsea daibuensis*, is a plant pathogen which infects soybean. *D. phaseolorum* var. *caulivora* was processed through solid-state fermentation, and its solid fermentate showed anti-inflammatory activity based on the preliminary screening. The phytochemistry and biological activities of *D. phaseolorum* var. *caulivora* have never been studied yet.

The 95% ethanolic extract of the solid fermentation was partitioned and afforded n-butanol and water-soluble layers. Bioassay-guided fractionation of the active n-butanol-soluble layer of the solid fermentate of *D. phaseolorum* var. *caulivora* led to the isolation of one new geranylcylohexenetriol, phaseolotoxin (1), two compounds isolated from nature source for the first time, 3-O-desmethyl phomentrioloxin (2)[1] and 2-hydroxyl peribysin A (3)[2], along with six known compounds, including one geranylcylohexenetriol which is found specifically in the genus, *Diaporthe*, phomentrioloxin (4), one sesquiterpene, peribysin A (5), two isocoumarins, mellein (6) and de-O-methyldiaporthin (7), one steroid, ergosterol peroxide (8), one amino acid, adenosine (9), and one fatty acid, palmitic acid (10). The structures of these compounds were elucidated by spectral analysis. The isolation of the active fractions is still in progress and the isolates are further evaluated with regard to anti-inflammatory activity.

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Poster Session-PO-126:

The therapeutic usefulness of the herbal preparation STW 5 in functional dyspepsia and ulcerative colitis is partly through affecting gut microbiota

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The herbal preparation STW5 (Iberogast®) is a multi-component multi-target herbal preparation consisting of hydro-alcoholic extracts of bitter candytuft, lemon balm, chamomile, caraway fruit, peppermint leaf, Angelica root, milk thistle, celandine herb, and licorice root. It has been shown to be clinically effective in irritable bowel syndrome¹ and functional dyspepsia² as well as experimentally in inflammatory bowel disease³. Since many clinical gastrointestinal conditions have been linked with changes in intestinal microbiota, we have studied such changes in models of functional dyspepsia and ulcerative colitis. The effect of STW5 on gut microbiota in normal rats and in established functional dyspepsia and colitis rat models was studied by assessing changes in selected major bacterial phyla using quantitative Real Time-PCR (qPCR). Ulcerative colitis was induced in rats by feeding them 5% DSS while functional dyspepsia was induced by subjecting rats first to neonatal maternal separation followed by restrained stress at maturity. In normal animals STW5 dramatically increased the relative abundance of Bacteroidetes, Bacteroides and Prevotella phyla of rat gut microbiota but a decrease in Bifidobacteria, Actinobacteria and Clostridium. Treatment with STW5 induced a dramatic increase in Lactobacillus and Methanobrevibacter populations. Changes in the microbiota Prevotella and Enterococcus induced by the stress models were prevented by STW5 administration. The findings lend support to the use of STW5 in the gastrointestinal conditions mentioned by influencing favourably the derangements in the intestinal microbiota caused by them.

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Poster Session-PO-127:

Novel formulations of curcumin, boswellia and xanthohumol extracts markedly enhance their individual and combined anti-inflammatory activity

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The chronic anti-inflammatory activity of novel formulations of extracts of Curcumin, Boswellia and Xanthohumol as stable solubilisates have been tested in the rat adjuvant induced arthritis model and compared with the native extracts. Female Wistar rats were inoculated with sub-plantar injections of Freund's complete adjuvant. Native and solubilized extracts of curcumin, boswellia, and xanthohumol were given orally daily in doses of 5 and 10 mg/Kg for 21 days. Diclofenac (3 mg/Kg) was run parallel as a reference drug. The rat paw edema was measured every 4 days. After 21 days, the rats were sacrificed and serum was used to measure relevant parameters to the inflammatory process. The combination of solubilisates of curcumin and boswellia extracts showed a better anti-inflammatory effect than either one alone. The reduction in paw volume was reflected in corresponding changes in relevant parameters for mediators of inflammation: C-Reactive Protein level, myeloperoxidase activity, total anti-oxidant activity, and thiobarbituric acid reactive substances as well as the inflammatory cytokines, TNF- α and IL-6. The findings show that the solubilisates of curcumin and boswellia extracts have a much more potent anti-inflammatory effect than the native forms. Moreover the combination of curcumin and boswellia solubilisates show that they potentiate one another to produce a therapeutic effect equivalent to if not more potent than diclofenac. Much better results were obtained with the combination of solubilized xanthohumol and curcumin, which revealed an even more potent anti-inflammatory effect than diclofenac, and better than the combination of solubilized curcumin and boswellia extracts. The findings open new perspectives in anti-inflammatory therapy allowing the use of smaller doses of herbal extracts with a lower risk of side effects.

Poster Session-PO-128:

Evaluation of phytochemical constituents and activity of South African plants used to treat inflammation and animal diseases

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Five plant species (*Ricinus communis*, *Carpobrotus edulis*, *Senna italica*, *Cotyledon orbiculata* and *Gomphocarpus fruticosus*) were selected in this study based on their use in traditional medicine against inflammation and helminthiasis in South Africa. The leaves from each plant were extracted using different solvents of varying polarity (hexane, ethyl acetate, acetone, ethanol and methanol) and tested for their total phenolic content, total flavonoid content, in vitro antioxidant activity using diphenyl-1-picrylhydrazyl (DPPH), and anti-inflammatory activity against the 15-lipoxygenase (15-LOX) enzyme. The extracts were also tested against the parasitic sheep nematode *Haemonchus contortus* in the egg hatch assay (EHA) and larval development assay (LDA). The *C. edulis* acetone extract had the highest phenolic content (107.85 mgGAE/g where GAE = gallic acid equivalent) and total flavonoid content with 31.12 mgRE/g (where RE = rutin equivalent). *C. edulis* also had very good antioxidant activity with an IC_{50} of 1.11 µg/mL compared to the positive controls vitamin C (IC_{50} = 0.32 µg/mL) and Trolox (0.69 µg/mL), as well as very good activity against 15-LOX with an IC_{50} value of 9.84 µg/mL compared to the positive control quercetin (IC_{50} = 24.6 µg/mL). Anthelmintic activity testing against *H. contortus* showed that *R. communis* had the best activity with an LC_{50} = 18.55 µg/mL for EHA compared to the positive control albendazole (LC_{50} = 1.99 µg/mL). *R. communis* also had good activity in the LDA with an LC_{50} value of 10.64 µg/mL compared to albendazole (LC_{50} = 5.48 µg/mL). *C. edulis* extracts were not active against *H. contortus*. This study revealed that *C. edulis* had good antioxidant and anti-inflammatory activity, and these results showed a good correlation with the phenolic and flavonoid contents. *R. communis* had good activity against *H. contortus* in the EHA and LDA. Therefore *C. edulis* and *R. communis* could be a potential source of new compounds with anti-inflammatory and anthelmintic activities.

Poster Session-PO-129:

Safety assessment of herbal products: Potential shortcomings

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Introduction:

The interest in safety assessments of herbal products is increasing, but not their quality, as limitations of available data are often not sufficiently taken into account. To adress the issue, a classification of sources of bias and ways out is aimed to.

Materials and Methods:

A systematic data base search for reviews in this field, combined with hand searching in text books, was conducted. Sources of bias were classified according to data types involved.

Results and Discussion:

Depending from the data involved, different sources of bias were identified:

- Data on quality: Often the great differences of the composition of herbal products prepared from the same plant are neglected, so leading to flaws when transferring data.
- Non-clinical data: Common pitfalls are the transfer of data from in vitro studies to the clinical setting, without taking into account the influence of ADME. Often also effects from sublethal high-dose settings are used without sufficiently taking into account dose dependency or, especially e.g. in carcinogenicity studies, methodological ambiguities [1].
- Data from clinical studies and post marketing surveillance: Lack of differentiation between negative studies and failed studies leads to wrong conclusions on inefficacy, the evaluation of safety data is often flawed by neglecting background incidences as e.g. in case of hepatotoxicity [2], by protopathic bias, and by the awareness and views of authors of case reports [3].

Conclusions:

A higher awareness of common pitfalls in the assessment of safety data on herbal products is needed, e.g. in case of hepatotoxic risks, if we want to avoid that methodological artefacts and misperceptions of the generalizability of data continue to influence our view of the safety of herbal products, both by neglecting risks, as, more abundant, by exaggerating non-existing risks.

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Poster Session-PO-130:

New phenanthrenes from *Paraleucobryum longifolium*

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The bryophytes, with more than 20 000 species, can be found everywhere in the world except in the sea. In the Hungarian flora, 659 species are present, with the predominance of mosses. Although not applied in human nutrition, several bryophytes have been widely used as medicinal plants, especially in China for various illnesses.

As part of a screening project, 168 aqueous and organic extracts of 42 selected bryophyte species were screened in vitro for antiproliferative activity on a panel of human gynecological cancer cell lines and for antibacterial activity. *Paraleucobryum longifolium* (Ehrh. ex Hedw.) Loeske (Dicranaceae) exerted remarkable bioactivity in both assays, therefore our aim was to perform an extensive phytochemical analysis of this species and to isolate its active components.

By using different chromatographic techniques, 3 compounds were isolated from the plant, the structures of which was determined by 1D and 2D NMR methods and HRMS. Compound 1 was identified as a phenanthrene dimer (paraleucobrin A). The other two compounds (paraleucobrin B and C) were identified as derivatives of paraleucobrin A. This is the first report of phenanthrenes from this genus, and considering the bioactivity profile of these types of compounds, the phenanthrenes isolated by us may play role in the bioactivities of *P. longifolium*.

Acknowledgements

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Poster Session-PO-131:

Isolation and structure determination of phenanthrenes from *Juncus compressus*

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In the past few decades, phenanthrenes have become of great interest from phytochemical and pharmacological points of view. To date, up to 90 phenanthrenes were isolated from eight Juncaceae species (*Juncus acutus*, *J. effusus*, *J. inflexus*, *J. maritimus*, *J. roemerianus*, *J. setchuensis*, *J. subulatus*, and *Luzula luzuloides*). These compounds are important chemotaxonomic markers, as the presence of a vinyl group

in the molecule is characteristic only for Juncaceae phenanthrenes. The most important pharmacological effects of phenanthrenes are the antiproliferative, anti-inflammatory, antibacterial and spasmolytic activities. Previously, 15 phenanthrenes were isolated from *J. inflexus* and *L. luzuloides* by our group, several of them showed antibacterial and anti-inflammatory activities. In continuation of our studies on Juncaceae species, in the present paper we report on the investigation of *J. compressus*.

The dried plant material was extracted with methanol. After evaporation, it was subjected to solvent–solvent partition with n-hexane, CH₂Cl₂ and ethyl acetate. The CH₂Cl₂ fraction was chromatographed by combination of different methods, including polyamide CC, VLC, preparative TLC, Sephadex LH-20 gel filtration and HPLC. The structure elucidation was carried out by extensive spectroscopic analysis, using 1D and 2D NMR spectroscopy and HRMS experiments.

The results allowed the identification of nine phenanthrenes [1 phenanthrene (dehydroeffusol), 6 dihydrophenanthrenes (compressin A, effusol, effusol A, juncusol, 7-hydroxy-1-methyl-2-methoxy-5-vinyl-9,10-dihydrophenanthrene, 2-hydroxy-7-hydroxymethyl-1-methyl-5-vinyl-9,10-dihydrophenanthrene, and 2 phenanthrene dimers (compressin B, and effusol A)]. Compressins A and B are new natural products. The isolated compounds are substituted with methyl, hydroxy, methoxy, hydroxymethyl, and vinyl groups. In cases of dimers, two juncusol or two effusol monomers are linked together via C-3 and C-3'. The position of vinyl group is C-5 in all cases. All compounds were isolated for the first time from *J. compressus*.

Acknowledgement:

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Poster Session-PO-132:

Phytochemical investigation of *Juncus atratus* Krock

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Juncaceae is a relatively small plant family with approximately 500 plant species worldwide. It can be divided into seven genera of which *Juncus* L. is the largest and by far the most important one from phytochemical and pharmacological points of view. Various *Juncus* species are used in the traditional Chinese medicine for the treatment of numerous disorders (e.g. fidgetiness, insomnia, painful urination, pharyngitis, and aphtha). *Medulla Junci*, the dried stem pith of *J. effusus*, is official in the Pharmacopoeia of the People's Republic of

China (2005).

Juncaceae species accumulate different secondary metabolites, e.g. phenanthrenes, flavonoids, triterpenes, steroids, coumarins and phenolic acid derivatives. According to the literature data, the major bioactive components of Juncaceae species are phenanthrenes. Almost 20% of currently known natural phenanthrenes were described from *Juncus* species.

In continuation of our work dealing with the isolation of biologically active secondary metabolites from Juncaceae species, *Juncus atratus* was investigated. The dried plant material was extracted with methanol. After evaporation, it was subjected to solvent–solvent partition with n-hexane, CH₂Cl₂ and ethyl acetate. The CH₂Cl₂ fraction was chromatographed by a combination of different methods, including CC, VLC, gel filtration, preparative TLC, and HPLC. The structure elucidation of the compounds was carried out by extensive NMR spectroscopic analysis, and HRMS experiments.

The results allowed the identification of five phenanthrenes [1 phenanthrene (dehydroeffusol), and 4 dihydrophenanthrenes (juncatrin A and B, effusol, and juncuenin B)], two flavonoids (apigenin and luteolin), one diterpene (phytol), and a fatty acid. Juncatrin A and B are new natural products, substituted by an acetyl and an acetylene group, respectively, instead of a vinyl group. This was the first time that a phenanthrene with acetylene moiety was isolated from natural source. All compounds were isolated for the first time from *J. atratus*.

Acknowledgement:

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Poster Session-PO-133:

Crude extracts of *Piper guineense*, rich in piperamide alkaloids, show promising antibacterial activity

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Bacterial infections are currently a threat in Sub-Saharan Africa. *Piper guineense* is a commonly used spice and medicinal plant in West Africa. Fruits and leaves from this plant are used to treat infectious diseases [1]. *P. guineense* extracts and its bioactive alkaloids, such as piperine are currently paving way as therapeutic agents in antimicrobial drug discovery, and are potential antibacterial agents for multiple human infectious diseases [2,3]. Due to increasing microbial resistance to the current antibiotics, there is a need to search for new natural antibacterial agents from *P. guineense*.

In our study, sequential extraction was conducted on the extracts of *P. guineense*, and twelve crude extracts and fractions of various polarities (hexane, chloroform, ethanol, methanol, cold water macerations and hot water decoctions) were screened for antibacterial activity against three Gram-positive and five Gram-negative bacteria. Piperine and piperlongumine were screened as representatives of the piperamide alkaloids in *P. guineense*. HPLC-DAD and UHPLC/Q-TOF MS analysis were conducted to characterize and identify the bioactive alkaloids present in the extracts.

Our result indicates that the extracts contain piperamide alkaloids. The extracts and pure compounds were active against *P. aeruginosa*, *E. aerogenes*, *S. aureus*, *E. coli*, *S. enterica*, *P. mirabilis* and *B. cereus* with MIC values ranging from 39 µg/mL- 1250 µg/mL. The piperine alkaloid-rich hexane leaf extract gave the lowest MIC of 19 µg/mL against *Sarcina* sp. The water extracts were not active against most of the bacterial strains. The result demonstrates that *P. guineense* contains antibacterial compounds, which are mostly piperamide alkaloids and which could be relevant for the discovery of new antibiotic scaffolds.

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Poster Session-PO-134:

Detection of infection markers in some *Allium* species

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The genus *Allium* covers more than 750 species. [1] *Allium cepa* L. (common onion) and *Allium sativum* L. (garlic) are considered as the most common and vital *Allium* species. The importance of some other useful species depends on the local cultivation and use. [2] Bulbs can be usually stored over several months. However, some bacteria and fungi can infect the stored *Allium* bulbs and spoil them within a short period of time. [3] In this project, *A. cepa*, *A. sativum*, *A. altaicum*, *A. pskmense*, *A. cornutum*, *A. fistulosum*, and *A. ampeloprasum* were infected by *Fusarium oxysporum*, and tested on development of two volatile chemical markers after infection. Healthy and infected bulbs were extracted by ethyl acetate. The extracts were analysed by HPLC/MS to define 2-hexyl-5-methyl-3(2H)-furanone and 2-octyl-5-methyl-3(2H)-furanone. The investigated markers appeared in all infected *Allium* species except *Allium sativum*. Because these compounds only occur after infection, they can be considered as infection markers in many *Allium* species as well as cultivars.

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Poster Session-PO-135:

Herbal medicinal products in irritable bowel syndrome: Mechanisms of action of STW 5

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Herbal treatment options are increasingly used in functional gastroenterological disorders (FGID) like irritable bowel syndrome (IBS), while an understanding of their mechanisms of action is often lacking. According to recent national and international therapeutic guidelines (e.g. 1,2,3), STW 5, a combination product of nine herbal extracts, is an evidence-based treatment option for IBS. This poses the question as to what the mechanisms of action are and the utility for its therapeutic efficacy.

To warrant completeness, a systematic search according to the PRISMA statement was conducted in order to retrieve all data on STW 5 or its trade name (Iberogast), using PubMed, Toxlit and BIOSIS. Identification of data on the mechanisms of action was then done manually. In addition hand searching was done and text books were screened to get a complete picture.

The search identified 468 publications. A considerable number of publications on spasmolytic as well as prokinetic activities could be identified, as well as on prosecretory effects. Furthermore data showing that the product can counteract inflammatory changes as well as an intestinal hypersensitivity and hyperpermeability were also found. Even a beneficial effect on the microbiota was described. Accordingly, the product has a multitude of mechanisms of action.

In IBS, a number of therapeutic options with different mechanisms of action are used. A search for the mechanisms of action of a herbal treatment used in this indication (STW 5) revealed not just one, but a multitude of mechanisms of action. This confirms for this product, that its action in IBS can be classified as multi-target, and makes its therapeutic efficacy in this indication plausible.

References

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- [2] Ivashkin et al. 2017. Ross z gastroenterol gepatol koloproktol 27:76-93
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Poster Session-PO-136:

Structure-dependent cytotoxicity of different pyrrolizidine alkaloids in primary rat hepatocytes and HepG2 cells: Role of cytochrome P450 3A

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Background:

Pyrrolizidine alkaloids (PAs) are secondary metabolites occurring in a wide range of plant species. Some 1,2-unsaturated PAs exert toxic effects through metabolic activation which form the corresponding dehydropyrrolizidine derivatives, primarily in the liver, catalyzed by cytochrome P450 monooxygenases. Due to their hepatotoxicity, genotoxicity and carcinogenicity, the accidental presence of PAs in food, feed and herbal medicinal products can be, depending on the dose, a cause for safety concerns.

Objectives:

In order to assess potential risks and confirm the connection between structure and in vitro toxicity [1], we generate data firstly concerning cytotoxicity of some food-relevant PAs. In addition, we also wanted to test in vitro the role CYP3A plays in metabolic activation of PA-induced cytotoxicity.

Methods:

After 24 h and 48 h exposure, cytotoxicity of the selected PAs was determined at concentrations ranging from 1 to 300 μ M by the Alamar blue assay in primary rat hepatocytes and in the human HepG2 cell line. A kinetic assay analyzing 7-benzyloxyresorufin-O-dealkylation (BROD) was used for measuring the activity of CYP3A enzymes.

Results:

A structure dependent cytotoxicity was demonstrated with rat hepatocytes in primary culture. Lasiocarpine, an open-chained di-ester with 7S-structure, proved to be the most cytotoxic followed by the other di-esters echimidine, retrorsine, seneciphylline and senecionine. The mono-esters heliotrine, indicine, europine and lycopsamine were much less cytotoxic. On the contrary, failure to detect cytotoxicity in HepG2 cells is possibly due to the lack of CYP3As. In primary rat hepatocytes, the CYP3A activity decreased rapidly during

the culture, therefore, the time to incubation significantly affects the cytotoxicity. These data confirm that CYP3A plays a critical role in PA-induced toxicity.

Reference:

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Acknowledgement:

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Poster Session-PO-137:

Natural products and the liver: Hepatoprotection as a key

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Introduction:

While in Europe the scientific awareness in the field of liver and natural products is presently mainly focused on herb induced liver injury (HILI) (1), in Asia, the focus of a number of recently published reviews is on hepatoprotective effects of herbs (2). But also in Europe, there is a high prevalence of hepatic disturbances due to different causes, so that an overview of the hepatoprotective potential of herbs in use in Europe would be of therapeutic relevance, e.g. in herbs used in the therapy of gastrointestinal diseases.

Materials and Methods:

For identifying these plants, the HMPc monographs of the European drug agency EMA and in addition leading textbooks were evaluated, followed by a systematic literature search on hepatoprotective effects.

Results and Discussion:

There are HMPc monographs describing a use in gastrointestinal diseases for 50 medicinal herbs, others are described in textbooks. For the majority of these plants there are published data on hepatoprotective effects. In most cases these are data from pharmacological models of different types of liver diseases, as are e.g. for caraway, peppermint, lemon balm; liquorice, gentian, angelica root, but also fennel and greater celandine. In specific cases there exist also data from clinical trials, as e.g. case of artichoke and milk thistle. In addition there are data on herbal constituents as e.g. quercetin und sulforaphane available, which points

to hepatoprotective effects of further plants, as e.g. bitter candytuft or horseradish.

Conclusions:

The herbs used in Europe have in many cases a hepatoprotective action, as e.g. in gastrointestinal diseases. By means of pharmacoepidemiological studies it could be evaluated whether this action is reflected in the respective patients also in a lower incidence of hepatitis of different origin.

References:

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- [2] Sultana et al. J. Ethnoph 2018, 216:104

Poster Session-PO-138:

The advantages of TLC as a screening and crosscheck method for natural products using α - and β - acids in hop

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Hops are a very important base material for the production of beer. The substance Lupulin, a yellowish powder isolated from the hops cones, is especially responsible for the bitterness and unique taste of a beer. That is why the amount of bitter acids in hops is very important for breweries. Lupulin contains various bitter acids [1], α - (humulone) and β - (lupulone) acids, which vary in different types of hops. Caused by the content of bitter acids, hops are divided into aromatic hops (< 10 % of α -acids) and bitter hops (> 10 % α -acids) [2]. In general, the α - and β -acids are also divided into five homologues. The quantitative analysis of their contents in different hops, as well as the screening for other ingredients was done with TLC and HPLC to compare both methods.

The investigation of different hop types has shown that the amount of α -acid is both dependent on the hop category as well as on the region. Most of the bitter hop samples, except one, have a much higher percentage of α -acid than the other samples. Furthermore, the same hops from different regions do not all have the same amount of acid.

It could also be determined that the β -acid is not related to these criteria and is similar in all hop samples regardless of whether the HPLC or TLC method was used. The amount of α -acid, but not the β -acid, depends on the kind of hop (bitter or aromatic) and the region. Both methods, TLC and HPLC, were used in comparison to quantify α - and β -acids.

Salicylate and polyphenol based phytopharmaceuticals exert adaptive cyto- and chemokine network responses in human fibroblast cultures¹

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Cyto- and chemokines play a central role in immunoregulatory and inflammatory processes. Neutralizing antibodies for single proinflammatory cytokines have developed into powerful therapeutic strategies for several autoimmune diseases. Considering the redundancy of cyto- and chemokine functions, network rather than single target approaches may support the development of more targeted therapies. Phytopharmaceuticals, common adjuvant therapies, are cyto- and chemokine modulators, but are not systematically investigated. This work explored the in vitro modulation of cyto- and chemokine networks (CCN) of clinically established phytopharmaceuticals alone or in combination under non-inflammatory and inflammatory conditions for potential co-medication strategies. Human skinfibroblasts were treated with standardized extracts (E) of *Populus tremula* L., *Solidago virgaurea* L., *Fraxinus excelsior* L., the combined multiextract mixture STW1 or the reference drug acetyl salicylic acid (ASA), alone or with lipopolysaccharides (LPS). CCN-profiles were determined in cell lysates by proteome profiler. Cell culture medium was investigated for IL-6 (ELISA). RNA gene-expression profiling was undertaken. *P. tremula* E and ASA increased cellular IL-8 and IL-10; *S. virgaurea* E modulated Groa, IL-1a, IL-10 and IL-15. *F. excelsior* decreased IL-1a and IL-15. The mixture of the three E - STW1 - modulated IL-1a, IL-3 and TNF- β . LPS stimulation increased cellular Groa, IL-8, MCP-1 and RANTES and IL-6 secretion into the media. Except for *F. excelsior*, none of the single extracts reversed LPS-induced rises of cytokines. STW1 inhibited IL-1a, Groa, IL-8, and MCP-1. IL-6-secretion was reduced by STW1 and ASA. Gene expression profiles supported non-additive CCN-profiles. Salicylate and polyphenol based phytopharmaceuticals modulate specific cyto- and chemokine networks rather than inhibit single pro-inflammatory cytokines. Pro- and anti-inflammatory cytokine responses adapt to anti-inflammatory responses after LPS-stimulation. A simultaneous activation of pro- and anti-inflammatory cytokines might improve the immunological reactivity status of a cell.

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Multi-target and synergistic effects of STW5

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STW5 is a herbal multicomponent mixture which is recommended in the management of functional dyspepsia. It is a combination of 9 different plant extracts (*Iberis amara* (L.), *Menthae piperitae* (L.), *Chamomilla recutita* (L.), *Glycyrrhiza glabra* (L.), *Angelica archangelica* (L.), *Carum Carvi* (L.), *Silybium marianum* (L.) Gaertn. *Melissa officinalis* (L.) und *Chelidonium majus* (L.). We reported earlier that STW5 targets the fatty acid receptor GPR84 [1], receptors involved in pain sensing (ASIC4, 5-HT) and multiple chemokine families on genome and proteome levels. Based on these data we proposed IL-8 and the TREM-1 signaling pathway to play an important role in the pathogenesis of GERD. We therefore investigated the influence of STW5 and its component on the IL-8 release in human esophageal epithelial cells (HET1A) alone or in combination and under stimulation of Capsaicin (80µM). As reported [2] the selected plant composition in STW5 lead to a downregulation of IL-8. But the IL-8 releases provoked by the different plant extracts were not simply additive. For the determination of synergistic, additive and antagonistic effects of the different plant extracts in combination we used the Chou-Talalay method and determined the Combination index (CI). The CI values of the triple combinations tested ranged from high synergies (CI<0.3) up to antagonism (CI>480). Within the combination *Menthae piperitae* (L.) and *Silybium marianum* (L.) were identified as the most effective combination partners in the inhibition of IL-8 releases. We further investigated the Ca-release in human intestinal smooth muscle cells (HISMC). Here STW5 did induce an increase in the Ca-release. In this cellular model a single plant extract – *Melissa officinalis* (L.)- was identified to provoke the increased Ca-release. The identification of multi-target and synergistic effects in phytopharmaceuticals will improve our ability to rationally compose effective and safe plant combinations.

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Rapid characterization of chemical constituents and rats metabolites of Deng-Zhan-Xi-Xin injection by UPLC-Q-TOF/MSE coupled with UNIFI software

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Deng- Zhan-Xi-Xin injection , a famous traditional Chinese medicine prescription (TCMP), is widely used for the treatment of cardiovascular and cerebral vessel diseases, such as ischemic stroke, coronary heart disease and angina pectoris . It was made from the whole herb of *Erigeron breviscapus* (Vant.) Hand.-Mazz.. It was well known that both chemical constituents and metabolites in vivo played a key role in the clinical efficacy of TCMP [1] . Owing to the complexity of chemical constituents in TCMP [2] and the lower intensity of prototypes and metabolites in rats biofluids , the detection and identification of chemical constituents and their metabolites became a great challenge [3]. In this work, an integrative strategy based on ultra performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry and UNIFI™ software combined with multiple data processing approaches was proposed to identify the chemical constituents and metabolites of Deng-Zhan-Xi-Xin injection. As a result, a total of 110 compounds were identified or tentatively characterized, including 40 compounds in vitro, 25 prototypes and 45 metabolites in rat plasma, urine and feces. It was the first time that the metabolic profiling of Deng-Zhan-Xi-Xin injection was systematic researched, glucuronidation and sulfation conjugation were the main metabolic pathway. This study provided significant information for its further research of Deng-Zhan-Xi-Xin injection. Moreover, it provided valuable strategy for rapidly screening and identifying chemical components and metabolites of other TCMP s.

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Poster Session-PO-142:

New malonylginsenosides from the leaves of *P. notoginseng* as Q- markers for the roots of *P. notoginseng*

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UPLC-QTOF was used to primarily characterize the ginsenosides in the leaves of *P. notoginseng*, and novel aglycone malonylated ginsenosides were targeted according to neutral loss of CO₂ and the product ions of acetylated aglycones, m/z 517.39 protopanaxatriol (PPT) and m/z 501.40 for protopanaxadiol (PPD). Guided by LC-MS, the target compounds were enriched by liquid-liquid extraction, and isolated by column chromatography (MCI-gel and ODS) and further purified by RPLC and orthogonal HILIC. High-resolution MS, 1D and 2D-NMR were applied for structural elucidation. Eight new compounds were obtained, named as notoginsenoside L5-L12. They are all 3-OH malonylated ginsenosides, and amongst them, notoginsenoside L9-L12 are of PPT type, while notoginsenoside L5-L8 are of PPD type. Further analysis of different parts (roots, leaves, stems, and flower buds) of *P. notoginseng* displayed the distribution specificity, as the target compounds are detected mainly in the aerial non-medicinal parts (flowers, leaves and stems) and rarely in the underground medicinal parts (roots), indicating the targets are potential quality markers (Q-markers) to differentiate the medicinal and non-medicinal parts of *P. notoginseng*. This study provides a solution to discovering and isolating the aglycone malonylated ginsenosides that are Q-markers for quality control of notoginseng products.

Poster Session-PO-143:

An enhanced strategy integrating offline two-dimensional separation and step-wise precursor ion list-based raster-mass defect filter for characterization of indole alkaloids

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Comprehensive chemical profiling is of great significance for understanding the therapeutic material basis and quality control of herbal medicines, which is challenging due to its inherent chemical diversity and complexity, as well as wide concentration range. In this study, we introduced an enhanced strategy integrating offline two-dimensional (2D) separation and the step-wise precursor ion list-based raster-mass defect filter (step-wise PIL-based raster-MDF) scan by tandem LTQ-Orbitrap mass spectrometer. A comprehensive analysis of indole alkaloids in five botanical origins of *Uncariae Ramulus Cum Unicis* (Gou-Teng) was used as an exemplary application. A positively charged reversed phase (PR) × conventional RP LC system in different pH conditions was constructed with the orthogonality of 74%. A theoretical step-wise PIL among 310 - 950 Da with the step-size of 2 Da was developed to selectively trigger fragmentations and extend the coverage of potential indole alkaloids. Simultaneously, by defining parent mass width (PMW) of the step-wise PIL to ± 55 mDa, a raster-MDF screening was achieved in the acquisition process. Additionally, subtype classification and structural elucidation were facilitated by a four-step interpretation strategy. As a

result, a total of 1227 indole alkaloids were efficiently exposed and characterized from five botanical origins of Gou-Teng, which showed high chemical diversity. A systematic comparison among five species was first performed and only 66 indole alkaloids were common. For method validation, three new alkaloid N-oxides were isolated and unambiguously identified by NMR. The present study provides a novel data-dependent acquisition method with improved target coverage and high selectivity. The integrated strategy is practical to efficiently expose and comprehensively characterize complex components in herbal medicines.

Poster Session-PO-144:

Improved TCM fingerprinting by benefiting from high robustness and matrix tolerance of monolithic HPLC columns

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When developing a new method for fingerprinting, people traditionally use one long column to obtain high peak capacity. However, long column with small particle often bring high backpressure, constitute a practical problem and limit the use of more viscous solvents in mobile phase. Monolithic column with its unique pore structure, comprising macro- and meso- pores provide lower column backpressure compared to particle packed column, even at elevated flow rate and even with more viscous mobile phase solvents, offer high peak capacity and shorter analysis running time. Besides this, robustness and resistance toward sample matrix make monolithic column to be very suitable for TCM fingerprinting. Under

Certain situation, coupling two, three and up to four Chromolith® columns in series for fingerprint analysis of herbal material has been used and published by Vander Heyden et al. in two papers.

Poster Session-PO-145:

Salvia multicaulis Vahl. versus Salvia hispanica L. – A Comparison

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Salvia L., the largest genus of the family Lamiaceae, represents an enormous and cosmopolitan assemblage

of nearly 1.000 species displaying a remarkable range of variation, of which 36 only occur in Europe. The phytochemistry of the genus *Salvia* consists mainly of volatiles, fatty acids, flavonoids, terpenes among other secondary metabolites. Due to the wide occurrence, folk medicinal use of various species is well documented and known. An ancient staple crop “chia” (*S. hispanica* L.) has become a popular novel food, due to its valuable nutritional content, especially fatty acid composition. In this present study, *S. multicaulis* seeds were evaluated also for the fatty acid composition, total protein amount and swelling index among other properties, which is known to be consumed such as in Turkey. Linolenic acid (69.7%) and linoleic acid (19.7%) were the major fatty acids in chia oil. Chia seeds showed a relatively low n-6 PUFA/ n-3 PUFA ratio. On the contrary, *S. multicaulis* seed oil was rich in linoleic acid (68.9%), palmitic acid (10.3%) and oleic acid (14.1%), where the amount of linolenic acid was very low (1%), respectively. The results were compared with chia seeds, not only due its chemical composition but also for its antimicrobial, antioxidant as well as lipoxygenase inhibitory properties.

Poster Session-PO-146:

Influence of St. John's wort extract and its components on gene expression in neuronal cells after induction of stress

S Verjee ¹, A Weston ², Olaf Kelber ³, Christiane Kolb ⁴, Heba Aziz-Kalbhenn ⁴, Veronika Butterweck ¹

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Introduction:

It is well known that dysregulation of the HPA axis plays an important part in the development and maintenance of depressive symptoms. Glucocorticoids affect cellular and molecular events in brains by modulating the expression of many genes during stress. In the present study we evaluated the effects of a St. John's wort extract (STWE, extraction medium ethanol 80 %, DER 3-6:1), hyperforin, miquelianin and the SSRI citalopram on the expression of genes relevant to HPA axis function in human neuronal cells.

Material and Methods:

SH-SY5Y cells were treated with STWE (20 µg/mL), hyperforin (10 µM), miquelianin (10 µM) or citalopram (10 µM) in the presence or absence of the glucocorticoid receptor agonist dexamethasone (DEX, 10 µM) for 6 h and 48 h, respectively. Quantitative real time PCR was used to determine the expression of FKBP5, CREB,

GRIK4, VEGF, NET, and ARRB, which have been shown to be meaningful biomarkers in the treatment response for depression. Relative expression values were determined by using the $-\Delta\Delta C_t$ method.

Results and Discussion:

Using DEX to mimic stress conditions, we were able to show the responsiveness of the selected genes. It was shown that the gene expression pattern of FKBP5, CREB, GRIK4, VEGF, NET, and ARRB2 in SH-SY5Y neuronal cells is time and treatment dependent. Most pronounced effects were observed for FKBP5, which was upregulated after 6h (1.3 fold) but an even stronger increase in mRNA expression was observed after 48h (1.8 fold). While after 6h of co-incubation only STWE could reverse the dexamethasone induced increase in FKBP5 expression, after 48h citalopram, miquelianin and hyperforin also reversed the glucocorticoid induced increase in FKBP5 mRNA expression.

Conclusion:

The effects observed on FKBP5, CREB, GRIK4, VEGF, NET and ARRB2 are in good correlation with published data, suggesting that this in vitro model can be used to screen the responsiveness of antidepressants under stress conditions.

Poster Session-PO-147:

Hypericum perforatum L.: Anti-inflammatory and cytoprotective effects in neuronal cells

Anna Schwendler ¹, Julian Hüther ¹, Gabriel Bonaterra ¹, Andrea Cordes ¹, Heba Aziz-Kalbhenn ², Olaf Kelber ³, Christiane Kolb ², Ralf Kinscherf ¹

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Introduction:

In psychic depression, St. John's wort (*Hypericum perforatum* L.) extracts are an established herbal treatment option. As depression has been linked to an inflammatory response, the combined antioxidant and anti-inflammatory properties [1] are suggested to contribute to the antidepressant effects [2] by normalization of an overactive hypothalamic-pituitary-adrenal axis [3].

Objective:

Thus, the aim of our investigations was to determine the effects of a *Hypericum* extract, STW 3-VI (extraction

medium ethanol 80%, DER 3-6:1), on the protection of differentiated mouse hippocampal HT22 cells against the cytotoxic effects of glutamate or NMDA and the possible anti-inflammatory properties on LPS-activated macrophages (MΦ).

Material and Methods:

Differentiated HT22 cells were pre-treated with STW 3-VI to investigate the protective effects against glutamate or NMDA cytotoxicity. The anti-inflammatory properties of STW 3-VI were evaluated by quantification of the TNF release on LPS activated PMA-differentiated THP 1 MΦ using ELISA assay and the mRNA expression of TNF and IL-6 by qRT-PCR. Glutamate or NMDA (0.1mM) induced 30% cytotoxicity in HT22 cells.

Results and Discussion:

Pre-incubation (24h) with STW 3-VI improved the viability by 30% compared to the control. Pre-treatment (48h) of LPS activated MΦ with STW 3-VI induced a significant lowering (54%, 64% and 53%) of TNF release. QRT-PCR revealed that 48 h pre-treatment with STW 3-VI inhibited the mRNA expression of IL-6 and TNF respectively by LPS-activated MΦ.

Conclusion:

STW 3-VI protects hippocampal cells from glutamate or NMDA induced cytotoxicity and activates the anti-inflammatory defense by inhibition of the cytokine production by MΦ. These effects are in accordance with the therapeutic use of STW3-VI in depression.

References:

1. Breyer A et al. Phytomedicine 2007, 14: 250-255
2. Denke A et al. Drug Res 2000, 50: 415-419
3. Gastpar M, Singer A, Zeller K. Pharmacopsychiatry 2006, 39: 66-75
4. Grundmann O, Kelber O, Butterweck V. Planta Medica 2006, 72: 1366-1371

Tuesday, 28th August, 2018 - Pavilion - 09:45 - 12:00

Plenary Lecture - Plenary Lecture

Plenary Lecture-KNL-01:

Nano-bioeffects and nanomedicine

Yu-liang Zhao

National Center for Nanoscience and Technology of China, and CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Chinese Academy of Sciences (CAS), Beijing, China

The changes of chemical and biological activities of materials at a nanoscale may result in unique interactions with biological systems. This fundament directly relates to many newly emerging frontier sciences in multidisciplinary fields, e.g., nanomedicine, nanobiotechnology, nanotoxicology, nanobiomedical engineering, cancer nanotechnology, etc. So far, the key discoveries for nanomedicines (NMs) and NMs-interacting with biosystems include, (i) NMs maybe a best candidate for development of the next generation of medicine (called as nanomedicine), (ii) NMs easily penetrate biological barriers, (iii) NMs easily adsorb blood proteins (protein corona), (iv) NMs easily cross cell membrane to enter cells and induce intracellular ROS generation, (v) nanosizes and nanosurface chemistry largely determine functions and fates of NMs in vivo or in vitro, etc. In the talk, we will discuss the new achievements of smart nanomaterials targeting and regulating tumor microenvironment to improve the therapeutic outcomes for cancer treatment.

Reference:

- [1] Li S.P., Jiang Q., Liu S.L., Zhang Y.L., Tian Y.H., Song C., Wang J., Zou Y.G., Anderson G.J., Han J.Y., Chang Y., Liu Y, Zhang C., Chen L, Zhou G.B., Nie G.J., Yan H., Ding B.Q., Zhao Y.L., *Nature Biotechnology*, 2018, 36, doi:10.1038/nbt.4071.
- [2] Han X.X; Nie G. J., Zhao Y.L., et al., *Nature Communication*, 2018, in press.
- [3] Li S.P., Zhang Y.L., Wang J., Zhao Y., Ji T.J., Zhao X., Ding Y.P., Zhao, X.Z., Zhao R.F, Li F., Yang X., Liu SL., Liu Z.F., Lai J.H., Whittaker A. K., Anderson G.J., Wei J.Y., Nie G.J., *Nature Biomedical Engineering*, 2018, doi: 10.1038/s41551-017-0125-6 .

Plenary Lecture-KNL-02:

Beyond malaria: The anticancer and antiviral activities of artemisinin-type drugs

Thomas Efferth

Department of Pharmaceutical Biology, Johannes Gutenberg University,, Mainz, Germany

Chinese medicine commands a unique position among all traditional medicines because of its 5000 years of history. Our own interest in natural products from traditional Chinese medicine was triggered in the 1990s, by artemisinin-type sesquiterpene lactones from *Artemisia annua* L. As demonstrated in recent years by us and others, this class of compounds has activity not only against malaria but also against cancer and viral diseases. To translate the experimental results from tumors in vitro and in vivo to the bedside, we will report the compassionate use of artesunate in single cancer patients as well as our efforts to initiate several clinical phase I/II trials in veterinary tumors as well as in human cervix or colorectal carcinoma. These pilot studies

have indeed indicated that artesunate is not only useful as an anti-malarial drug, but also exerts activities against cancer and viral diseases. Clinical results will also be presented which shows that artesunate is not only the semisynthetic chemical derivative of Artemisinin, but they are also in herbal extracts from *A. annua* which are active in veterinary and human tumor patients. Interestingly, the bioactivity of artemisinin and its semisynthetic derivative artesunate is even broader and includes the inhibition of certain viruses, such as human cytomegalovirus (HCMV) and other Herpesviridae (e.g. HSV1 and EBV), HBV, HCV and BVDV. We will also present clinical data on the anti-HCMV activity of artesunate in pediatric organ transplant or cancer patients. References: [1] Saeed M. et al. (2016) *Pharmacol. Res.* 110:216-226. [2] Efferth T. (2017) *Semin. Cancer Biol.* 46:65-83. [3] Efferth T (2017) *Biochem. Pharmacol.* 139:56-70. [4] Efferth T. (2018) *Biotechnol. Adv.* [Epub ahead of print]

Plenary Lecture-KNL-03:

A SWOT Analysis of Chemoinformatics in Natural Products Research

Judith M. Rollinger

University of Vienna, Vienna, Austria

virtual screening, pharmacophore modeling, drug discovery, caveats

Computer-aided techniques, such as molecular modeling, docking, virtual screening, machine learning, are well approved for their usefulness in drug discovery, design and development of therapeutically relevant small molecules. Although delayed by several decades, their application in natural product research has led to outstanding findings [1].

The number of small molecules from nature and their occupied chemical space is constantly increasing by the discovery of taxonomically diverse producing organisms and the diligent exploitation of already known material [2, 3].

Additionally, historical information from traditional medicine and findings from observational studies, and the increasing knowledge we observe in structural and biological data from new chemical entities, macromolecular targets and their physiological role in humans on the other hand provide an infinite source of data. Combining information derived from all these heterogeneous sources, structuring big data and not getting lost within it will be a future challenge in our society.

Strategies and examples will be presented on how to integrate chemoinformatics in pharmacognostic workflows to benefit from these two highly complementary disciplines by streamlining experimental efforts. Awareness concerning data reliability, a critical view on and an unbiased attitude towards predicted results are indispensable prerequisites for successful projects. Considering their limits, pitfalls and exploiting

their potential, computer-aided strategies will successfully guide future studies and thereby augment our knowledge on bioactive natural lead structures.

[1] Kaserer TK, Schuster D, Rollinger JM. Chemoinformatics in Natural Products Research. In: Applied Chemoinformatics. A Textbook; ed. Gasteiger, J.; Engel, T. 2nd ed., Wiley, 2018. doi: 10.1002/9783527806539.ch6c

[2] Pye CR, Bertin MJ, Lokey RS, Gerwick WH, Linnington RG (2017) Retrospective analysis of natural products provides insights for future discovery trends. PNAS USA 2017; 114: 5601-5606. doi: 10.1073/pnas.1614680114

[3] Boufridi A, Quinn RJ. Annu Rev PharmacolToxicol 2018; 58: 451-470. doi:10.1146/annurev-pharmtox-010716-105029

Tuesday, 28th August, 2018 - Bibo BallroomAB - 14:00 - 16:00

Invited & Short Lecture - Session 4-1

Session 4-1-IL-01:

Chemistry and bioactivity of medicinal plants

Hong-xi Xu

School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Garcinia species, which have been studied for more than 70 years, comprise a large genus of tropical evergreen trees and shrubs that are widely distributed throughout Southeast Asia and Southern Africa. This genus consists of 450 species, of which 22 species are found across China. Xanthonenes, polycyclic polyprenylated acylphloroglucinols (PPAPs) and benzophenones are the major compounds obtained from these species, and exhibit anti-tumor, anti-inflammation and anti-virus activities. The research on Garcinia has become one of the hotspots in the field of natural compounds in recent years. Our in-depth comprehensive and systematic research on Chinese Garcinia plants could be traced back to 2005. So far, we have isolated more than 300 compounds, including over 150 components novel in structure. Through technologies and means of Analytical Chemistry, Molecular Biology, Proteomics, Computational Chemistry and Modern Pharmacology in our previous study, we have confirmed that PPAPs such as Oblongifolin C (OC) and Guttiferon K (GUTK) isolated from *Garcinia yunnanensis* Hu and Cambogin isolated from *Garcinia esculenta* have significant anti-tumor and anti-inflammation activities. In the past decade, we have published over 85 SCI papers on Garcinia species. We have also obtained 6 US patents and 15 Chinese patents and our research is supported by 10 of National Natural Science Foundation of China (NSFC). At

present, we are focusing resources on large-scale preparation, chemical structure modification, anti-tumor and anti-inflammation molecular mechanism research of some bioactive compounds. Our study provides theoretical guidance for the comprehensive utilization of natural compounds and lays the ground work for the development of new drugs.

Session 4-1-IL-02:

Natural products-induced hepatotoxicity—Shall we seriously consider the safe use of them?

Ge Lin

1. *School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR,*
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The safe use of orthodox drugs and natural product-derived healthy supplements is always a public concern. Comparing with the orthodox drugs, there are less awareness and investigation on the adverse effects/toxicity induced by natural products. Natural products consist of multiple ingredients acting on multiple targets, which makes the investigation of their adverse effects/toxicity much more difficult and challenging. Among drug-induced toxicities, drug-induced liver injury (DILI) is one of the leading causes of acute liver failure. Unlike well-recognized DILI caused by orthodox drugs, the hepatic impairments caused by natural products, including herbal remedies, often have less public awareness and are also lack of confirmative diagnosis in clinic, although herb-induced liver injury (HILI) has been reported as one of the major causes of DILI worldwide. The diagnosis of HILI is challenging due to the lack of characteristic clinical features and specific biomarkers for the diagnosis. This has subsequently hindered the development of specific and efficacious treatment. Among differential natural products caused HILI, pyrrolizidine alkaloids (PAs) are one of the most significant and responsible phytotoxins. To date, over 660 PAs have been identified in approximately 3% of flowering plants, and about half of PAs have been reported to be hepatotoxic. Yearly, numerous incidences of PA-induced liver injury (PA-ILI) associated with the intake of PA-producing herbal products and/or PA-contaminated foodstuffs have been reported worldwide. In this presentation, using PA-ILI as an example, our methodical translational study, from the identification of clinical problems, to the laboratory basic science for delineating toxic mechanism and developing the mechanism-based biomarker, and then the transformation of the biomarker to clinical diagnosis of PA-ILI will be illustrated. [The study was supported by GRF Grants (Ref No.: 14110714, 14111816 and 14160817), and CUHK Direct Grant (Ref No.: 4054376)]

Session 4-1-IL-03:

The rising star molecule from a special Chinese tea plant, theacrine, is a fat burner by promoting fatty acid oxidation

Rong-Rong He, Guo-En Wang, Hiroshi Kurihara

Jinan University, Guangzhou, China

Ectopic fat storage was caused by metabolic disorder and resulted in metabolic diseases such as nonalcoholic fatty liver disease (NAFLD), obesity and hyperlipidemia. Fat burning was considered as an effective way for ameliorating metabolic diseases. Herein, fat mobilization failed to improve fat consumption effectively, promoting fatty acid oxidation, thus was considered as the key step of fat burning process. After searching for the natural activators, we found that theacrine, obtained from *Camellia assamica* var. Kucha, a special tea plant in Yunnan province of China, improves palmitate oxidation and induces a subsequent inhibition of fatty acid elongation by using fatty acid flux analysis. In addition, theacrine reduced body fat by increasing the mid-dorsal temperature in obese mice and focal uptake of ¹⁸F-FDG in brown adipose tissues (BAT). Moreover, theacrine ameliorated triglyceride metabolic disorder in restrained mice through increasing lipoprotein lipase activity, thereby promoting fatty acid metabolism. Our findings also demonstrated theacrine protected against diet-induced or stress-susceptible NAFLD. As acylcarnitine metabolism disorder contributed a lot to the development of NAFLD, the reductions of long-chain or short-chain acylcarnitines and the increased ratios of longer/shorter-chain acylcarnitines by theacrine could explain its improvement involved in fatty acid oxidation. In molecular mechanism, theacrine increased the activity of hepatic long-chain acyl coenzyme A dehydrogenase (LCAD) through deacetylation, and the deacetylation involved theacrine's activation of the mitochondrial deacetylase Sirtuin 3 (SIRT3). On the basis of theacrine's promoting fatty acid oxidation and energy expenditure, theacrine ameliorated acylcarnitine metabolism disorder in diet-induced or stress-susceptible NAFLD through the SIRT3/LCAD signaling pathway, thereby promoting fat burning in liver and adipose tissues. SIRT3 is confirmed as a target involved in theacrine's fat burning effect. Finally, it will be worth to develop a theacrine-containing medication to prevent ectopic fat storage in metabolic diseases like NAFLD, obesity and hyperlipidemia.

Session 4-1-SL-04:

Assessment of antimalarial properties and potential genotoxicity of African medicinal plants traditionally used in western and central Uganda

Fabien Schultz ^{1,2,5}, Godwin Anywar ³, Ogechi Favour Osuji ², Anh Nguyen ², Luc Pieters ⁴, Leif-Alexander Garbe ^{1,2,5}

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Many plant and insect species of the tropical rainforests in western Uganda and eastern DR Congo have not yet been discovered; about 90% have never been screened for bioactivity. Approx. 60% of the world's population relies almost entirely on plants for medication. The knowledge of African plants and their traditional uses are mainly transferred orally from one generation to the next by traditional healers, leading to the loss of vital information due to lack of records. Our study provides documentation of 16 different African medicinal plants, which are claimed to possess antimalarial, anti-cancer and antibiotic properties, amongst others. A possible methodology for the discovery of novel pharmacologically active drugs is the screening of selected plant extracts for a broad array of pharmacological activities. We present results of diverse bioassays performed with 61 different plant extracts from these 16 selected plant species, including: 1. Antimalarial heme biocrystallization assay as a pre-screen for upcoming in vitro and in vivo evaluation, 2. Evaluation of antiplasmodial activity against chloroquine-resistant *Plasmodium falciparum* K1 strain, 3. Cytotoxicity testing with human MRC-5 lung fibroblast cells, 4. GC-MS-assisted Ames test with human S9 liver fractions for investigation of mutagenic / potential carcinogenic effects of the extracts. Of all plants tested, diethyl ether extracts of *Warburgia ugandensis* showed the lowest IC₅₀ value against *P. falciparum* K1 with 0.5 µg/ml. In many cases, the traditional use of the plant species could be scientifically validated. Bioassay-guided fractionations combined with GC/LC-MS techniques enabled identification of bioactive compounds in some of the tested African plants. For instance, extracts of *Zanthoxylum chalybeum* contained 8% of lupeol that is known for its antiprotozoal activity in literature. This study was performed according to the international and national rules considering the Convention on Biodiversity and the Nagoya Protocol.

Session 4-1-SL-05:

Traditional medicine to bedside: in vivo-enabled discovery of potential antiseizure drugs from *Boswellia sacra*

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Epilepsies affect 1% of the population and include a variety of syndromes which have in common recurrent seizures [1]. Despite the availability of over 25 anti-seizure drugs (ASDs), 30% of epilepsy patients do not respond to or have serious side effects with current treatments. Moreover, 60% of people with epilepsy living in low- and middle-income countries have no access to ASDs and consequently rely on medicinal plants. Frankincense, the resin of *Boswellia* trees, is traditionally used for its relaxant properties in Middle East and India and has demonstrated anticonvulsant activity in a rat epilepsy model [2]. We recently used a zebrafish epilepsy model with seizures induced by the GABAA antagonist pentylenetetrazole (PTZ) [3] to monitor the isolation of anticonvulsant principles by bioassay-guided fractionation. The most active compound isolated, β -boswellic acid (β BA), reduced up to 70% of PTZ-induced seizure activity. Additionally, the comparison of six isolated boswellic acids revealed a specific structure-activity relationship, with bioactivity found only for β BA. In preparation for a future clinical trial, a mouse seizure model is being used to validate the zebrafish results and to compare the anticonvulsant activity of the pure β BA and a formulated extract of *Boswellia serrata*. This work reports for the first time the *in vivo* anticonvulsant activity of an isolated compound from *Boswellia sacra*, highlighting its potential to be developed as a novel ASD, and also the potential of frankincense as a botanical treatment for epilepsy.

[1] WHO (2018) Epilepsy Fact Sheet No. 999, World Health Organization (WHO)

[2] Ziyaurrahman AR, Patel J. *Der Pharmacia Lettre* 2012; 4(4):1308-1326

[3] Challal S, Buenafe OEM, Queiroz EF, Maljevic S, Marcourt L, Bock M, Kloeti W, Dayrit FM, Wolfender JL, Crawford AD, et al. *ACS Chem Neurosci* 2015; 15: 993 - 1004

Session 4-1-SL-06:

6-Dihydroparadol, a ginger constituent, enhances cholesterol efflux from THP-1-derived macrophages

Dong-dong Wang ^{1,2,3}, Verena Hiebl ¹, Angela Ladurner ¹, Simone Latkolik ¹, Franz Bucar ⁴, Elke HeiB ¹, Verena Dirsch ¹, Atanas Atanasov ^{1,2}

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Ginger is reported to be used for the prevention and treatment of cardiovascular diseases (CVD) [1, 2]. Cholesterol efflux from macrophage foam cells is an important process in reverse cholesterol transport, whose increase may help to prevent or treat CVD [3]. In this study, we investigated the effects of 6-dihydroparadol, a ginger constituent, on macrophage cholesterol efflux. We show that 6-dihydroparadol enhances concentration-dependently both apolipoprotein A1- and human plasma-mediated cholesterol

efflux from cholesterol-loaded THP-1-derived macrophages. 6-Dihydroparadol increases protein levels of both ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) according to Western blot analysis. Application of probucol (ABCA1 inhibitor) revealed that the 6-dihydroparadol-promoted efflux mainly correlates with increased ABCA1 protein levels. Application of probucol (ABCA1 inhibitor) revealed that the 6-dihydroparadol-promoted efflux mainly correlates with increased ABCA1 protein levels. Furthermore, increased ABCA1 protein levels in the presence of 6-dihydroparadol were associated with both increased ABCA1 mRNA levels and increased ABCA1 protein stability. Enhanced ABCG1 protein levels were only associated with increased protein stability. Increased ABCA1 protein stability appeared to be the result of a reduced proteasomal degradation of the transporter in the presence of 6-dihydroparadol. We identified 6-dihydroparadol, a ginger constituent, as a novel promoter of cholesterol efflux from macrophages that increases both ABCA1 and ABCG1 protein abundance. This newly identified bioactivity might contribute to the antiatherogenic effects of ginger.

[1] Kim HJ, et al. J Cell Biochem 2018; 119(1): 260-268.

[2] Wang J, Ke W, Bao R, Hu X, Chen F. Ann N Y Acad Sci 2017; 1398(1): 83-98.

[3] Rohatgi A, et al. N Engl J Med 2014; 371(25): 2383-2393.

Session 4-1-SL-07:

Immunomodulatory Activities of Maca on Spleen-Deficiency Syndrome Mice

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Maca (*Lepidium meyenii* Walp.) is consumed both as a sports supplement by strength and endurance athletes as well as a medical food in Peru for thousands of years. Its significant introduction in China has transformed its use within Traditional Chinese Medicine (TCM) and with the guidance of TCM theory. Immunosuppressive activity is closely related and quite similar with the syndrome of spleen-deficiency in TCM. This study aimed to investigate the immunomodulatory activities of Maca on spleen-deficiency syndrome in mice, and to verify the TCM property of strengthening the spleen function of Maca. A spleen-deficiency model was established by intraperitoneally injecting cyclophosphamide. Ginseng, a major spleen strengthening herb was used as a control. Maca powder and Ginseng granules were intragastric administrated to different groups of mice. The bodyweight, thymus and spleen indices were measured. The number of blood cells from peripheral blood were counted. The lymphocyte proliferation inhibition rate of each group was observed by the MTT method. The rate of CD4⁺T cells, CD8⁺T cells proliferation and the ratio of CD4⁺/CD8⁺ in peripheral blood were analyzed, and the lymphocyte cycle was detected by flow cytometry.

Maca significantly increased the numbers of peripheral blood cells and reversed the atrophy of the thymus and spleen. The proliferation of lymphocytes increased. Furthermore, Maca increased the rate of CD4+T cell proliferation and the ratio of CD4+/ CD8+, but decreased the rate of CD8+ T cell proliferation. The proportion of cells in the G2/M phase and S phase increased, but the proportion of cells in the G0/G1 phase decreased. Our results suggested that Maca has immunomodulatory activities on spleen-deficiency syndrome in mice and has the property of strengthening spleen function in TCM.

Keywords:

Maca; Immunomodulatory; Spleen-Deficiency Syndrome; Lymphocyte; TCM Property

Tuesday, 28th August, 2018-Bibo BallroomC+Room1-14:00- 15:50

Invited & Short Lecture - Session 1-2

Session 1-2-IL-01:

Protective effects of anticomplement agents from traditional Chinese medicines on mice with influenza virus infection

Dao-feng Chen

School of Pharmacy, Fudan University, Shanghai, China

The complement system plays a crucial role in the elimination of invading pathogens and generation of an optimal host response. However, the inappropriate activation of complement system may cause some life-threatening symptoms including acute respiratory distress syndrome (ARDS). In order to demonstrate the effective constituents of traditional Chinese medicines (TCMs) with functions of heat-clearing and detoxification and to obtain natural anticomplement agents from TCMs for prevention and treatment of the complement-associated diseases, bioactivity-guided fractionation and isolation was thus performed and led to the isolation and characterization of some valuable anticomplement constituents from several TCMs.

The Bupleurum polysaccharides showed anti-complementary activity and exhibited beneficial effects on the acute lung injury (ALI) in rats and mice. The polysaccharides obtained from *Houttuynia cordata* (HCP) exhibited anti-complementary and antipyretic activities, and attenuated the lipopolysaccharide-induced ALI in mice and rats as well. The interesting finding is that HCP exhibited protective effect on mice infected by influenza A virus (H1N1), although it had no direct inhibition against the virus. In addition, the total flavonoids obtained from *Houttuynia cordata* showed antiviral activity against influenza virus in vitro and exerted synergistic protective effects on the mice with virus infection in combination with HCP. The flavonoids enriched from *Scutellariae Radix* showed beneficial effects on the H1N1-induced ALI in mice as well, with their in vivo metabolic activation by gut microbiota.

Our studies demonstrated that the polysaccharides and flavonoids from the traditional Chinese medicines are valuable anticomplement agents for prevention and treatment of ARDS, severe acute respiratory syndrome (SARS), influenza and bird flu.

Acknowledgements :

This work was supported by the National Natural Science Foundation of China (NO. 81330089 and 30925042) .

Session 1-2-IL-02:

Native Mass Spectrometry in Drug Discovery: Anti-TB Natural Products and Their Targets

Asmaa Boufridi ¹, Miaomiao Liu ¹, Ali Elnaas ¹, Tin Mak ¹, Angela Di Capua ¹, Yang Yang ¹, Carl Nathan ², Ruslana Bryk ², Peter Myler ³, Ronald Quinn ¹

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Native Mass Spectrometry using an electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer (ESI-FTICR-MS) can detect protein in its native folded state and can also detect non-covalent protein-ligand complexes.

We have harnessed the chemical diversity of natural products for fragment-based drug screening. We have reported 96 low molecular weight natural products identified as binding partners of 32 putative malarial targets. Seventy-nine (79) fragments have direct growth inhibition on *Plasmodium falciparum* at concentrations that are promising for development of fragment hits against these protein targets. This adds a fragment library to the published HTS active libraries in the public domain.¹ Subsequently, we identified 26 low molecular weight natural products that bind to 9 proteins from *Mycobacterium tuberculosis*.

Phenotypic screening against *M. tuberculosis* H37Rv and the TB target Lipoamide Dehydrogenase (Lpd) produced 452 fractions showing a MIC following 11-point dose response analysis. The Lpd screen gave 169 fractions that inhibited Lpd in a duplicate point assay following a single point HTS.

We are using the phenotypic active fractions to conduct target identification using a panel of cloned and expressed *Mycobacterium* proteins.

[1] Vu, H.; Pedro, L.; Mak, T.; McCormick, B.; Rowley, J.; Liu, M.; Capua, A. D.; Williams-Noonan, B.; Pham, N. B.; Pouwer, R.; Nguyen, B.; Andrews, K. T.; Skinner-Adams, T.; Kim, J.; Hol, W.; Hui, R.; Crowther, G. J.; Voorhis, W. C. V.; Quinn, R. J., *ACS Infect. Dis.* 2018, 10.1021/acsinfecdis.7b00197.

Session 1-2-SL-03:

Surface Waxes of Northern Berries: a comprehensive study of cuticular wax composition

Linards Klavins, Jorens Kviesis, Maris Klavins

University of Latvia, Department of Environmental Science, Riga, Latvia

Investigation of plant surface waxes began nearly 100 years ago with the isolation of C₃₁ (hentriacontane) alkane from spinach leaves. Number of publications on plant waxes have rapidly grown since the first studies, which is due to development of new analytical techniques and mainly as a result of advancement of gas chromatography (GC). In the present day, gas chromatography is coupled with a mass spectrometer (MS), which allows efficient identification of unknown substances in complex mixtures. Plant surface waxes have a crucial role in the interaction between biotic and abiotic factors- the outermost layer of plant surface protects the plant from dehydration, extreme temperatures and other varying environmental factors as well as insect attacks, fungi infestation and bacteria. The most recent studies on plant waxes are motivated by the interest to increase shelf life of produce and reduce the risks of microbial infections in fresh fruit and vegetables. The main compound classes in plant surfaces waxes are triterpenoids, which are derivatives of squalene- these compounds possess various biological and pharmaceutical activities. In this study, 16 different berries grown in Latvia (Europe) were investigated and the differences were compared. Berry surface wax extracts were analysed as TMS esters using GC/MS. In the highest concentrations various phytosterols, alkanes, alcohols (including secondary alcohols) and fatty acids were found. In total 80 different substances were found of which 42 were identified and quantified. It was found that the same species of berries, but different varieties have similar wax profiles, however, berries within the same genus (*Vaccinium* spp.) have significantly different surface wax composition. Further investigation of wild berry surface waxes can give an important insight into the possibilities to prolong shelf life and quality of fruits, which, in turn, reduces losses to suppliers due to spoilage of produce and pest attacks.

Session 1-2-SL-04:

Metabolite profiling based evaluation of natural products from *Hypericum*

Katrin Franke ¹, Serge A. Fobofou ¹, Pauline Stark ¹, Sarah Scharfenberg ¹, Andrea Porzel ¹, Paride Rizzo ², Tim Sharbel ², Ludger A. Wessjohann ¹

¹ *Leibniz Institute of Plant Biochemistry, Halle, Germany*

² *Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

The genus *Hypericum* comprises more than 450 species widely occurring in temperate regions and tropical

highlands. Many species are used locally as traditional medicine against a variety of diseases. However, only a small proportion has been phytochemically characterized. The Common St. John's wort (*Hypericum perforatum* L.) is a well-known medicinal herb used for the treatment of mild to moderate depressions. Prominent secondary metabolites include flavonoids, naphthodianthrone such as hypericin, and polyprenylated phloroglucinols such as hyperforin.

We developed untargeted metabolomic approaches to detect a high number of metabolites with different physical and chemical properties simultaneously. Metabolite profiles obtained by 1D and 2D NMR and MS were evaluated by multivariate data analysis and can be furthermore correlated with various bioassay results to identify the bioactive chemical constituents.

This approach was successfully applied to compare and evaluate commercial *Hypericum* preparations, to investigate the secondary metabolite diversity within the genus [1] or species and to select species or samples for the discovery of unknown natural products [2, 3]. Detailed investigation of species selected by this method resulted in the isolation and structure elucidation of approximately 30 novel natural products including compounds with promising anthelmintic or anti-HIV activities [2, 3].

Currently, comparative metabolite profiles of *H. perforatum* accessions with different genetic background are investigated to estimate the intraspecific variance. The results can be used to characterize breeding lines and to select genotypes with an optimized compound composition with respect to specific pharmacological applications.

References:

- [1] Porzel A, Farag MA, Mülbradt J, Wessjohann LA. *Metabolomics* 2014; 10: 574-588.
- [2] Fobofou SAT, Franke K, Porzel A, Brandt W, Wessjohann LA. *J Nat Prod* 2016; 79: 743-753.
- [3] Fobofou SAT, Franke K, Sanna G, Porzel A, Bullita E, La Colla P, Wessjohann LA. *Bioorg Med Chem* 2015; 23: 6327-6334

Acknowledgements:

The Leibniz Association (SAW-2015-IPB-2), Saxony-Anhalt and EFRE (ZS/2016/05/78617) and the DAAD are acknowledged for financial support.

Session 1-2-SL-05:

Accurate compound identification of complex traditional herbal medicine using a novel mass spectrometry acquisition method

Hans Vissers, Giorgis Isaac ¹, Jimmy Yuk ¹, Lee Gethings ¹, Rob Plumb ¹, Rudolf Bauer ²

¹ *Waters Corporation, Milford, United States*

² *Department of Pharmacognosy, University of Graz, Graz, Austria*

Yu Ping Feng San (YPFS) is a three herb TCM formulation which has been traditionally used for treatment of immune system related diseases. The LC-MS data generated from such multiple herbs contain fragments from co-eluting multiple precursor ions. This makes the fragment data more complex and hence difficult to make correct compound identification. Here we describe the application of a novel data-independent acquisition (DIA) approach called SONAR™ for improved spectral clarity and confident compound identification from complex samples such as TCM.

SONAR™ utilizes a low resolution quadrupole mass filter, which is scanned continuously and both precursor and MS/MS data are acquired. Data were also collected for comparison purposes using a traditional DIA method such as MS^e, which provides both precursor and fragment ion information but without a resolving quadrupole. From the results, the specificity of SONAR™ provides cleaner precursor and fragment ion spectra compared to the traditional DIA acquisition method. As an example, the identification of prim-O-glucosylcimifugin acquired using a traditional DIA and SONAR™ was compared. When using the traditional DIA there are multiple co-eluting compounds which could confound the structural analysis. The presence of these co-eluting precursor ions with prim-O-glucosylcimifugin provided a high complexity with 97 high energy fragment ions, making compound identification very complex and challenging. On the other hand when the data were acquired using SONAR™, cleaner precursor and fragment ion spectra were generated. The selected narrow precursor mass window from SONAR™ provided specific fragment ions and contained only the parent ions prim-O-glucosylcimifugin [M+H]⁺ and [M+Na]⁺. Eight clean relevant fragment ions were generated only from the parent ion prim-O-glucosylcimifugin which led to correct and confident compound identification. In summary, compared to the traditional DIA method, the specificity of SONAR™ provided cleaner precursor and high energy fragment ion spectra, which resulted in confident compound identification.

Session 1-2-SL-07:

Dissecting the Quality Traits of Medicine Plants by Deep Phenotyping, Metabolic, and Transcriptome Analysis

Jun-feng Chen

TCM Resources and Biotechnology Center, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Plant is the major source of active chemicals for human health. We developed an integrated research strategy to investigate the quality traits and the underlying molecular mechanism using *Salvia miltiorrhiza* as a model. Morphological traits were investigated by high-throughput image analysis. Accordingly, high-resolution metabolic analyses were performed to demonstrate the metabolic variation of active compounds and general metabolic flux. Subsequently, phenotypic and metabolic components were integrated for

analyses of phenotypic variance, heritability, growth modeling, and traits correlation. Parameters generated by above analyses are promising for subsequent genetic mapping to uncover the genetic basis of quality traits by genome re-sequencing. The study strategy presented here will be useful to advance our views of medicine plants and key components underlying their quality traits. Meanwhile, it will provide opportunities to discover efficient genetic elements for metabolic engineering.

Tuesday, 28th August, 2018 - The Chapel - 14:00 - 16:00

Invited & Short Lecture - Session 3-2

Session 3-2-IL-01:

Traditional Decoction Slices vs Modern Ultrafine Granular Powder: Greatly different, or not?

Wan-Ying Wu

Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

TCM is developing to modernization and internalization based on inheriting the essential tradition. In modern studies of TCM decoction slices, ultrafine granular powder (UGP), which are shivered into ultrafine powder ($D_{90} < 45 \mu\text{m}$ before granulation) from single species of TCM decoction slices and then reshaped as granules, is a novel type. UGP overcomes the disadvantages such as poor homogeneity, unsure safety and quality in traditional decoction slices (TDS). After cells-cracked, the utility of ingredients can be maximized. With convenient and diverse application ways, UGP can be brewed, decocted or stirred into a suspension (just like the coffee form).

Comparative studies on traditional decoction slices and UGP of Chinese Salvia is an example research including chemical analysis, contents of major components, in vitro dissolution and in vivo pharmacokinetics, pharmacology, safety and stability. The chemical profiles of TDS and UGP were compared via similarity analysis and discriminated via multivariate analysis. The mean similarity correlation coefficient (> 0.987) revealed that UGP and TDS of Chinese Salvia were consistent between each other and stable between different batches. Compounds were further characterized by ESI-MS/MS. Eventually, 62 compounds selected as characteristic markers for evaluation were characterized or tentatively characterized. Meanwhile, compared with TDS, UGP showed significant cardio-protection against myocardial remodeling with therapeutic treatment. Results show that UGP also holds the advantages of uniform, convenience and efficiency. Undoubtedly, UGP can bring about great changes for clinical treatment.

Quality standard system of UGP has been established and developed gradually. In the near future, UGP with Chinese cultural characteristics will definitely step into the international medical and healthcare market, being an important natural and innovative category for human health, intervention in sub-health and clinical use.

Session 3-2-IL-02:

Identification of effective combination in the extract of Ginkgo biloba

Hua Yang

China Pharmaceutical University, Nanjing, China

Nowadays, combination drugs shows increasing advantageous merit in treating complex disease than single target drug, but rapid discovery of combinatorial bioactive components as drug candidates is a much greater challenge. In this work, a metabolic distribution-oriented network regulation strategy was developed for the identification of effective combination. The extract of Ginkgo biloba (EGB) was taken as a case study. Firstly, comprehensive chemical profiling and metabolic exposure of EGB in a pathological state were conducted. Then the effective combination was screened by combining network regulation and the metabolic exposure level of EGB. Finally, a combination of 12 active compounds was found for treatment of ischemia stroke, showing bioactivity equivalence with original EGB. The results also indicated that beside the well-known ginkgolides and flavonoids, trace compounds might also play an important role of the holistic effect of EGB.

Session 3-2-SL-03:

Adulteration and adverse events due to complementary health products

Hwee-Ling Koh

National University of Singapore, Singapore, Singapore

With aging population, sedentary lifestyle, physical and psychological stresses from everyday life and work, increasing prevalence of both communicable and non-communicable diseases and increasing healthcare costs, it is timely to explore the use of Traditional Chinese Medicine and other medicinal plants for health promotion, disease prevention and treatment purposes. Quality, safety and efficacy of herbs and herbal products are closely inter-related. The quality control and safety considerations of herbs and complementary health products are of paramount importance. A brief overview of the factors affecting the quality of herbs and herbal products will be given. In particular, the presence of undeclared drugs and drug analogues pose a challenge to health regulators and healthcare professionals. Unknowing users are at risks of adverse events associated with the use of such illegal and poor quality preparations. The issues and challenges related to the adulteration of complementary health products with phosphodiesterase Type 5 inhibitors and their analogues will be discussed. Concerted efforts to screen for undeclared drug adulterants and the adverse events associated with complementary health products from 1998 to 2016 in Singapore will be shared.

Session 3-2-SL-04:

Complexity in Chinese herbal medicine supply chains: Exploration and evaluation using thematic analysis.

Martin Fitzgerald

University of Westminster, London, United Kingdom

Chinese herbal medicine, supply chains, complexity analysis, thematic analysis.

Increasing demand for Chinese herbal medicines (CHM) has led to expanding supply chains. Additionally, the incidence of CHM adverse events including fatalities has necessitated increased compliance. [1] As regulators focus safety and suppliers on volume, a risk of divergence between these presents with increasing complexity.

Aim:

To explore and evaluate factors which render CHM supply chains particularly complex and thus potentially vulnerable.

Methods:

Literature search using keywords; “Chinese herbal medicine, complexity, problems, toxicity and supply chain” (11th April 2018), yielded 325 relevant publications. 302 were excluded, 23 were included. The model of A Booker-and M Heinrich on value chains was used in the analysis. [2]

Computer-aided thematic generation using (NVivo 11). Coding and thematic analysis with of Braun and Clark methodology. [3]

Results and Discussion:

Findings show that 9 complexity factors impact CHM supply chains which can be represented by three major themes; human, product and conceptual complexity. They contribute 21, 28 and 51% of the complexity factors respectively.

Conclusion:

Most complexity in CHM supply chains is contributed by conceptual factors (such as medicinal material status, regulation and value chain elements), followed by product and human complexity aspects. There is a need to further explore the relationship between conceptual complexity and the risk to product quality and safety.

Funding was received from the Brion Research Institute of Taiwan (Sun Ten Group) and Herbprime Co., Ltd for a PhD Studentship.

- [1] Izzo A, Hoon-Kim S, Radhakrishnan R, Williamson E. *Phytother Res* 2016; 30: 691-700.
- [2] Booker A, Johnston, D. and Heinrich, M. 2012. Value chains of herbal medicines—Research needs and key challenges in the context of ethnopharmacology. *Journal of ethnopharmacology*, 140(3), pp.624-633.
- [3] Braun V, Clarke V. Using thematic analysis in psychology. *Qualitative research in psychology*, 3(2), pp.77-101.

Session 3-2-SL-05:

Direct Visualization of botanicals Using Mass Spectrometry for discovery and quality control

Jin-jin lu ¹, zheng-wei jia ¹, wei rao ¹, Thanai Paxton ², Jimmy yuk ³, Giorgis Isaac ³, Jose Castro-Perez ³, jiang xu ⁴, Shilin Chen ⁴, Hirofumi Enomoto ⁵

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³ *Waters Corporation, Milford, MA, 01757, USA, Milford, United States*

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Ambient mass spectrometry provides a comprehensive approach in obtaining rapid chemical profiles and also spatial distribution of a large variety of endogenous and exogenous analytes from a natural product without sample extraction or addition of any external label. Distribution of molecules in natural products (botanicals, microbial, and traditional medicines) can provide an understanding of spatial metabolomics, sample-environment interactions, and aid in compound screening. For example, location information of medically important compounds within a natural product will strengthen fundamental understanding of their metabolic origins, as well as, improve their extraction. For quality control, a detailed chemical profile of natural product can give valuable insight to a natural product's geographical origin or species-specific information. Molecular images provided by MS are complementary to the structural information provided by optical microscopy.

Here, we share advances in ambient mass spectrometry such as multimodal molecular imaging of natural products using desorption electrospray ionization (DESI) and Rapid Evaporative Ionization Mass Spectrometry™ (REIMS™) for various botanical studies. From understanding the distribution of ginsenosides in panax ginseng, plant hormones in *Phaseolus vulgaris* L. to conducting rapid identification of different herbs using multivariate modeling of the chemical profiles, ambient mass spectrometry can be a very powerful analytical technique for natural product discovery and quality control.

Definition of Content of Cyanoglucosides in Raw Material of Sambucus Nigra

Dmitriy Kruglov, Tatiyana Shinko

Novosibirsk state medical university, Novosibirsk, Russia

Elder flower is used in scientific medicine by a lot of countries as a diaphoretic remedy. Herb raw material "Elder flower" consists of the dried flowers of *Sambucus nigra* L. The flavonoids are the main group of biological-active compounds of this herb and precisely for this reason the identification of authenticity of herb raw material is carried out by using chromatography [1]. At the same time any flavonoids can't determine the similar pharmacological activity (diaphoretic action). It is known also that one of the secondary metabolites synthesized in *Sambucus nigra* is cyanoglucoside (sambunigrin). Sambunigrin can be hydrolyzed by using acid or enzyme and as a result the cyano-groups evolve which have the diaphoretic action.

The aim of this work was research and determining of cyanoglucoside amount in herb raw material. The collected inflorescences were dried up and crushed. Then the water extraction has been taken from the researched samples. At the following stage the cleaning from carbohydrates has been made and the received filtrate was subjected to acid hydrolysis. After hydrolysis picric acid was added and solution was heated on the boiling water bath for 15 minutes

.Later on UV-spectra was investigated (see Fig 1)

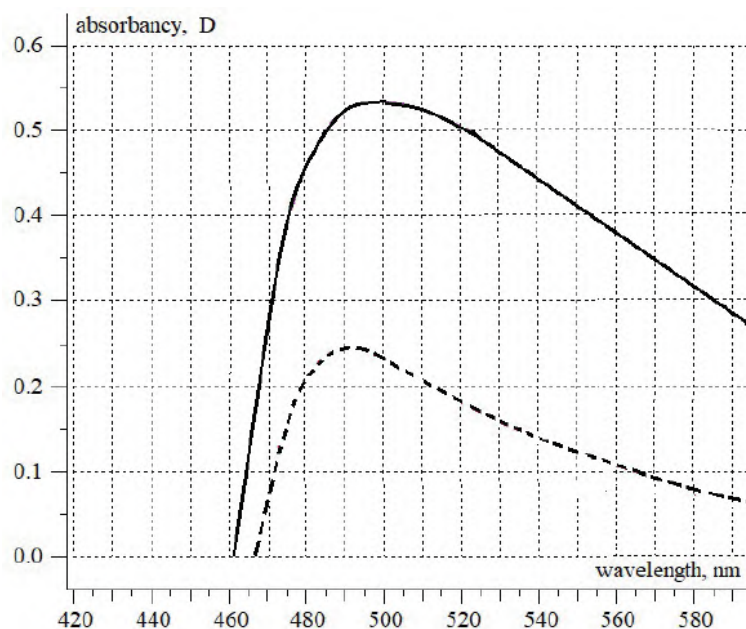


Fig .1 UV-spectra researched solution (—) and standard cyanid-ion (---)

UV-spectra of the investigated solution and of standard sample of cyanid-ion are close and have a

characteristic maximum of absorption at 495 nm. Using the calculating formula given in work [2] it has been established that the amount of cyanoglycosides in flowers of elder has totaled $23,0 \pm 5.0$ ppm.

Reference:

1. British herbal pharmacopeia. 4th edition - 1996, BHMA.-212p.
2. Rezaul Haque M., Howard Bradbury J. Total cyanide determination of plants and food using the picrate and acid hydrolysis method// Elsevier., - Food chemistry 77 (2002) .- pp.107-114

Tuesday, 28th August, 2018-Poster Area-Poster Shed-16:00-18:00

Poster session-PO-01:

Study on Biological Effects of Açai and Açai extracts on Kidney-Yin and Kidney-Yang deficiency Syndrome Mice

Yan Qu, Yujie Wang, Wenting Fei, Jianjun Zhang, Cheng He, Chun Wang, Linyuan Wang

Beijing University of Chinese Medicine, Beijing, China

Açaí (*Euterpe Oleraceae* Mart.) is a South American herbal medicine which has been introduced into China. The aim of this study is to investigate Açai's Traditional Chinese Medicine(TCM) properties by comparing with *Radix Rehmanniae Recens* and *Cinnamomi Cortex*, which are traditionally accepted as Cold and Hot drugs and determine the material basis of Açai's TCM properties by studying the biological effects of Açai and Açai extracts on Kidney-Yin and Kidney-Yang deficiency Syndrome Mice . Firstly, we split Açai into oil, alcohol extract and water extract, then KM mice were randomly divided into blank, Kidney-Yin model, Kidney-Yin+Açaí, Kidney-Yin+oil extract, Kidney-Yin+alcohol extract, Kidney-Yin+water extract, Kidney-Yang model, Kidney-Yang+Cinnamomi Cortex, Kidney-Yang+Açaí, Kidney-Yang+oil, Kidney-Yang +alcohol extract, Kidney-Yang+water extract groups; the mice were administered orally at the dose of 160mg/kg thyroxine and 25mg/kg hydrocortisone per day respectively for 14 days to induce Kidney-Yin and Kidney-Yang models; temperature trend of mice were investigated by cold/hot plate differentiating assay; the levels of cAMP, cGMP, cAMP/cGMP, T3, T4, CORT, E2, T, IgG, IgM, C3, C4, TC, TG, TP, FFA, Alb, 5-HT and NE in serum were detected. Cold medicine mostly has central inhibition, which can weaken the respiratory, circulatory, metabolic activities, etc, while hot medicine has the opposite effect. From the analysis of cyclic nucleotides, thyroid hormones, endocrine hormones, metabolism, neurotransmitters, we verified that Açai is cool, alcohol extract is cool, while the oil and water extract are warm, thus alcohol extract is the material basis of the cool properties. The properties of Açai is the result of the combined effects of the different extracts.

Açaí ; material basis; Açai oil ; Açai alcohol extract ; Açai water extract ; Hot/Cold TCM Properties

Poster session-PO-02:

Anti-Influenza Virus Activity of Lanostane Triterpenes from Polypores

Julia Zwirchmayr ¹, Ulrike Grienke ¹, Martina Richter ², Christina E. Mari ¹, Ursula Peintner ³, Michaela Schmidtke ², Judith M. Rollinger ¹

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² *Institute of Medical Microbiology, Section Experimental Virology, Jena University Hospital, Jena, Germany*

³ *Institute of Microbiology, University of Innsbruck, Innsbruck, Austria*

The constant increase of viral resistance and the limited number of effective antiviral agents emphasizes that the current portfolio of anti-influenza drugs needs extension^[1]. To identify new anti-influenza agents from nature, locally grown European polypores belonging to the polyphyletic group of Agaricomycetes (Basidiomycota) were collected and identified via rDNA ITS phylogenetic analyses^[2]. Fruit bodies from ten species were collected and mycochemically investigated, i.e. *Fomes fomentarius*, *Fomitopsis pinicola*, *Ganoderma lucidum*, *G. applanatum*, *Gloeophyllum odoratum*, *Ischnoderma benzoinum*, *Laetiporus sulphureus*, *Phellinus robustus*, *Piptoporus betulinus*, and *Trametes gibbosa*. The generated ethanol extracts were screened in a cytopathic effect (CPE) inhibition assay against H3N2 influenza virus A/Hong Kong/68 (HK/68) in MDCK cells. Especially the extract of *G. odoratum* dose-dependently inhibited the CPE with an IC₅₀ of 15.0 µg/mL^[3]. A fluorescence-based neuraminidase (NA) inhibition assay excluded that the antiviral activity was based on the inhibition of the surface protein NA. HRESIMS, 1D and 2D NMR spectroscopy were used for the identification of eight isolated lanostane triterpenes with two novel natural compounds and three undescribed so far for *G. odoratum*. The most potent activity was determined for trametenolic acid B against HK/68 and the 2009 pandemic H1N1 strain A/Jena/8178/09 with IC₅₀s of 14.1 and 11.3 µM, respectively. Additionally, this compound was able to bind to cell-free viruses and to neutralize their infectivity in a plaque reduction assay.

[1] U. Grienke, M. Schmidtke, S. von Grafenstein, J. Kirchmair, K. R. Liedl, J. M. Rollinger, *Nat Prod Rep* 2012, 29, 11-36.

[2] P. Dresch, D. A. MN, K. Rosam, U. Grienke, J. M. Rollinger, U. Peintner, *AMB Express* 2015, 5, 4.

[3] U. Grienke, C. E. Mair, J. Kirchmair, M. Schmidtke, J. M. Rollinger, *Planta Med* 2018. doi 10.1055/a-0590-5153

Poster session-PO-03:

Fusicoccane diterpenes from *Hypoestes forskalii* (Vahl) R.Br.

Massimiliano D'ambola ¹, Ammar Bader ², Lorenzo Fiengo ^{1,3}, Fabrizio Dal Piaz ⁴, Antonio Vassallo ⁵, Roberta Cotugno ¹, Nunziatina De Tommasi ¹

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⁴ *Dipartimento di medicina, chirurgia e odontoiatria "Scuola Medica Salernitana", Università di Salerno, Via Giovanni Paolo II 132 - 84084, Fisciano (SA), Italy*

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In order to obtain bioactive secondary metabolites, we investigated the chemical constituents of *Hypoestes forskaoilii* Vahl. Roem. & Schult. (Acanthaceae) roots. *H. forskaoilii* is a perennial herb widely distributed in many African countries as well as in the Arabian peninsula [1]. The whole plant is popularly used as natural insecticide, moreover fresh leaves are used by locals to accelerate the healing process. Various biological properties have been attributed to the plant, including antiplasmodial, antifungal, antiparasitic and cytotoxic properties [2]. Several *Hypoestes* species have been chemically investigated before establishing that diterpenes belonging to the class of fusicoccane, isopimarane, and labdane are the main skeleton synthesized by these species [3].

Fusicoccanes characterized by a complex 5-8-5 dicyclopenta-cyclooctane nucleus, are powerful phytotoxins in *Hypoestes* and different fungi species [3].

Eleven new fusicoccane diterpenes were isolated from the dichloromethane extract of *H. forskaoilii* roots by CC, MPLC and RP-HPLC chromatography, together with two known lignans. All structures were elucidated on the basis of NMR and HR-MS spectroscopic methods. Additionally, the affinity of isolates towards Hsp90, one of the most promising targets for anti-cancer therapy, was tested by surface plasmon resonance. Results demonstrated that 17-hydroxy-hypoestenone efficiently interacted with the protein (K_D 0.32 mM). The study of the activity of 17-hydroxy-hypoestenone by means of a panel of biochemical and cellular approaches was achieved. 17-hydroxy-hypoestenone showed an antiproliferative activity with an EC_{50} of 26 μ M on the HeLa cell line by inducing a G₂/M cell cycle block through the down-regulation of pCdc2 protein levels.

[1] Andriamihaja B, Martin MT, Rasoaivo P, Frappier F. J Nat Prod 2001, 64: 217-218.

[2] Pettit G R, Goswami A, Cragg GM, Schmidt M, and Zou JC. J Nat Prod 1984, 47: 913-16

[3] de Boer AH, de Vries-van Leeuwen IJ. Trends Plant Sci 2012; 17: 360-368.

Poster session-PO-04:

Discovery of new GPBAR1 agonists by combined in silico - in vitro screening

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The G protein-coupled bile acid receptor (GPBAR1) is a possible new drug target for the treatment of metabolic and inflammatory diseases, including type 2 diabetes. Previously, it has been shown that a number of herbal remedies and natural small molecule metabolites were able to activate this receptor and thus may exert antidiabetic and anti-inflammatory effects. The fast-forward identification of GPBAR1 agonists is highly relevant, as metabolic diseases like type 2 diabetes have become an epidemic, and novel treatments are urgently needed [1].

The aim of this study was to generate reliable prediction models for the fast-forward assessment of GPBAR1 activating natural compounds. A cheminformatics workflow including ligand-based pharmacophore- and shape-based virtual screening was set up. The workflow was validated theoretically and employed for the prospective virtual screening of open-source and in-house molecular databases. From 34 chemically diverse virtual hits subjected to experimental testing, 14 were confirmed as GPBAR1 activators, including new scaffolds from natural and synthetic origin. Triterpenes previously isolated from the South African tree *Burkea africana* [2] and coumarins from the middle-eastern spice *Ferula assa-foetida* [3] showed activities comparable to the endogenous ligands chenodeoxycholic acid and lithocholic acid.

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Poster session-PO-05:

¹ H-NMR and UPLC-MS metabolomics: functional tools for exploring chemotypic variation in *Sceletium tortuosum* from two provinces in South Africa.

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Sceletium tortuosum is widely recognised for the treatment of stress, anxiety and depression. A comprehensive intraspecies chemotypic variation study, using samples harvested from two distinct regions of South Africa, was done using both proton nuclear magnetic resonance (¹H-NMR) spectroscopy of methanol extracts (N=145) and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) of acid/base extracts (N=289). Chemometric analysis of the ¹H-NMR data indicated two main clusters that were region-specific. Two dimensional (2D) NMR was used to identify analytes that contributed to the clustering as revealed by the S-plot. The *sceletium* alkaloids, pinitol and two alkylamines, herein reported for the first time from *S. tortuosum*, were identified as markers that distinguished the two groups. Relative quantification of the marker analytes conducted by qNMR indicated that samples from the Northern Cape generally contained higher concentrations of all the markers than samples from the Western Cape. Quantitative analysis of the four mesembrine alkaloids using a validated UPLC-UV method confirmed the results of qNMR with regard to the total alkaloid concentrations. Samples from the Northern Cape Province were found to contain, on average, very high total alkaloid ranges. Regarding the Western Cape samples, the total yields of the four mesembrine alkaloids were substantially lower. Hierarchical cluster analysis of the UPLC-MS data, based on the alkaloid chemistry, revealed three branches, with one branch comprising samples primarily from the Northern Cape that seemed somewhat chemically conserved, while the other two branches represented mainly samples from the Western Cape. The construction of an orthogonal projections to latent structures-discriminant analysis model and subsequent loadings plot, allowed alkaloid markers to be identified for each cluster. The diverse *sceletium* alkaloid chemistry of samples from the three clusters may facilitate the recognition of alkaloid profiles, rather than individual compounds, that exert targeted effects on various brain receptors involved in stress, anxiety or depression.

Poster session-PO-06:

Methyl- β - cyclodextrin and coronatine effect on hyoscyamine and scopolamine content of hairy root cultures of *Atropa acuminata*

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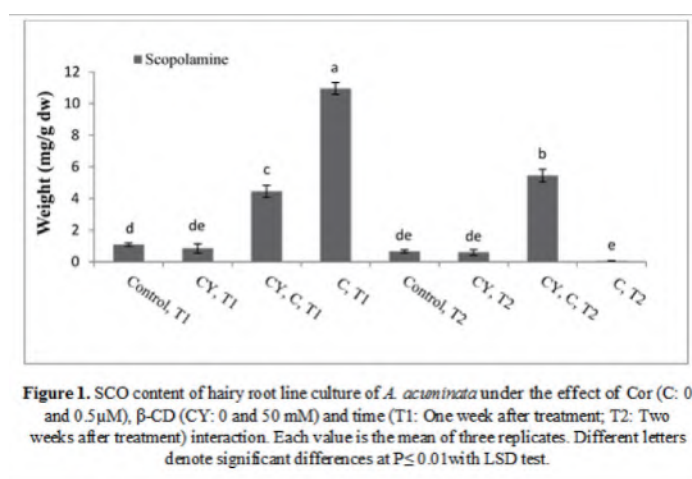
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Cyclodextrins and coronatine (Cor) are new elicitors and permeabilizing agents which are applied in plant cell and hairy root cultures to increase phytopharmaceuticals production (1). In present research the effect of two biotic elicitors, methyl- β - cyclodextrin (β -CD) and Cor, on hairy root cultures of *A. acuminata* Royle ex Miers was evaluated in order to develop a sustainable and high production system of hyoscyamine (HYO) and

especially scopolamine (SCO) tropane alkaloids. In this regard, the *Agrobacterium rhizogenes* A4 was used for direct infection of 2 month pot plant leaves of *A. acuminata*. Inoculums as 1 gr fresh weight of selected hairy root line were inoculated in glass jars which contained 30 ml of liquid half-strength B5 (B5/2) medium supplemented with 3% sucrose and placed in a shaker incubator (110 rpm) at 25 C° in dark situation. On day 14th the old medium was replaced with 30 ml fresh liquid medium as the same explained above which containing the elicitor treatments including: 50 mM β -CD, 0.5 μ M Cor and joint treatment of 50 mM β -CD and 0.5 μ M Cor with 3 replications. The sampling was fulfilled on day 21 (T1) and 28 (T2) of culture. HPLC analysis showed that Cor had negative effect on HYO content and also β -CD alone or in combination with Cor didn't have any significant effect on HYO content in hairy root cultures. On the other hand, the maximum amount of SCO was achieved by Cor alone at T1 and it was about 10 times more than controls at both sampling times. Also joint treatment of β -CD and Cor at T1 and T2 had positive effect on SCO quantity (Fig. 1).



Key words:

Cyclodextrin, Coronatine, Hairy root, *Atropa acuminata*

Reference

1- Khojasteh A, Mirjalili, M. H, Hidalgo D, Corchete P, Palazon J. *Biotechnol Lett* 2014; 36: 2393-2406

Poster session-PO-07:

In vitro antisickling activity of *Alafia barteri* Oliv. (Apocynaceae)

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ABSTRACT

Alafia barteri Oliv. (Apocynaceae) leaves and stems are used in ethnomedicine in South Western Nigeria for the management of sickle cell disease (SCD).

The in vitro anti-sickling activities of crude ethanolic extracts (CEE) and fractions of *Alafia barteri* powdered leaves and stems were evaluated using Vanillin and Phosphate Buffered Saline as positive and negative controls respectively.

The methods involved the inhibition and reversal of sodium metabisulphite induced sickling of HbSS red blood cells, obtained from confirmed non-crisis sickle cell patients.

The anti-sickling assay showed that the CEE of *A. barteri* leaves and its fractions (Petroleum ether and Ethyl acetate fraction) have significant inhibitory activity ($p < 0.05$) with percentage inhibition of 88.8, 67.3 and 53.1% respectively; CEE of *A. barteri* leaves and its fractions, (Ethyl acetate and Chloroform fractions) have significant reversal activity at 4 mg/ml with % reversal of 84.6, 90.2 and 86.2% respectively; while the CEE of *A. barteri* stems and its fractions, (Chloroform and Ethyl acetate fractions) have significant reversal activity ($p < 0.05$) at 4 mg / mL with percentage reversal of 59.3, 77.8 and 89.2% respectively but little inhibitory activity. Phytochemical screening of the powdered leaves and stems samples revealed the presence of alkaloids, saponins, tannins, and glycosides; with flavonoids present in leaf but absent in the stem. Further purification of the leaf extract ethyl acetate fraction using preparative thin layer chromatography (PTLC) and ultraviolet analysis revealed a major spot having absorbance of 0.265 at 404 nm wavelength, indicating a conjugated polyphenolic which might be responsible for the anti-sickling activity of the leaf.

The study has provided scientific validation for the traditional use of *Alafia barteri* leaf extract in the management of sickle cell anaemia.

Anti-sickling activity, *Alafia barteri* leaves and stems, Phytochemical screening

Poster session-PO-08:

Scale up fermentation of *Streptomyces* sp. (CA-129531 strain) - a potential source of bioactive compounds with skin-whitening activity

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In the frame of MICROSMETICS EU project 56 potential candidate actinobacteria strains of global biodiversity were selected to be studied using OSMAC strategy. In total 614 extracts were produced

and cell-free bioassays have been used for the evaluation of their skin-whitening bioactivity. Among the initial 614 extracts, the actinomycete strain CA-129531 of the genus *Streptomyces*, originated from Martinique and cultivated in the fermentation medium DNPM, exhibited the most promising skin-whitening activity (i.e. tyrosinase inhibition). This activity was confirmed in cell-based assays in mouse melanocytes (B16F10 cell line). Preliminary study of this strain, including the scale up fermentation in 1lt and bioguided fractionation of the active EtOAc extract, led to the isolation and identification of trichostatin A and trichostatic acid. Scale-up process optimization was performed in a bioreactor Biostat C+ (total volume 30 kg). Direct liquid/liquid extraction of the culture medium with EtOAc was performed and the yield of trichostatin A was measured in different experiments performed under modified media. The highest amount of this marker was observed when using media DNPM*3, a modified formulation of DNPM that used as carbon source dextrose in agreement with requirements of cosmetic legislation. After confirming the tyrosinase inhibition in cell-free assay of the EtOAc extract of this broth (IC₅₀=63.27µg/ml), preparative HPLC was used in order to bioguided isolate the active compounds. The full set of spectroscopic data (HRMS and NMR) was recorded for all active isolated compounds in order to unambiguously elucidate their structure, while it was also identified the main metabolite responsible for this activity (IC₅₀=3.07µg/ml). Therefore, this extract can be considered as potential candidate for industrial development and this optimized small-scale process can then be transferred to pilot scale following established scale-up strategies in cosmeceutical industry.

Poster session-PO-09:

Phytochemical analysis of antioxidant extracts obtained from the aerial parts of Greek *Cistus* species

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The majority of published research on *Cistus* species focuses on the aromatic resin labdanum, which demonstrates high antimicrobial activity. However, the aerial parts of rockrose plants have been used in Greek traditional medicine as anti-inflammatory agents for rheumatism and skin diseases [1]. This study emphasizes on the exploration of the phenolic content and anti-oxidant potential of five species (*C. monspeliensis*, *C. salvifolius*, *C. parviflorus*, *C. creticus* spp *creticus*, *C. creticus* spp *eriocephalus*) belonging to the Greek flora. After successive extraction with c-hexane, ethylacetate, methanol and water by Accelerated Solvent Extraction, all extracts were tested for their phenolic content and antioxidant capacity by

TPC, ABTS and DPPH assays and their chemical profile was compared with HPTLC.

The methanolic extract of *C. monspeliensis* as well as methanolic and ethylacetate extracts of *C. parviflorus* were further analysed. However, the challenge faced for the isolation of the bioactive compounds was the presence of high amount of tannins. For this purpose, two methodologies were implemented, FCPC chromatography using a three-phase solvent system (*C. monspeliensis*) and adsorption resin XAD-4 (*C. parviflorus*), resulting to removing of tannins and fractionation of the extracts. Further purification of compounds was accomplished with MPLC, Sephadex CC and prep-TLC. Their purity and identity was confirmed by NMR and LC-MS. The abovementioned process afforded to the isolation of over 40 compounds, among them 5 novel metabolites and over 15 known compounds isolated for the first time from the genus *Cistus*.

To the best of our knowledge, this is the first thorough phytochemical investigation of the methanolic extracts of the aerial parts of Greek *C. monspeliensis* and *C. parviflorus*, demonstrating simultaneously that the enrichment of crude extracts in polyphenols increase their antioxidant properties, while wise-chosen methodologies simplify the separation of natural compounds from complex matrices.

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Poster session-PO-10:

Optimization of silymarin extraction from milk thistle fruit using water/glycerol mixtures

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Milk thistle (*Silybum marianum* (L.) Gaertn., Asteraceae) fruit is a traditionally used hepatoprotective agent. Silymarin, a complex of flavonolignans, consisting of silybin A, silybin B, isosilybin A, isosilybin B and others, is considered to be the active component of this herb. In order to effectively extract the flavonolignans from milk thistle fruit, the ultrasonication-assisted extraction was carried out using water/glycerol as a non-toxic biodegradable solvent mixture. Firstly, for selection of independent variables that have the strongest influence on the silymarin extraction, the two-factor interaction model was used. Silymarin content was analyzed using the HPLC-DAD method. Analysis of variance (ANOVA) has shown that the most important extraction factors were glycerol concentration, temperature and extraction time. Subsequent Box Behnken analysis, performed by varying the selected factors, has shown that silymarin content was linearly dependent on time

and temperature, as well as on their interaction. Glycerol concentration, on the other hand, influenced the extraction process as a quadratic factor. Numerical optimization has revealed that the best conditions for silymarin extraction were 40% glycerol, 80 °C and 60 min. The experimental silymarin content corresponded well with the expected one (105 microg/mL vs. 102 microg/mL) thus confirming the validity of the quadratic model. The results indicate that the extraction using water/glycerol mixtures is an eco-friendly procedure for extraction of silymarin from milk thistle fruit.

Poster session-PO-11:

Bioactive polysaccharides from *Codonopsis pilosula*

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Radix *Codonopsis*, the root of *Codonopsis pilosula* (Franch) Nannf, *C. pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen, and *C. tangshen* Oliv, is a traditional herbal medicine in Asian countries, with various pharmacological effects, such as heart protection, lowering blood pressure, anti-ulcer, etc [1]. It was used as the replacement of ginseng because of the similar pharmacological activities. Polysaccharide is one of the major contributors to the biological activity. In our group, we aimed to find out different bioactive polysaccharides from three species of radix *Codonopsis*. *C. pilosula* from Gansu province, *C. pilosula* Nannf. var. *modesta* (Nannf.) L. T. Shen from Sichuan and *C. tangshen* from Guizhou province, were collected and the polysaccharides were hereby obtained. Three crude polysaccharide fractions and more than 20 purified polysaccharide fractions were obtained from those three species using anion exchange chromatography and gel filtration. The structural elucidation of some of the polysaccharide fractions were finished and the results indicated both neutral fractions and acidic fractions are present in all three species. The crude polysaccharides showed activity in regulating intestinal flora and immunity. Complement fixing activity, antioxidant activity, prebiotics activity [2] and immunomodulating activity in intestinal epithelial cells were performed in all purified polysaccharide fractions obtained. The results from biological tests indicated that polysaccharides from different species of *Codonopsis* showed different activity. The further study aimed structure-activity relationship analysis would be performed.

Reference:

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Poster session-PO-12:

Phytochemical composition and biological activity of selected antidiabetic plants used in Croatian ethnomedicine

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Diabetes is a chronic disease with growing prevalence worldwide. In addition to conventional therapy, many patients use phytotherapeutic preparations for the management of diabetes. The goal of this study was to assess the phytochemical composition and biological activity of species most used for phytotherapy of diabetes in Croatia: areal parts of yarrow (*Achillea millefolium*), wormwood (*Artemisia absinthium*) and common centaury (*Centaureum erythraea*), elderflower (*Sambucus nigra*), white mulberry (*Morus alba*) and sage (*Salvia officinalis*) leaf, as well as hulls of beans (*Phaseolus vulgaris*). The TXRF analysis has shown that the plant samples did not contain elevated amounts of heavy metals. In addition to that, chromium, the metal important for combating insulin resistance, was found in the wormwood sample. Aqueous and ethanolic (80 %) extracts were prepared from the selected plants, and the chemical composition and biological activity of the extracts compared. Polyphenolic content was higher in the ethanolic than in the aqueous extracts. Rutin and chlorogenic acid were the most abundant phenols in the extracts. The richest in total phenols, flavonoids and phenolic acids was ethanolic mulberry leaf extract (154.51 ± 22.64 mg QE/g). The extracts have shown excellent antiradical activity, as well as the ability to inhibit beta-carotene degradation in presence of linoleic acid, with some being as active as the positive control, BHA. Chelating activity, on the other hand, was significantly weaker. alpha-glucosidase-inhibiting activity of the investigated extracts was excellent. The most remarkable example were the mulberry leaf extract, as well as yarrow extract. Their activity did not differ from the activity of acarbose. The presented results may explain the extent and popularity of the selected medicinal plants use as a supportive therapy of diabetes in Croatia.

Poster session-PO-13:

Preparation of B-ring saturated nontoxic protoflavones as novel xanthine oxidase inhibitors

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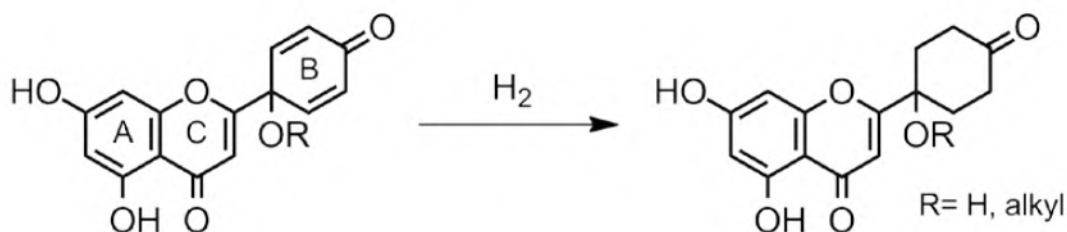
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Protoflavones express a non-aromatic, p-quinol B ring that confers them a unique 3D structure among natural flavonoids. Anticancer activity of these compounds is intensively studied, but their cytotoxicity is a

strong limitation for investigating their other possible uses [1].

Our group identified protoapigenone 1'-O-propargyl ether as the first non-planar flavonoid that is a strong inhibitor of xanthine oxidase (XO), however its cytotoxicity was similarly strong. XO plays a crucial role in the pathomechanism of gout, and it also significantly contributes to oxidative stress [2]. Recently, our group reported a selective method to saturate the protoflavone B ring of protoapigenone and its 1'-O-butyl ether through continuous flow chemical hydrogenation to obtain non-cytotoxic derivatives with the rare, naturally occurring tetrahydroprotoflavone moiety [3].



In our present work, we aimed to investigate the structure-activity relationships of differently substituted 1'-O-alkyl tetrahydroprotoapigenone derivatives concerning their potential to inhibit XO. We utilized both batch and continuous flow chemical approaches for the preparation. With an aim to investigate the isotope effects on the XO-inhibition protoapigenone analogues, selective deuteration of the B ring was also achieved. Our preliminary studies on the obtained derivatives indicated that saturation of the p-quinol B ring could not only eliminate the cytotoxicity, but it could also further increase the XO-inhibition potential of protoflavones as compared to their parental compounds. Related pharmacological studies are currently ongoing, and results of these will also be presented.

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[2] Hunyadi et al., *Tetrahedron Letters* (2013) 54 6529-6532.

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Acknowledgments

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Poster session-PO-14:

Camellia sinensis (L.) Kuntze Sekonder Metabolites and microRNA Content

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Camellia sinensis (L.) Kuntze, popularly known as tea, is the one of the oldest, most commonly consumed beverages after water. It is generally recommended to use two leaves and a bud cut off from the shoot tip in tea production. This is done to prevent the decrease of some chemical entities in the leaves as they age (Kacar 1997). Secondary metabolites in plants are produced for special purposes including natural defense and are called as xenobiotics when taken by humans through diet (Silva Pinto 2013). Tea chemistry is quite complicated. The biological effects of tea has been associated with active substances like tannin, flavonoids and alkaloids. In recent years, there are some very surprising studies that possibly suggest the association of microRNAs with plants' medicinal activities. It was reported that plant miRNAs might enter human bloodsystem and play an important role in gene expression (Xie et al 2017). In this study, it was aimed to investigate the relation between tea microRNAs and their possible role in human health.

Key words:

Tea, *Camellia sinensis*, microRNA, biological activity

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Poster session-PO-15:

***Salvia multicaulis* Vahl. versus *Salvia hispanica* L. – A Comparison**

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Salvia L., the largest genus of the family Lamiaceae, represents an enormous and cosmopolitan assemblage of nearly 1.000 species displaying a remarkable range of variation, of which 36 only occur in Europe. The

phytochemistry of the genus *Salvia* consists mainly of volatiles, fatty acids, flavonoids, terpenes among other secondary metabolites. Due to the wide occurrence, folk medicinal use of various species is well documented and known. An ancient staple crop “chia” (*S. hispanica* L.) has become a popular novel food, due to its valuable nutritional content, especially fatty acid composition. In this present study, *S. multicaulis* seeds were evaluated also for the fatty acid composition, total protein amount and swelling index among other properties, which is known to be consumed such as in Turkey. Linolenic acid (69.7%) and linoleic acid (19.7%) were the major fatty acids in chia oil. Chia seeds showed a relatively low n-6 PUFA/ n-3 PUFA ratio. On the contrary, *S. multicaulis* seed oil was rich in linoleic acid (68.9%), palmitic acid (10.3%) and oleic acid (14.1%), where the amount of linolenic acid was very low (1%), respectively. The results were compared with chia seeds, not only due its chemical composition but also for its antimicrobial, antioxidant as well as lipoxygenase inhibitory properties.

Poster session-PO-16:

Nanostructured Lipid Carriers of Silymarin: Design, Characterization and In Vitro Studies

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Silymarin is the main constituent of the extract from seeds of *Silybum marianum* L. Gaertn. and has been used for decades as hepatoprotectant. Recently it has been proposed to be beneficial in type 2 diabetes patients. However, silymarin is a poorly water soluble drug with limited oral bioavailability [1]. Nanoparticles-based delivery systems resulted a promising strategy to resolve these issues. In this work, nanostructured lipid carriers (NLCs) with two different lipid combinations were prepared through emulsion/evaporation/solidifying method [2]. Stearic acid:Capryol 90 (NLCs-SA) and cetyl palmitate:Lauroglycol 90 (NLCs-CP) were selected as lipid mixtures. Brij S20 was used as surfactant. The optimized formulations were of 210-270 nm in particle size and with zeta potential between -31 and -35 mV. Surface morphology was determined by TEM. NLCs showed good encapsulation efficiencies (80% for NLCs-SA and 93% for NLCs-CP). No degradation phenomena were observed in simulated gastrointestinal fluids. Storage stability of suspensions and lyophilized products was also investigated. Glucose and mannitol were selected as cryoprotectant agents for freeze drying and it was observed that glucose was superior to mannitol especially with regard to the physical stability. About 60% of silymarin was released in 24 h in PBS. Invitropermeation experiments with artificial membranes and Caco-2 cells revealed that both NLCs enhanced the permeation of entrapped

compound. Cellular uptake studies indicated that active processes are involved in the internalization of developed formulations.

Acknowledgments: Financial support was granted by Ente Cassa di Risparmio di Firenze 2016, financing n. 2016.0802.

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Poster session-PO-17:

A metabolomic approach to investigate metabolization of a medicinal plant extract in the gastrointestinal tract in-vitro

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Introduction

Metabolization of medicinal plant extracts in the gastrointestinal tract may lead to the formation of new active compounds and could therefore be highly relevant for the explanation of the mode of action. STW-5 is a well-known liquid fixed 9-herb combination, which is effective in treating functional dyspepsia and irritable bowel syndrome (IBS) [1] . It was pre-digested by a static in-vitro digestion method and thereafter incubated with human gut bacteria to mimic human digestion and microbial fermentation in the gastrointestinal tract.

Method

Two concentrations of STW-5 were predigested in-vitro according to the InfoGest consensus method [2]. After protein precipitation, all samples were analyzed by UHPLC-HRMS and data were processed with Compound Discoverer 2.1 (Thermo Fisher Scientific). In order to assess metabolization during in-vitro digestion, UHPLC chromatograms of samples taken after simulated digestion were compared to respective STW-5 dilutions.

For bacterial fermentation, fecal samples of one donor were anaerobically incubated with InfoGest predigested STW-5 as well as with non-predigested STW-5 (two concentrations). Samples were taken after 30min, 4h and 24h of incubation, and analyzed by UHPLC-HRMS. After data processing, peak areas were compared to respective STW-5 dilutions incubated with PBS-buffer only.

Results

The majority of STW-5 main constituents were not significantly changed by in-vitro digestion. Microbial fermentation, however, led to fast metabolization of the major compounds detected in STW-5, such as flavonoids, caffeic acid derivatives, and triterpene glycosides.

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Poster session-PO-18:

liWRKY34 transcription factor is essential for novel phenotypes of induced autotetraploid *Isatis indigotica* Fort

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Polyploidy is recognized as a prominent force shaping plant evolution. The autotetraploid *I. indigotica* with better yield, enhanced resistance and higher lignans accumulation had been obtained in our previous study. However, the causes of novel variation in autotetraploid *I. indigotica* are not well understood. In the present study, liWRKY34, which expresses significantly higher in tetraploid *I. indigotica* than in diploid progenitor, was screened as a major contributor to the control of quantitative traits. Over-expression and RNAi analysis in transgenic *I. indigotica* hairy roots demonstrated that liWRKY34 significantly improves root development, salt and drought tolerance, as well as the contents of lignans such as lariciresinol, which represents an important efficacious compound for the antiviral effect of *I. indigotica*. Integrated analysis of transcriptome and metabolome profiling demonstrated the far-reaching consequences of lignan pathway perturbations on carbon metabolism, phenylpropanoid biosynthesis, and hormone signal transduction, etc. Moreover, liWRKY34 interacts with li4CL3, a key catalytic enzyme involved in lignan pathway of *I. indigotica*. Meanwhile, liWRKY34 interacts with liNAC31, which is predicted as a regulatory factor of stress tolerance. Our study discloses a valuable mechanism for the significantly qualitative difference between tetraploid and diploid *I. indigotica* by liWRKY34, and manipulation of this gene should facilitate improvements in yield, stress tolerance and lignan accumulation in *I. indigotica* and other crops.

Cytotoxicity of natural halimane and labdane diterpenes by mitochondrial dysfunction in human lung cancer cells

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Medicinal plants from the *Plectranthus* genus are a valuable source of natural products such as diterpenes [1,2]. Mitochondrial dysfunctions (MD) have been associated with several pathologies such as ROS increase and uncontrolled *Mycobacterium tuberculosis* (Mtb) replication [3,4]. The electrochemical gradient produced by mitochondria generates the mitochondrial membrane potential (MMP), which is a key parameter for evaluating MD [4]. Previous works have reported the cytotoxicity of *Plectranthus* diterpenoids and pointed their potential against *M. smegmatis* [2,5]. In this work, diterpenoids from *P. ornatus* Codd. (previously isolated [1,2]) were evaluated for their cytotoxicity and for the mechanisms of cell death associated with MD in A549 cell line (human lung adenocarcinoma). One halimane HAL: (11R*,13E)-11-acetoxylhalima-5,13-dien-15-oic acid) and two labdane diterpenes PLEC: Plectornatine C and the MRC: 1,6-di-O-acetylforskolin:1,6-di-O-acetyl-9-deoxyforskolin (1:1). Our pioneer study showed that only HAL and PLEC were cytotoxic (IC_{50} =60 and 8 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively). Also, the ROS level observed after 1h was significantly higher ($p < 0.01$) with HAL and this effect was maintained for up to 48h. All compounds were able to decrease mtDNA copy number, but only HAL increased MMP and exhibited DNA damage of 8.78 lesions per 10 kb (ND5 region). In conclusion, HAL has a cytotoxic effect associated with MD on lung cancer cells, that may be further evaluated on the Mtb replication mechanism. Additional studies are ongoing, aiming to unveil the coexistence of tuberculosis and lung cancer that has remained controversial, since the middle of the 19th century.

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Prediction of specioside-drug interactions by its in vitro inhibition effect on human cytochrome P450 enzymes

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Background : Specioside (SP – Fig 1) is a glycosylated iridoid esterified with a phenylpropanoid moiety present in *Tabebuia* genus (Bignoniaceae). The hydroethanolic extract of *Tabebuia aurea* inhibit leukocyte infiltration and paw edema, reduce hemorrhage, myotoxicity and hydrogen peroxide production induced by *Bothrops neuwiedi* venom [1]. Besides, the outstanding pharmacological potential of SP is due its modulation on the Hsp90 protein activity, associated with the development of almost all the types of cancer [2]. Therefore, since SP is a drug candidate, it is important to evaluate its potential to interact with the metabolism of other drugs. In this context, the present work aimed to evaluate the in vitro inhibitory effect of SP on human CYP450 major isoforms to assess SP-drug interactions. **Methods :** Specific reactions catalyzed by CYP450 isoforms were monitored in the presence and absence of SP (100 μ M) employing human liver microsomes. The inhibition of each probe substrate metabolism was determined and IC₅₀ values were obtained for the CYP450 isoforms significantly inhibited. **Results :** SP at 100 μ M presents weak inhibitory potential for CYP1A2 (50% of inhibition). No significant inhibition by SP at 100 μ M was observed for CYP2C9 (25%), as well as for CYP2C19 (6%), CYP2D6 (5%), CYP2E1 (3%), and CYP3A4/5 (20% for midazolam 1'-hydroxylation and no inhibition for nifedipine oxidation). **Conclusion :** These results present important information about SP-drug interactions. The very low inhibitory potential of SP against the major CYP450 isoforms indicates that its concomitant intake with other drugs metabolized by these isoforms may not be a problem due to the low SP-drug interactions. **Acknowledgments :** We are grateful to FAPESP, CNPq and CAPES for financial support.

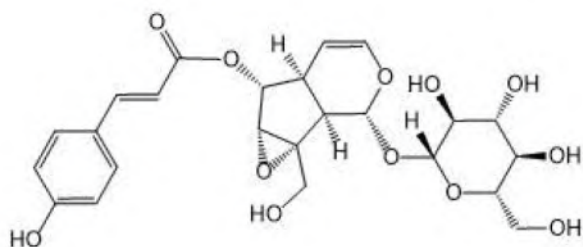


Fig 1. Chemical structure of specioside (SP)

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Poster session-PO-21:

Correlation of immuno-modulatory activity with beta-glucan content of medicinal mushrooms

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beta-Glucans are an important contributor to the biological activity of medicinal mushrooms including Reishi, Shiitake and Maitake. However, measuring the level of glucans is complicated by their molecular size, structural diversity, solubility and linkage specificity between alpha- and beta-glucans. The latter is important in assessing mushroom quality, as they are inherently low in alpha-glucans and rich in 1,3:1,6-beta-glucans. Enzymatic analysis of beta-glucans in mushrooms has become an accepted method of choice, however it has limitation with formulated products. An alternative is through bioassays that focus on immunomodulatory effects by quantifying the expression of cytokines in immune cells.

Nine different mushroom powders and extracts (0.1 mg/mL) were assayed for their effects on a suite of cytokines (IL-1alpha, IL-6 and TNF-alpha) in human macrophages (+/- LPS stimulation). We found with the exception of IL-1alpha, cytokine expression was up regulated for the majority of samples. That is, both TNF-alpha and IL-6 were up regulated in non-LPS-stimulated macrophages and for the most part up regulated for the majority of extracts and powders in LPS-stimulated macrophages. Some variation in cytokine expression was evident between the mushroom powders and extracts. We therefore determined the alpha- and beta-glucan content to assess if this correlated with cytokine expression.

beta-Glucan content across nine different samples ranged from 8-40% whilst the alpha-glucan content was lower (0.75-13.8%). A suite of growth media and formulation excipients invariably led to interferences with the glucan data. An inverse correlation between IL-1alpha expression and beta-glucan content was observed, however the correlations were inconsistent across the other cytokines monitored. Our data suggests that further investigations are essential to characterise the structural diversity of beta-glucan rich medicinal mushroom products, particularly with respect to detailed beta-glucan composition and how this correlates with specific immuno-modulatory responses.

Current pharmacopoeial method greatly underestimates procyanidins in Hawthorn (*Crataegus* spp.)

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Procyanidin content of Hawthorn berries sourced from either *Crataegus monogyna* Jacq. (Lindm.) or *C. laevigata* (Poir.) DC. is defined in the European Pharmacopoeia monograph[1]. Procyanidin content is determined by the HCl-butanol spectrophotometric method which has a long history of use. More recent work has shown that this method tends to underestimate the procyanidin content and can be improved by the addition of acetone to the solubilisation step [2]. More detailed data can also be obtained using thiolysis and subsequent HPLC-MS analysis [3].

Comparing a suite of commercial berry (6) and leaf (4) *C. monogyna* samples analysed by the BP method and the modified acetone-HCl-butanol method confirmed that the former underestimated procyanidin content by at least one order of magnitude. Leaf samples by the BP method ranged from 0.25-0.30% whilst the same samples gave 4.1-6.8% by the acetone-HCl-butanol modified method. Similarly, the procyanidin content of berry samples was in the range 0.035-0.23% by the BP/EP method but much higher by the modified acetone-HCl-butanol method (0.8-2.1%).

Results on the same suite of samples by the thiolysis-HPLC-MS method were consistent across the leaf samples (3.6-4.5% procyanidin), but significantly and consistently lower for the berry samples (0.2-1.5%). Thiolysis-HPLC-MS analysis also provides more detailed chemical information on the mean degree of polymerisation, procyanidin:prodelphinidin ratio, cis:trans flavan-3-ol ratio as well as information on various aspects of A-type procyanidins present.

We conclude that the current EP method greatly underestimate the concentration of procyanidins in *Crataegus* and suggest the method be revised.

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Poster session-PO-23:

Phenolic composition and antioxidant activities of *Porophyllum ruderale*

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Porophyllum ruderale (Pr), known in Brazil as “couve-cravinho”, is an aromatic herb widely distributed in Brazil and Latin America. Its leaves have a characteristic aroma and flavour and are used for food and medicinal purposes. Leaves of Pr were harvested at São Paulo state (Brazil), lyophilized, grinded, and extracted with methanol 80%. A phytochemical screening (HPLC-DAD) of this extract was performed, followed by evaluation of its antioxidant activity. Compounds identified by HPLC-DAD were caffeoylquinic acid derivatives and flavonoids (major constituents). The antioxidant activity was evaluated using several approaches including: free radical scavenging (DPPH), nitric oxide (NO) and superoxide scavenging (SOD), iron chelating activity (ICA), and ferric reducing antioxidant power (FRAP). Pr extract exhibited high antiradical activity in all methodologies used, namely DPPH, SOD, ICA, NO and FRAP with EC₅₀ values of 47.8, 120, 229, 10.5 and 133 µg/mL, respectively. We concluded that Pr leaf extract has good antioxidant properties, probably due to its high content in phenolics, showing potential to be used as functional food or functional ingredient.

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phenolic compounds, antioxidant activity, medicinal plants

Poster session-PO-24:

Comparison of NMR and LC-MS for metabolic profiling of Citrus-type crude drugs

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Introduction: The importance of quality evaluation and control of crude drugs using metabolomics is

increasingly recognized as a way of providing herbal medicines with predictably reliable quality and efficacy. In crude drugs, most metabolomics studies are conducted using a single analytical method. However, each analytical method has advantages and disadvantages that can influence the analytical coverage of the metabolome. In this study, we used both NMR and LC-MS analyses for a more thorough analysis of the components of Citrus-type crude drugs. Furthermore, we compared NMR analysis with LC-MS analysis for metabolic profiling.

Methods: Five Citrus-type crude drugs (39 samples); Kijitsu, Touhi, Chimpi, Kippi and Seihi, were used. The methanolic extracts of samples were analyzed using ^1H - and ^{13}C -NMR and High Resolution LC-ESI-MS. The NMR data were processed using the Alice2 for metabolome (JEOL). LC-MS data were processed using Progenesis (Waters). The resulting data sets were then imported into SIMCA ver. 14.0 (Umetrics) for further multivariate statistical analysis.

Results: The PCA score plots indicated the classification of the Citrus-type crude drugs into the same four groups in both NMR and LC-MS analyses. In the loading plots, differences were found in components contributing to the discrimination of the groups. The metabolites that were identified by the NMR and the LC-MS were three flavonoids such as naringin as contributors. The metabolites that were identified by the NMR only were primary metabolites; α - and β -glucose and sucrose, limonene and synephrine. However, the metabolites that were identified by the LC-MS only were three flavonoids such as hesperidin. The high dynamic range of the NMR provided broad coverage of the metabolome in a single experiment. LC-MS may be superior to detect secondary metabolites with high sensitivity. The combined NMR- and LC-MS-based metabolomics may provide useful information for the quality evaluation and control of crude drugs.

Poster session-PO-25:

Indigo naturalis suppresses proinflammatory Th1/Th17 responses in dextran sulfate sodium-induced murine colitis

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Ulcerative colitis (UC) is a disease that cause chronic inflammation in colon, characterized by abdominal pain and diarrhea mixed by bloody stool. Indigo naturalis (referred to as Qing-Dai), a traditional Chinese medicine, has been widely used as an anti-ulcerative colitis regimen in clinical practice of Chinese Medicine since 1960s (Feagan et al., 2013) . However, the precise mechanisms behind its efficacy remain unknown. In this

study, we aimed to investigate the effects of indigo naturalis on Th1/Th17 cell responses in UC. A dextran sulfate sodium (DSS)-induced colitis mouse model was established, and the clinical and immunologic modulations of indigo naturalis in this model were investigated. It was found that indigo naturalis attenuated DSS-induced colitis as represented by suppressing Th1 and Th17 cytokine profiles and associated genes expression, as well as reducing Th1 and Th17 cells in mesenteric lymph nodes. Indigo naturalis treatment also abated the expression of p-p38, p-ERK and p-JNK, and inhibited degradation of I κ B- α in the colon of DSS-treated mice. In vitro studies also showed that indigo naturalis suppressed Th1 and Th17 cells differentiation and inhibited the phosphorylation of STAT1 and STAT3 in T cell. These findings suggest that indigo naturalis could attenuate colonic damage by modulating Th1/Th17 cell responses and indicate that indigo naturalis is an effective regimen in treatment of inflammatory bowel disease.

Keywords : Indigo naturalis; Qing-Dai; Ulcerative colitis; Th1/Th17; Mucosal healing

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Poster session-PO-26:

Effect of *Dendrobium Huoshanensis* on Energy Metabolism and Anti-fatigue in mice with Kidney-Yin Deficiency

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Dendrobium Huoshanense (DH) is regarded as the best quality variety of *Dendrobium* and has been recorded in the classics of all past generations. However, due to the factors of its special growth conditions and over exploitation in the past, the yield of DH was less, leading to the lack of pharmacological research and the reduction of clinical application, and it has not been included in the Chinese Pharmacopoeia yet. With the improvement and increase of cultivation technique and yield of DH, it is necessary to strengthen the study of its modern pharmacology. According to the Chinese herbs property of DH about sweet, slightly cold and channel tropism of kidney and the basis of previous studies, the kidney-yin deficiency mice induced by thyroxine were used as the model. *Dendrobium candidum*, collected in the Chinese Pharmacopoeia, and Liuwei Dihuang pill, a clinical common medicine for treatment of kidney-yin deficiency, were used as

control drugs. After 14 days of administration, the general behaviors were observed. The thermotropism behaviors and weight-loading swimming time were recorded. The level of cAMP , cGMP , T3 and T4 in serum were measured by Enzyme immunoassay and radio immunity. The BLA,BUN,FFA,TG,CK,LDH in serum were detected by colorimetric methods. The hepatin, Na⁺,K⁺ATPase and Ca²⁺ATPase activities in liver were estimated by spectrophotometry. The experimental results show that DH can enhance the anti-fatigue function of kidney - yin deficiency mice, and its mechanism may be related to regulate the activities of energy metabolism enzymes, decrease metabolites accumulation, and then reduce unnecessary energy consumption, which is consistent with its TCM effect of nourishing yin and supplementing kidney. This study reveals the anti-fatigue effect of DH from the correlation of nature and efficacy, which is beneficial to promot rational clinical application and the development of health care products.

Key words:

Dendrobium Huoshanense(DH); Anti-Fatigue Effect; Energy Metabolism; Thyroxine; Kidney-Yin Deficiency

Poster session-PO-27:

Investigating mechanism of rapid screening the tyrosinase inhibitory activities by tandem mass spectrometry

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Mass spectrometry is the best and dominant technique for the analytical investigation of molecules and complex mixtures in modern life sciences and bio-analytical approaches. Especially, the high-resolution mass spectrometry (HRMS) that is important in determining the elemental composition of a molecule and in gaining partial structural insights using mass spectral fragmentations. Furthermore, mass spectral library searches to achieve the structure elucidation. And through the data processing and statistical analysis can understand the drug metabolic pathway.

Tyrosinase (EC 1.14.18.1), a copper-containing oxidoreductase, also commonly called polyphenol oxidase, has two catalytic activities: o-hydroxylation of monophenols and aerobic oxidation of o-diphenols. It is mainly involved in the formation of pigments such as melanins. The activity of mushroom tyrosinase can be measured by monitoring the conversion of phenolic compounds into quinone derivatives using the spectrophotometer. But this method cannot directly distinguish a single active compound.

In this aim, we used the inhibitor kojic acid treatment with tyrosinase. The reactant via ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) to analysis, software processed and statistical analysis. We found some ions in chromatography were from kojic acid treated with tyrosinase. Next step, we will use HRMS to elucidate the structure and clear the metabolic pathway in compound between tyrosinase.

This method is potential in finding the tyrosinase inhibitory activities compounds in complex natural product extract and accelerate the activities compounds finding in natural material research.

Keyword:

tyrosinase, HRMS, UPLC-MS

Poster session-PO-28:

Study on the Protective Effect of Simiao Pills on Vascular Endothelial Function in Hyperuricemia Model Rats Based on NOS Uncoupling

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The incidence of hyperuricemia is increasing year by year, and it is becoming younger, which brings great burden to family and society. In recent years, Chinese medicine has achieved certain results in the clinical treatment and basic experimental research in hyperuricemia. It has unique advantages, and has become a hot spot in the study of hyperuricemia. Chinese medicine has always had obvious advantages in the treatment of chronic diseases. Simiao pills can clear damp-heat, and it is common Chinese patent medicine, which is used to treat hyperuricemia and gouty arthritis. Rats were randomly divided into blank group, model group, benzbromarone group, and Simiao Pills group, a total of 4 groups. A hyperuricemia rat model was prepared with yeast extract 10g/(kg · d) combined with adenine 100mg/(kg · d). The rat serum and kidney tissues were collected for 14 consecutive days after successful modeling. The experimental results show that: Simiao Pills could reduce serum uric acid, increase the content of UUA, 24hUUA and UCr, reduce the content of Scr, BUN and XOD activity in hyperacidemia rat, inhibit the expression of URAT1 protein and enhance MRP4 and ABCG2 protein expression in kidney tissue of hyperuricemia rats. Simiao pills can not only inhibit uric acid production, but also promoted uric acid excretion by upregulating the expression of MRP4 and ABCG2 protein in renal tissue.

hyperuricemia ; MRP4; ABCG2; URAT1; Simiao pills

Acknowledgements: The National Natural Science Foundation of China(No. 81273632)

Poster session-PO-29:

Study on the effect and mechanism of SiMiao Pills on reducing serum uric acid in model rats with Hyperuricemia

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Hyperuricemia is a chronic disease that seriously threatens human health. Vascular endothelial dysfunction is an important early stage in the development of chronic diseases such as hypertension, metabolic syndrome, and cardiovascular disease. Elevation of serum uric acid and impairment of vascular endothelial function occur at the same time. NOS uncoupling is the key link of uric acid damaging to endothelial function. Therefore, the countermeasures against the eNOS uncoupling and/or triggering factors may be an effective means to protect the vascular endothelial function and prevent and treat vascular diseases in patients with hyperuricemia, which has become a research hotspot. Chinese medicine has always had obvious advantages in the treatment of chronic diseases. The Simiao Pills is a traditional Chinese medicine that treats hyperuricemia and gouty arthritis, and it can clear damp-heat. Rats were randomly divided into blank group, model group, benzbromarone group, and Simiao Pills group, a total of 4 groups. A hyperuricemia rat model was prepared with yeast extract 10g/(kg · d) combined with adenine 100mg/(kg · d). The rat serum and kidney tissues were collected for 14 consecutive days after successful modeling. The experimental results show that: Simiao Pills can reduce the serum levels of UA, ET-1, ROS, MDA in hyperuricemia model rats, increase serum NO, BH4 levels, can effectively up-regulate the expression of eNOS mRNA and down-regulate the expression of Nox4 mRNA in the aorta of hyperuricemia rats. Therefore, Simiao Pills can reduce the serum uric acid in rats with hyperuricemia and protect the vascular endothelial function. The mechanism may be related to the NOS uncoupling.

hyperuricemia ;NOS uncoupling; Endothelial function; Simiao pills

Acknowledgements: The National Natural Science Foundation of China(No. 81273632)

Poster session-PO-30:

Drying Effects on the Volatile Compounds of Kumquat, Limequat and Mexican Lime Fruits

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Citrus fruits are produced and used as in natura or juices in large scale around the World. These fruits are of great interest as raw materials for pharmaceutical, cosmetics and food industries due to their aromatic properties [1]. Residues of the production of citrus juice, such as peels, membranes, and seeds, possess a

large variety of phytochemicals [2]. In this study, it was aimed to prepare whole fruits powders of kumquat, limequat (a biogeneric hybrid) and mexican lime excluding the seeds. The volatile compound profiles were determined by two methods and also the effect of the heat treatment on was evaluated. Mature fruits were harvested from Subtropical Fruits Research and Experimental Center at Çukurova University, Adana in December 2017. The sliced mature and fresh fruits were freeze-dried. For the heat treatment, the sliced mature fruits were subjected to hot air drying in an oven at 130°C for 30 min. The obtained dried sliced fruits were then freeze-dried. Then, each sample was pulverized in a blender and passed through a 100 µ sieve to obtain powders. To trap volatile compounds in the each sample, Headspace-SPME technique was employed. The manual SPME device with a fiber precoated of a 65 µm thick layer of PDMS/DVB-blue was used. The extraction conditions at 40°C for 15 min. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds. Each sample was also subjected to microdistillation. The volatile compounds were analyzed by GC-FID and GC-MS, simultaneously. Limonene was found to be major in all samples. The effects of drying on the compositions were discussed.

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Poster session-PO-31:

Chemical constituents from branches of *Cercidiphyllum japonicum* Sieb. et Zucc

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Cercidiphyllum japonicum Sieb. et Zucc. also known as Katsura, is a tree in *Cercidiphyllum*, the sole genus of the family *Cercidiphyllaceae*. This species is well represented in the fossil record, with occurrence in the late Cretaceous and Tertiary of Europe and North America. Unfortunately, *C. japonicum* is currently only distributed in China, Japan and Korea, where its raw extracts have long been used in folk remedies to treat various of disorders or diseases. In the current work, chemical constituents of *C. japonicum* branches were studied for the first time and seven constituents were isolated, including a new galloylflavonol glycoside, namely 8-methoxykaempferol-4'-O-galloyl-3-O- α -L-rhamnopyranoside (VII), and six known phenolics [two anomeric galloyltannins (3,4,6-tri-O-galloyl- β -D-glucopyranoside (I) and 2,2',5-tri-O-galloyl- α / β -D-hamamelose (III)), one anomeric ellagitannin, pedunculagin (II), one flavonol, kaempferol (V), and two

flavonol derivatives (kaempferol-3-O- α -L-rhamnopyranoside (IV) and 8-methoxykaempferol (VI)). Structure elucidation of the extractives I~VII were conducted mainly on the basis of their spectroscopic (UV, IR, NMR, MS) and chemo-physical analysis, as well as by comparison with literature values. Compound I, II, IV and VI have not yet been reported from the genus *Cercidiphyllum*. Compound VII, a previously undescribed flavonoid, was isolated and elucidated in this work for the first time.

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Poster session-PO-32:

Alkaloids From *Hypecoum Procumbens* L.

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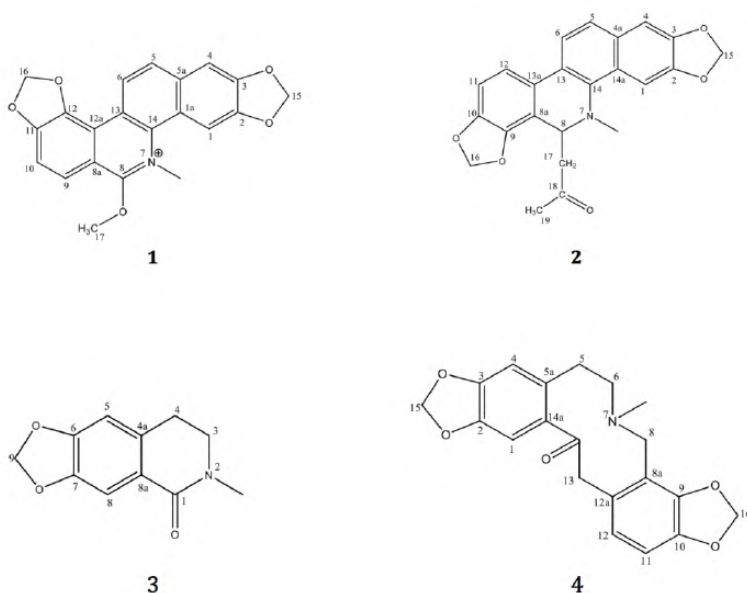
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In this study, the chemically investigation of *Hypecoum procumbens* L. was aimed. For this purpose, the plant material was soaked in EtOH for three weeks. Then EtOH extract acidificated and extracted with hexane after plant make basic and fractionated with dichloromethane, and ethyl acetate, respectively. Based on the results of TLC chromatography, it was observed that CH₂Cl₂ extract contain good amount of alkaloids in rich diversity. Several chromatographic methods have been used to isolate the alkaloids from this fraction.

Purification of dichloromethane extract of *Hypecoum procumbens* L. resulted in the isolation of four alkaloids including 8-methoxy-11,12-methylenedieter (1), 8-acetonyl dihydro sanguinarine (2), oxohydrastin (3), deoxy protopine (4). The structure determination of the compounds achieved by spectral methods such as IR, UV, ¹H-NMR, ¹³C-NMR, DEPT, HMBC, HSQC, COSY, ESI-MS.

The isolated compounds (1-4), were screened against AChE and BChE inhibitory activity. When tested against the AChE, compounds 1-4 displayed IC₅₀ values of 1.13 μ M, 1.80 μ M, 0.92 μ M, 0.88 μ M, respectively. The IC₅₀ values for BChE inhibition by compounds 1-4 were 6.81, 0.187, 4.9, 1.62 μ M, respectively. Standard inhibitor (galanthamine) exhibited AChE and BChE inhibition with IC₅₀ value of 3.5 μ M and 36.0 μ M, respectively.



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Poster session-PO-33:

Preparation of B-ring saturated nontoxic protoflavones as novel xanthine oxidase inhibitors

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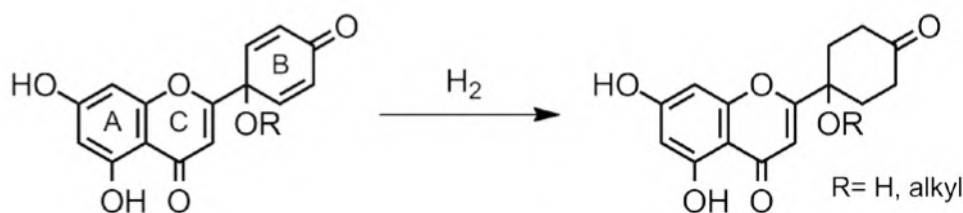
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Protoflavones express a non-aromatic, p-quinol B ring that confers them a unique 3D structure among natural flavonoids. Anticancer activity of these compounds is intensively studied, but their cytotoxicity is a strong limitation for investigating their other possible uses [1].

Our group identified protoapigenone 1'-O-propargyl ether as the first non-planar flavonoid that is a strong inhibitor of xanthine oxidase (XO), however its cytotoxicity was similarly strong. XO plays a crucial role in the pathomechanism of gout, and it also significantly contributes to oxidative stress [2]. Recently, our group reported a selective method to saturate the protoflavone B ring of protoapigenone and its 1'-O-butyl ether through continuous flow chemical hydrogenation to obtain non-cytotoxic derivatives with the rare, naturally

occurring tetrahydroprotoflavone moiety [3].



In our present work, we aimed to investigate the structure-activity relationships of differently substituted 1'-O-alkyl tetrahydroprotoapigenone derivatives concerning their potential to inhibit XO. We utilized both batch and continuous flow chemical approaches for the preparation. With an aim to investigate the isotope effects on the XO-inhibition protoapigenone analogues, selective deuteration of the B ring was also achieved. Our preliminary studies on the obtained derivatives indicated that saturation of the p-quinol B ring could not only eliminate the cytotoxicity, but it could also further increase the XO-inhibition potential of protoflavones as compared to their parental compounds. Related pharmacological studies are currently ongoing, and results of these will also be presented.

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Acknowledgments

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Poster session-PO-34:

In Vitro screening of Chinese edible mushrooms as antiobesity and antidiabetics with α -amylase and lipase inhibitory activities

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Obesity has become a major public global health concern. Pancreatic lipase plays important roles in the

digestion, transport and processing of dietary lipids in humans. Inhibition of pancreatic lipase leading to the decrease of lipid absorption may be used for treating obesity. The α -amylase inhibitors can prevent carbohydrate digestion or absorption can decrease calorie intake to promote weight loss and combat obesity. In order to find new pancreatic lipase and α -amylase inhibitors from mushrooms for the treatment of obesity, 18 different edible mushrooms families were investigated. Methanolic and water extracts of the plants were detected by using two in vitro testing systems. All the varieties of edible mushrooms reported to have inhibitory pancreatic lipase and α -amylase activities. Methanolic extract (95%) of Yellow silk mushroom showed strong anti-lipase activity (IC_{50} : $43 \pm 1.3 \mu\text{g/mL}$), the aqueous extract of Cordyceps mushroom shows high anti-amylase (IC_{50} : $7.42 \pm 1.03 \text{ mg/mL}$) activity. The results obtained indicated that the extracts of Yellow silk mushroom and Cordyceps mushroom could be good candidates for further studies.

Key words:

anti-obesity; anti-diabetes; pancreatic lipase inhibition; α -amylase inhibition; edible mushrooms

Poster session-PO-35:

Cytoprotective effect of Lycium extracts against oxidative insults

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Gogi berries are widely used in China both as food and as herbal medicines due to their variety of biological activities. Since oxidative stress is known to be a major cause of the development of several diseases, in this study we evaluated of the antioxidant potential of methanolic extracts of *Lycium ruthenicum* (Lr) and *Lycium barbarum* (Lb) in HepG2 cell line using t-BHP (0,5 mM) and paraquat (2 mM) as oxidative insults (4h). *Lycium* extracts, showed no significant cytotoxicity up to 100 $\mu\text{g/ml}$ and 24h incubation time with cells. Firstly, the extracts were pre-incubated for 2 and 6h before the application of t-BHP and paraquat, respectively. After, the extracts were pre-incubated for 20h before the application of insults. Cellular viability (MTT assay) was assessed immediately or 16h later, after oxidant insults. In short pre-incubation assays, Lr (100 $\mu\text{g/ml}$) showed significant cytoprotection against t-BHP and paraquat insults with 0,74- and 0,56-fold increases in cellular viability, respectively. In the long pre-incubation assay, both Lr and Lb showed significant protection against t-BHP toxicity with 9,9- and 1.7-fold increases in cellular viability at 100 $\mu\text{g/ml}$ and 250 $\mu\text{g/ml}$,

respectively. Using flow cytometry, Lr (100 µg/ml) triggered a significant decrease (less 38%) in apoptotic cells (FITC-Annexin) following long oxidant pre-incubation regimen. Additionally, in the short pre-incubation assay Lr showed a 0,55-fold decrease in reactive oxidative species (determined by DCFH-DA). Further studies, are necessary to understand the antioxidant and cytoprotective activities of Lr.

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Poster session-PO-37:

Determination of the content of Cyanidin-3-O-glucoside in extract of Açai with different processing methods by HPLC

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Açaí (*Euterpe oleracea* Mart.) is a round and purple well-known palm fruit in Brazil and a commonly used herb in South America. Modern studies have shown that Açai has various activities such as anti-oxidation, anti-inflammatory, anti-cancer and other activities. The Cyanidin-3-O-glucoside (C3G) is one of the anthocyanins were isolated from Açai. This work aimed to propose a analytical methods for the quantitative and qualitative analysis of Cyanidin-3-O-glucoside (C3G) in extract of Açai by HPLC, and to evaluate the different processing of extract of Açai. In this study, Açai was degreased with petroleum ether firstly, then we added ten times 80% ethanol to extract in ultrasound for three times and 1h for each time. The condition for concentrating-drying processing is that respectively freeze-drying, drying under reduced pressure at 50°C and drying at 100°C. The HPLC conditions is that Dikma Diamonsil C18 column (150mm × 4.6 mm, 5 µm) was used as chromatographic column to separate the C3G. by gradient elution using 0.5% phosphoric acid solution as mobile phase A and water: acetonitrile (50:50, V/V) as mobile phase B at a flow rate of 0.8 mL/min. The column temperature was set at 30°C and the detection wavelength at 520 nm. The results showed that, C3G basically reached the baseline separation with a good linearity when the concentration was 5.375 -172.000 µg/mL ($r > 0.9998$). The recovery rates were 99.84 % (RSD = 0.11 % , n=6). The C3G content and process transfer rate in extract of Açai with drying under reduced pressure at 50°C processing and freeze-drying processing were respectively 0.94 mg/g, 91.84% and 1.00mg/g, 97.86%. The C-3-G was not detected after drying at 100°C. These results indicated that, the method is simple and reliable, and may be used for the quality control of the extract of Açai, Which should be processed in low temperature environment. and Laying a foundation for further development and application of Açai.

[Key words] Açai ; Cyanidin-3-O-glucoside; HPLC; determination;different processing methods

Poster session-PO-38:

Antimicrobial natural products and their synthesized analogues from marine-derived fungus, *Trichoderma reesi*

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Marine organisms are taxonomically diverse yet unique, hence they are considered as a reservoir of potential bioactive secondary metabolites. Many of these marine secondary metabolites have already been successfully developed for medicinal usages. In our ongoing study to search new antimicrobial agents from marine resources by the established bioassays of antimicrobial and anti-biofilm formation activities, we have screened 65 crude extracts from marine invertebrates and marine-derived microbes toward *Staphylococcus aureus*. Among them, the extract of *Trichoderma reesi* showed apparent inhibitory effect against *S. aureus* by punch and paper disc plate tests. Furthermore, by the bioassay-guided fractionation isolation, a series of bioactive compounds, including 6-n-pentyl- α -pyrone, as well as a series of peptaibols, were isolated. We tried to synthesize a series of analogues of 6-n-pentyl- α -pyrone and evaluate their anti-microbial and anti-biofilm formation activities against *S. aureus*. In the poster, the structure-activity relationship of this type of compounds will be presented.

Poster session-PO-39:

Synthesis of 8-oxogeranial, a precursor of iridoid biosynthesis

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Iridoids are important natural products and the secoiridois derived thereof are universal precursors of all monoterpene indole alkaloids, including the anti-cancer drugs vinblastine and vincristine. Thus, the precise elucidation of their biosynthesis pathway is a significant step to maximize the biotechnological production of this natural product class. Iridoid synthases (IRIS) belong to the PRISEs among the SDR family of proteins and catalyze the decisive step in the biosynthesis of iridoids, namely the cyclization of 8-oxogeranial to nepetalactol, the common biosynthetic precursor for all iridoids [1]. 8-oxogeranial is not available commercially but can be synthesized chemically in four steps starting from geraniol [2]. We here optimized the synthesis of 8-oxogeranial. This allowed the investigation of the conversion of 8-oxogeranial

by various PRISE enzymes. The protocol involved the conversion of geraniol to geranyl acetate (steglich esterification; yield of 99 %). Microwave-assisted oxidation of the allyl group by selenium dioxide and flash column chromatography provided 8-oxogeranyl acetate (yield 42 %). The protecting group was removed by base-catalyzed hydrolysis to afford 8-oxogeraniol (yield 98 %). In the last step, swern oxidation and the subsequent column chromatographic treatment supplied the desired product 8-oxogeranial in a high purity with a yield of 50 %. The substrate was used to characterize recombinant forms of PRISEs from *Arabidopsis thaliana*, *Digitalis lanata* and *Plantago* species. The immediate availability of 8-oxogeranial will now help to elucidate still open questions in PRISE action, such as stereochemistry of bicyclic iridoid scaffold formation and mechanisms of substrate discrimination/ preferences of PRISES.

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Poster session-PO-40:

Systematic and Holistic Investigation of Pistacia Lentiscus Resin, A Unique Product With Exceptional Pharmacological Properties

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Chios mastic, is the resinous secretion obtained from the wounds of the trunk and branches of *P. lentiscus* L. var. *Chia*, which is endemic to the Greek island of Chios [1]. Since antiquity (500 BC), Chios Mastic has been well recorded for its medicinal and pharmaceutical properties. From 1997, Chios mastic has been identified as a product of Protected Designation of Origin (PDO) while cultivating mastic has been inscribed by UNESCO in 2014 in its Representative List of the Intangible Cultural Heritage of Humanity. In July 2015, mastic was recognized as a traditional

medicinal product by the European Medicines Agency (EMA) with two therapeutic indications (mild dyspeptic disorders & skin inflammation/ healing of minor wounds) [2]. In the frame of a continuation

study on *Pistacia* sp. an integrated, complementary bottom up approach has been designed. This approach includes isolation of active, marker compounds from starting material with fast and state-of-the-art techniques (CPC-UV, SFC-UV-MS); profiling and characterisation of composition via multiple analytical methods (HPTLC, HPLC-DAD, UPLC-HRMS & HRMS/MS & NMR); and validation of methods for quality control purposes. Additionally, pharmacokinetic characteristics of major mastic constituents have been determined after a human cohort and metabolomics approaches (LC-MS and NMR) have been implemented for revealing of biomarkers. The current work could be considered as an example of a complete workflow implemented in medicinal plants, from the natural entity to human organism.

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Poster session-PO-41:

Natural compounds with osteoprotective properties derived from Greek plant species

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Menopause-associated degenerative diseases such as osteoporosis, remain an increasingly significant problem affecting women in particular. Nowadays, numerous traditional herbal medicines are used worldwide for the treatment of osteoporosis and plant derived supplements are increasingly considered as a “natural” alternative to drugs in preventing post-menopausal disorders [1]. In this study, we exploited traditional medicine knowledge of ancient Greek doctors (e.g. Dioscorides) and Greek flora’s diversity for the detection of novel plant-derived selective estrogen receptor modulators (SERMs). To achieve this goal, 36 plant species were selected, based on traditional medicine sources and current literature [2] and 74 extracts were prepared and screened using well-validated cellular models of menopause-related diseases. Preliminary evaluation revealed 9 extracts (mainly obtained from plant species belonging to Leguminosae family) between the most promising osteoprotective candidates and were selected for isolation of their major constituents using several chromatographic techniques. In total, 62 compounds (terpenes, isoflavones,

phenolic acids, flavonols, flavones, coumestans, furanocoumarins, anthraquinones) were isolated and identified using NMR spectroscopy, whereas 5 of which were isolated for the first time. All these compounds were in vitro evaluated using a cell-based screening concerning: a) the differentiation of MC3T3-E1 cells to osteoblasts b) the inhibition of differentiation of RAW264.7 macrophages to osteoclasts c) their estrogenic properties using MCF-7 and Ishikawa cell lines. According to the combined results, phenolic acids (vanillic acid, gallic acid) terpenes (β -amyrin, lupeol, stigmastene), coumestans (coumestrol, 2- α , α -dimethylallylcoumestrol, isotrifoliol), coumarin and isoflavone derivatives exhibited potential osteoprotective properties.

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Poster session-PO-42:

Cytotoxicity of natural halimane and labdane diterpenes by mitochondrial dysfunction in human lung cancer cells

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Medicinal plants from the *Plectranthus* genus are a valuable source of natural products such as diterpenes [1,2]. Mitochondrial dysfunctions (MD) have been associated with several pathologies such as ROS increase and uncontrolled *Mycobacterium tuberculosis* (Mtb) replication [3,4]. The electrochemical gradient produced by mitochondria generates the mitochondrial membrane potential (MMP), which is a key parameter for evaluating MD [4]. Previous works have reported the cytotoxicity of *Plectranthus* diterpenoids and pointed their potential against *M. smegmatis* [2,5]. In this work, diterpenoids from *P. ornatus* Codd. (previously isolated [1,2]) were evaluated for their cytotoxicity and for the mechanisms of cell death

associated with MD in A549 cell line (human lung adenocarcinoma). One halimane HAL: (11R*,13E)-11-acetoxyhalima-5,13-dien-15-oic acid) and two labdane diterpenes PLEC: Plectornatine C and the MRC: 1,6-di-O-acetylforskolin:1,6-di-O-acetyl-9-deoxyforskolin (1:1). Our pioneer study showed that only HAL and PLEC were cytotoxic (IC_{50} =60 and 8 $\mu\text{g} \cdot \text{mL}^{-1}$, respectively). Also, the ROS level observed after 1h was significantly higher ($p < 0.01$) with HAL and this effect was maintained for up to 48h. All compounds were able to decrease mtDNA copy number, but only HAL increased MMP and exhibited DNA damage of 8.78 lesions per 10 kb (ND5 region). In conclusion, HAL has a cytotoxic effect associated with MD on lung cancer cells, that may be further evaluated on the Mtb replication mechanism. Additional studies are ongoing, aiming to unveil the coexistence of tuberculosis and lung cancer that has remained controversial, since the middle of the 19th century.

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Poster session-PO-43:

Semi-synthetic preparation of antitumor p-coumaric acid derivatives

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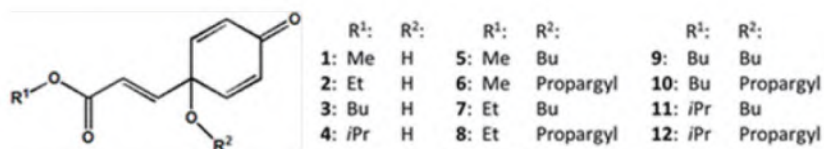
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In our previous experiments, a natural cinnamic acid derivative, graviquinone (1) was synthesized through the hypervalent iodine-catalyzed oxidative de-aromatization of p-coumaric acid methyl ester. Graviquinone proved to be a potent anticancer compound, similarly to the p-quinol flavonoid protoapigenone, expressing the same pharmacophore in its B-ring. Considering the promising pharmacological activity of 1'-O-alkyl protoflavones,^[1] the aim of the present study was to prepare a set of substituted graviquinone analogs, and to study their in vitro antitumor potential.

As a first step, various esters (methyl, ethyl, butyl, i-propyl) of p-coumaric acid were prepared with the corresponding alcohol and cc. sulfuric acid. The esters were then dissolved in acetonitrile:R-OH mixtures (9:1, v/v), and oxidation was performed with hypervalent iodine reagent PIDA or PIFA, to obtain compounds 1-12.

All compounds were purified with the help of combined chromatographic methods (Flash, HPLC), and their structures were confirmed by NMR spectroscopy.



The anticancer activity of the derivatives was investigated on a susceptible/multi-drug resistant mouse lymphoma cell line pair and on a human gynecological cancer cell line panel (HeLa, SiHa, MCF-7, MDA-MB-231). The compounds could efficiently bypass resistance conferred by the ABCB1 transporter. On the mouse lymphoma cell lines, 1 and 4 exerted very strong antiproliferative activity with sub-micromolar IC₅₀ values, while compound 3 was the most effective on the human cell lines with IC₅₀ values ranging from 1.7 to 5.8 μM.

Acknowledgements

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Poster session-PO-44:

Medicinal and Aromatic Plants by-products: Wastes or High-added value products with antioxidant properties?

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Southeastern European region is known for its rich biodiversity, especially in medicinal and aromatic plants (MAPs). Annually, significant quantities of cultivated and harvested wild MAPs are subjected to several processes to obtain essential oils and their commercially exploitable parts, yet resulting to a huge amount of by-products, such as herbal residues or hydrolats. As part of our research work, a systematic effort has been made over the last decade to exploit MAPs' by-products for the development of novel pharmaceuticals

and food supplements. Hence, within the framework of the European project “EXANDAS”, the production of bioactive extracts from Rose (*Rosa damascena*) and Lemon verbena (*Aloysia citrodora*) by-products has been investigated.

In more details, during the production of rose oil by hydrodistillation, significant amounts of waste water are produced and discarded in the fields. In this study, samples from different distillation apparatuses and production days were collected and processed with macroporous adsorption resin XAD-4 to produce enriched phenolic extracts with remarkable antioxidant activity. The most active extract was further studied using FCPC chromatography resulting to the identification of kaempferol and quercetin glucosides using NMR with LC-MS techniques. Furthermore, raw material of lower quality Lemon verbena leaves, obtained during herbal processing, was used to produce bioactive extracts. PLE was elaborated to maximise the content of acteoside, a phenylpropanoid glycoside with antioxidant and cytoprotective properties [1], through the optimisation of the extraction process in terms of temperature, ethanol-water ratio and extraction cycles. All extracts obtained from *Rosa damascena* and *Aloysia citrodora* by-products demonstrated high Total Phenolic Content (Folin-Ciocalteu method) and antioxidant potential (DPPH/ABTS assays), proving to become a readily available source of bioactive compounds for health nutrition and cosmetic industries.

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Poster session-PO-45:

Synthesis and biological activity of naringenin-oxime derivatives

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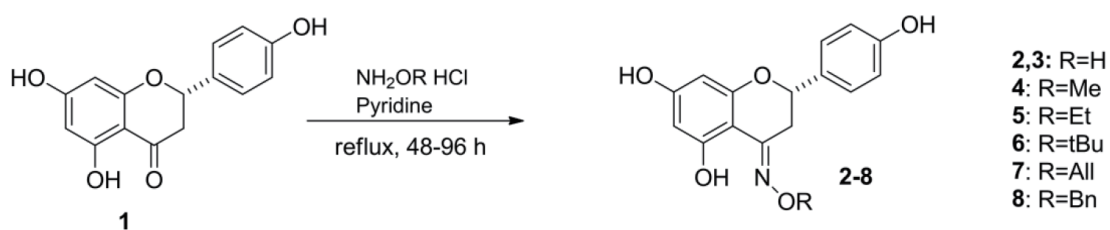
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Naringenin is one of the most abundant dietary flavonoids, predominantly found in citrus fruits and grapes. This compound exerts several pharmacological activities: its antioxidant, antiviral, anti-inflammatory, anti-carcinogenic and cardio-protective effects have been reported.[1-3] Due to its several beneficial biological activities, the design and synthesis of novel naringenin derivatives is of continuous interest.

During the current work our aim was to synthesize a compound library of oxime and oxime-ether derivatives of naringenin, and to test their in vitro antitumor activity on different cancer cell lines, as well as their antioxidant activity.

Seven compounds have been prepared. When using hydroxylamine as reagent, the formation of geometric isomers was observed, and the obtained E- and Z-oximes were separated by flash chromatography. On the other hand, stereospecific formation of the E-oxime ethers were observed with alkoxylamines. The structure of the prepared compounds was confirmed by NMR and HRMS measurements.



Antiproliferative activity of the prepared compounds was evaluated by MTT assay against human leukemia cells and a gynecological cancer cell line panel. Compound 6 exerted the most potent activity with IC_{50} values of 19.5, 23.5, 29.7, 31.8 and 35.4 μM against MCF-7, HeLa, MDA-MB-231, HL-60, and SiHa cell lines, respectively. Compound 6 was also found to exert apoptosis in HeLa cells through the activation of caspase-3. When testing the antioxidant activity of the compounds by ORAC and DPPH assays, compound 4 was found the most potent.

Acknowledgements

This work was supported by the National Research, Development and Innovation Office, Hungary (NKFIH; K119770 and K109293). A.H. acknowledges the János Bolyai fellowship of the Hungarian Academy of Sciences and the Kálmán Szász Prize.

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Poster session-PO-46:

New polyprenylated acylphloroglucinol derivatives from *Hypericum scabrum*

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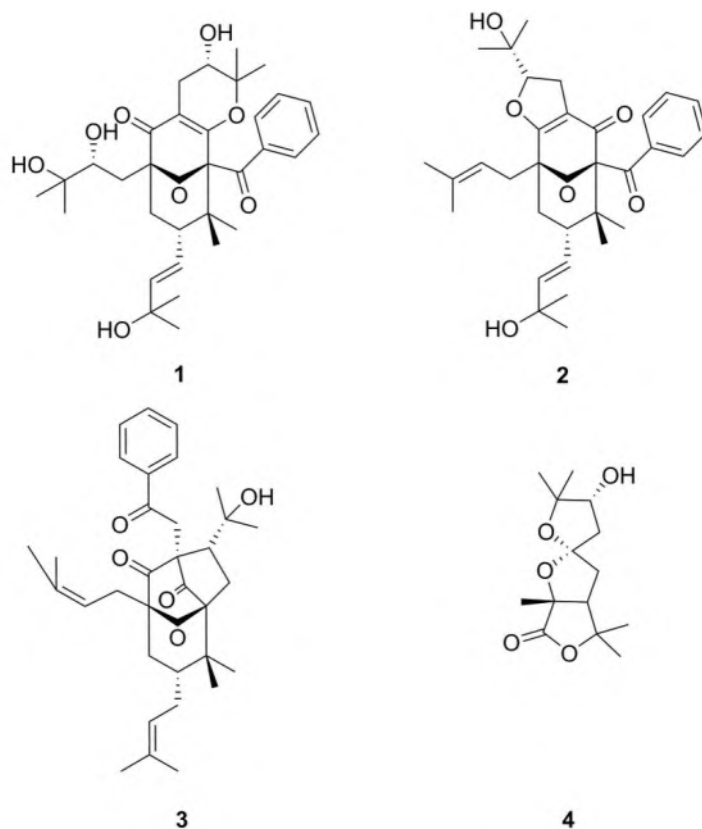
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Polycyclic polyprenylated acylphloroglucinols (PPAPs) are a structurally diverse group of natural products possessing neurotrophic and antiproliferative activities. PPAPs have been reported so far only from plants of the families Clusiaceae (Guttiferae) and Hypericaceae, and the majority has been isolated from the genus *Hypericum* [1-3]. In a systematic search for new PPAPs from Iranian *Hypericum* species we investigated *H. scabrum*. The species is commonly known in Persian as "Gol-e Raei deihimi", and is used

in traditional Iranian medicine for the treatment of wounds, gastric ulcers, and as an antiseptic, sedative, and antispasmodic drug. From the n-hexane extract of the aerial parts three (1-3) new polyprenylated acylphloroglucinol derivatives (1-3) and compound 4 were obtained. Isolation was achieved by a combination of column chromatography on silica gel, and preparative and semipreparative HPLC. The structures were established on the basis of extensive spectroscopic analysis, including 1D and 2D NMR, HRMS, and ECD for determination of absolute configurations. Compound 3 represents a new scaffold of PPAPs, and compound 4 possesses a new scaffold for natural products.



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Optimized methodology for the recovery of cannabinoids from fibre-type *Cannabis sativa* L.: Monitoring by UPLC-PDA

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Fibre-type *Cannabis sativa* L. (hemp) and related cannabinoids provide a considerable therapeutic potential, as revealed by on-going research [1]. More specifically, cannabidiol (CBD) and its acidic precursor cannabidiolic acid (CBDA) represent the major non-psychoactive cannabinoids in hemp, possessing numerous pharmacological properties [1]. Hence, an efficient extraction protocol followed by rapid and reliable quantification of CBD and CBDA is increasingly required. The aim of this work was the assessment of different extraction methodologies in terms of cannabinoid yield as well as the optimization of the extraction procedure based on the selected technique. Additionally, the development and validation of a simple and efficient analytical protocol employing ultra-high performance liquid chromatography coupled with photodiode array detection (UPLC-PDA) was also carried out to determine the principal cannabinoids. Samples of dried hemp inflorescences (*C. sativa* 'Futura 75') were studied by examining three different extraction techniques, namely ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and dynamic maceration (DM) [2]. As regards UAE, which proved to be the most promising technique, optimization of different parameters was performed to ensure the highest yield of CBDA & CBD by applying design of experiments (DoE) [3]. The variables investigated included sample-to-solvent ratio, time, and temperature. Appreciably higher recovery of cannabinoids was achieved by the optimized UAE method followed, enabling a simplified and more rapid extraction as compared to the recent literature [3]. The UPLC-PDA method developed herein is also suggested for the accurate and high-throughput determination of the major cannabinoids in fibre-type *C. sativa*.

P. Tzimas would like to thank Stavros Niarchos Foundation (SNF) for the financial support.

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Poster session-PO-48:

Abietane diterpenoids from *Plectranthus* spp. induce ROS-mediated cytotoxicity

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Plants from the *Plectranthus* genus are commonly known for their medicinal properties, and their applications are vast in traditional medicine [1,2]. Often accountable for these properties, bioactive secondary metabolites such as diterpenes are present in these plants, and some are cytotoxic. The abietane diterpenoids obtained from *Plectranthus* plants, 7 α -acetoxy-6 β -hydroxyroyleanone, 7 β ,6 β -dihydroxyroyleanone, 6,7-dehydroroyleanone, and Parvifloron D are examples of this class of bioactive diterpenes [3,4].

According with the previous studies, this work aimed to further evaluate the cytotoxic activity of these abietanes. This property was evaluated through MTT assays on A549 cells and CCRF-CEM cells. In addition, the level of intracellular Reactive Oxygen Species (ROS) in these cells was also measured. Our study has revealed that on CCRF-CEM cells, there is a slight increase of the ROS levels after exposure to all compounds. Nevertheless, on A549 cells the levels of ROS increase approximately 10-fold, with notorious increase after exposure to 7 β , 6 β -dihydroxyroyleanone, which can induce cytotoxicity due to ROS accumulation on cells. In conclusion, this study proves the therapeutic potential of *Plectranthus*-derived diterpenes and validates its use in traditional medicine.

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Poster session-PO-49:

Anti-diabetic Activity of Dendropanoxide Isolated from *Dendropanax moribifera* on Diabetic Zebrafish Models for Type 1 and 2

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Diabetes mellitus is a group of most common endocrine disease characterized by hyperglycemia. *Dendropanax morbifera* Leveille is a Korean endemic species in the family Araliaceae, and distributed in the south-western area and Jeju island, Korea. This plant has been used in traditional medicine for the treatment of headache, dysmenorrhea, infectious and skin diseases. We evaluated the anti-diabetic activity of the extract and solvent fractions of *D. morbifera* aerial part in zebrafish models for type 1 and 2 diabetes. Type 1 diabetic zebrafish model was induced by alloxan, which cause pancreatic β -cell necrosis. Type 2 diabetic zebrafish model was induced by insulin. Exposure to excess insulin can induce insulin resistance typical of type 2 diabetes. The CH₂Cl₂ fractions of *M. morbifera* which showed the strongest anti-diabetic activity was subjected to activity-guided fractionation, and dendropanoxide was isolated from the CH₂Cl₂ fraction. Dendropanoxide revealed potent ameliorative effect on pancreatic islet damage in zebrafish for type 1 and 2 diabetic models, respectively. The compound was investigated inhibitory activities on PTP1B, α -glucosidase and DPP-4. PTP1B was significantly inhibited by dendropanoxide.

Poster session-PO-50:

China's Medicine Road: A Material History of Medicinal Ingredients from the Southern Ocean

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University of Michigan, Ann Arbor, United States

The Tang (618-907 CE), Song (960-1279 CE), and Yuan (1271-1368 CE) periods witnessed a dramatic increase in the number of foreign medicinal ingredients entering China's written materia medica from abroad. Many ingredients that would emerge as essential to Chinese pharmacology by the Ming period (1368-1644 CE) were imported in these earlier centuries by sea at China's southern coast, and represent contributions of traditional medical systems from afar. An analysis of medicinal and organic materials recovered from shipwrecks dated from within this transition reveals a dynamic network of long-distance trade relationships that informed traditional Chinese medical practice. Examining recovered materials including star anise, Chinese olive, and multiple aromatic tree resins from the Belitung, Intan, and Java Sea shipwrecks within the evolution of Chinese materia medica and records of long-distance trade illuminates the role of the maritime trade routes in the Southern Ocean as China's premodern Medicine Road.

Poster session-PO-51:

Chamomile efficacy in pediatric ALL patients: a randomized placebo-controlled trial

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Background and Objectives:

Acute lymphoblastic leukemia (ALL) is the most common malignancy of childhood. Chemotherapy-induced neutropenia as a serious hematologic toxicity is one of the main treatment complications in these patients. We hypothesized that an herbal formulation of chamomile could be effective in management of neutropenia.

Methods:

A randomized triple-blind placebo controlled clinical trial was conducted with inclusion criteria of: 2-18 years old children, diagnosis of ALL (B-cell subtype), and admission in hospital. Those with sensitivity to chamomile were to be excluded. Participants in each group received 2.5 cc of either chamomile syrup or placebo syrup once daily for 30 days. Their white blood cell (WBC) count and absolute neutrophil count (ANC) was measured before and after the intervention as the primary outcome measures.

Results: A total number of 20 patients in each group (40 in total) finished the study. Comparison of longitudinal changes between the groups revealed higher level of WBC (P-value=.008) and ANC (P-value=.038) in the test group. No adverse effect was reported.

Conclusion :

Using chamomile syrup as a complementary treatment in pediatric oncology patients can improve their immunity and minimize their chemotherapy complications, specially neutropenia.

Matricaria recutita , Chamomile, Leukemia, Neutropenia, Integrative Oncology

Poster session-PO-52:

Arctium tomentosum and A.lappa from Sub-Carpatian Region of Poland. Comparison of antioxidant and enzyme inhibition activities, as well chemical composition

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As a source of *Bardanae radix* and *Bardanae folium* among other species *Arctium lappa* L. and *Arctium tomentosum* Mill. are mentioned. The plant materials in traditional medicine are used in gastrointestinal tract disorders, minor urinary tract complaints, as well in different skin diseases [1]. The aim of our study was determination and comparison of antioxidant and anti-inflammatory activities, and chemical composition of 70% ethanolic extracts of roots and aerial parts of above mentioned species collected from various positions in Sub-Carpatian Region of Poland. DPPH radicals, hydrogen peroxide, and superoxide anion scavenging activity of the tested extracts were evaluated. To examine the enzyme inhibitory activity of the studied extracts inhibition of lipoxidase and xanthine oxidase activity was tested. The range of the extracts concentration was: 10-250, 1-50, 5-125, and 200-500 µg/mL for DPPH, H₂O₂, O₂^{-•} and xanthine oxidase, and lipoxidase, respectively. All mentioned assays were made in in vitro cell-free systems. Studies of composition of the extracts were performed using HPLC coupled with DAD and Ion Trap Mass Detector. The content of total phenols was determined using colorimetric method. The results demonstrated the antioxidant activity of the tested extracts, while their enzymes inhibition activity was rather weak. Our studies showed that activity of extracts of *A.lappa* and of *A.tomentosum* are comparable. Also the chemical composition of both species is similar. The dominating compounds of aerial parts extracts are flavonoids - kaempferol and quercetin derivatives, while root extracts are rich in phenolic acid derivatives mainly caffeoylquinic acids and their conjugates. Determination of the polyphenols content showed that extracts from the roots contain more polyphenolic compounds than extracts from aerial parts. Therefore, the equivalent use of plant materials (roots and aerial parts) obtained from the two studied species seems to be justified.

1. EMA Community herbal monograph on *Arctium lappa* L., radix

Poster session-PO-53:

Wild Mushrooms, a Source for Bioactive Compounds

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Mushrooms (basidiocarps) are responsible for the production of sexual spores, which are the fundamental dispersal units of fungi. It is therefore not surprising that certain fungal species have evolved secondary metabolites to protect their fruiting bodies, e.g. against infections by widespread parasitic moulds, to ensure full development and distribution of their spores.

Field observations revealed that fruiting bodies of certain species of the genus *Hygrophorus* (Hygrophoraceae, Agaricales) are hardly ever attacked by mycophilic fungi. Consequently, several novel compound classes with pronounced biological activity were isolated from *Hygrophorus* spp. [1-3]. Compounds were examined for their antibacterial activity as well as against the human pathogenic fungus *Malassezia pachydermatis* (causing *Malassezia* Dermatitis) and the plant pathogenic fungi *Botrytis cinerea* (gray mold pathogen on many crops including strawberries and wine grapes) and *Septoria tritici* (causes septoria leaf blotch of wheat) as well as the oomycete *Phytophthora infestans* (causal agent of the late blight disease on potato and tomato) [2, 3]. Tested compounds exhibited pronounced activity against the pathogenes and may serve as lead structures for the development of novel fungicidal agents.

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Poster session-PO-54:

Effect of high hydrostatic pressure treatment on medicinal resources - petals of *Hypericum patulum* –

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Enzymatic reactions can be promoted by disrupting membrane structure using high-hydrostatic-pressure (HHP) treatment to obtain functional substances, because the enzyme protein component is not deactivated under HPP conditions . This makes it possible to control the metabolic response by HHP treatment of medicinal resources during HHP treatment, and thus, allows the examination of the effects of new functional constituents. Using this method, we have previously found that the HHP treatment of *Swertia japonica*, a traditional Japanese medicine, resulted in increased numbers of new functional constituents. In this study,

we investigate the change in the metabolism compounds of the petals of *Hypericum patulum* upon HHP treatment; these compounds have previously shown anti-allergic and anti-pruritic effects.

Fresh petals of the *H. patulum* (10 g) which had been stored in vacuum-packed pouches were treated by HHP of 0.1 (control), 200, 400 and 600 MPa for 10 min at 20 °C using an HHP device (KOBELCO). The treated samples were extracted with 50 mL methanol. Subsequent to the in vacuo evaporation of the methanol, the extracts were subjected to TLC and HPLC analyses.

The HHP treatment did not result in any appreciable changes to the outward appearance or the amounts of extracts. However, among the many spots observed in the TLC of the control extract, one major spot was absent following the HHP treatment at > 200 MPa. Upon comparison with spectroscopic methods, it was proposed that the missing structures belonged to a 1:2 mixture of catechin (1) and epicatechin (2). This present study indicates that the enzymatic reaction led to the metabolism of 1 and 2 by HHP treatment. The HPLC analyses and the change in the biological activities of the extracts are currently under investigation. HHP treatment is a promising technique to be applied in the development of new functions for medicinal herbs.

Poster session-PO-55:

Non-Cyanogenic cyanoglucoside derivatives from *Bauhinia holophylla* leaves possessing strong in vivo hypoglycemic activities

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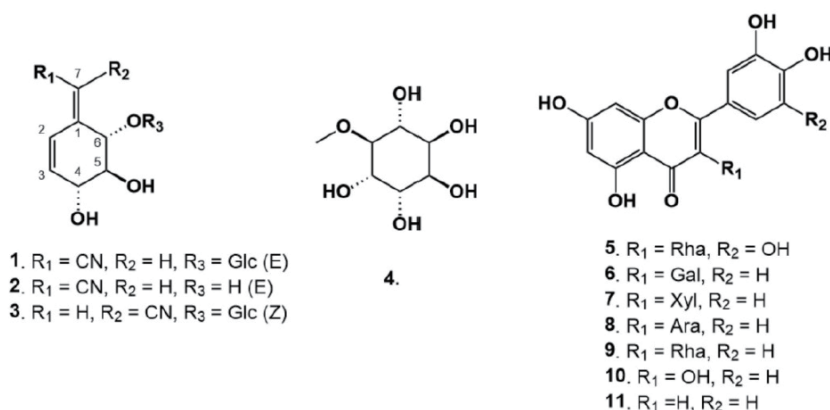
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The leaves of *Bauhinia holophylla*, also known as "pata-de-vaca", have been traditionally used in Brazil to treat diabetes.[1] While the hypoglycemic activity of the extract is known, the active compounds are not yet identified. To this end, the active hydroalcoholic extract was fractionated by medium pressure liquid chromatography bioguided by an in vivo glucose tolerance test in diabetic mice.[2] This led to the identification of three non-cyanogenic cyanoglucoside derivatives (1-3) and a series of glycosylated flavonoids (5-11). One of the main leaves constituent, lithospermoside (3), exhibited strong hypoglycemic activity. NMR quantitation revealed that the hydroalcoholic extract contains 1.4% of lithospermoside and 3.4% of flavonoids. Surprisingly this NMR profiling also showed the presence of a high amount of pinitol (4)

(12.5%), a cyclic polyol known to possess in vivo hypoglycemic activities. The glucose-lowering properties of the hydroalcoholic and traditional water infusion extracts of leaves of *B. holophylla* seems thus interestingly be the results of the cumulative/synergetic in vivo activities of three classes of unrelated compounds: glycosylated flavonoids (5-11), pinitol (4) and lithospermoside (3). These results support to some extent the traditional use of *Bauhinia holophylla* to treat diabetes.



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Poster session-PO-56:

Research of Changing of Tocopherol Content in Wheat during Germination

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The tocopherols and their derivatives - tocotrienols have the important meaning for the vital function of a body in total. Their sum is named «Vitamin E» which is a natural antioxidant and modulates the activity of several enzymes involved in neuromuscular transmission signals. Degenerative change in skeletal muscles and heart muscle are originated and the capillary permeability and fragility are increasing with a lack of vitamin E. The seeds of grain crops are natural sources of vitamin E and first the wheat. The germinated seeds of wheat are recommended to eat for the support of the vitamin E balance. The aim of this work was the research of changing of vitamin E content in wheat seeds in the process of their germination. The objects of research were the wheat grains which submerged for fixed time after this they were dried out very quickly. Then an oil extract was made using these dried seeds and liquid paraffin as a solvent.

Quantitative definition of the tocopherol sum was carried out by a spectrophotometry using absorption of UV-light on the characteristic wavelength of 292 nanometers in comparison with absorption of standard solution (see tab 1):

Tab 1 The content of tocopherol in the oil extracts C, %

The duration on germination, h	C
0	0,28±0,02
6	0,32±0,02
12	0,34±0,03
24	0,46±0,02
30	0,41±0,02
36	0,26±0,03
48	0,1±0,03
72	0,076±0,005
96	0,054±0,003

The similar dependence can be explained by the physiological processes proceeding in a seed - intensity of cell breath, which is necessary for development of germ, increases during an initial stage of ontogenesis. As a result, biosynthesis of tocopherols has to begin with using reserve substances of seed.

In conclusion we can say the content of vitamin E reaches the maximum later 24 hours after the beginning of germination.

Poster session-PO-57:

Research of Cardiac Glycosides in Herb Raw Material of *Convallaria Majalis* and *Polygonatum Odoratum*

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Medicines of cardiac glycosides are irreplaceable at in the chronic heart failure treatment especially if the violation of a heart rhythm is a concomitant disease. Nowadays only herbs are the sources of their manufacturing. This group of biologically active compounds is rather rare and is found only in limited number of plant families including the family Convallariaceae.

In this family there are two very widespread plants: a lily of the valley (*Convallaria majalis* L.) and a solomon's seal (*Polygonatum odoratum* Druce). Raw materials of *C.majalis* are used long since in scientific medicine as a source of cardiac glycosides of strophanthine group, mainly the convallatoxinum. At the same time *P.odoratum* is considered inadmissible impurity in raw material of *C.majalis*. The aim of this work was the comparative research of cardiac glycosides in *P.odoratum* and *C.majalis*.

The leaves of both plants which were collected in stage of flowering, were used as the research objects. The collected leaves were dried up, crushed and then the extraction has been taken from the researched samples with using 70% ethanol.

The TLC method with using solvent system - ethyl acetate:ethanol:water (81:11:8) and standard samples of cardiac glycosides has help to establish that the convallatoxinum contains in both plants.

Quantitative determination of convallatoxinum amount has been carried out by two methods:

- spectrophotometry - by using absorption of UV-light with the wavelength of 494 nanometers by a chromogen complex which has been formed by addition picric acid in the researched solution;
- by common HPLC method.

As a result it has been established that the convallatoxinum amount determined by photometry was 0,025% and 0,035%; by HPLC method – 0,021% and 0,028% for *C.majalis* and *P.odoratum* respectively.

In conclusion that the leaves of *Polygonatum odoratum* are disposable herb raw material for manufacturing cardiac glycosides of *Strophantus* group.

Poster session-PO-58:

Attenuation of *Pseudomonas aeruginosa* Virulence by *Terminalia catappa* leaves and *Artocarpus altilis* flower Ethanolic Extracts

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One strategy to address the emergence of infectious diseases supported by the increasing cases of microbial antibiotic resistance is the development of anti-pathogenic drugs, a quorum quenching compound(s) capable of inhibiting microbial communication (quorum sensing inhibitor) (1,2). Previous anti-infective studies on Indonesian medicinal plants have focused mainly on anti-microbial drug discovery perspectives. However, no systemic effort has been made to explore the anti-quorum sensing activity of these plants. Our objective is to investigate several Indonesian medicinal plants' ethanol extracts inhibitory activities against QS-mediated virulence factors in *P. aeruginosa*. Indonesian medicinal plant ethanolic extracts were tested for their capability to inhibit *P. aeruginosa* motility, biofilm formation using microtiter plate method, pyocyanin and LasA production using LasA staphylolytic assay. Statistical significance of the data were determined using one way ANOVA, followed by Dunnett's test. Ethanolic extract of *T. catappa* leaves and *A. altilis* flower capable to inhibit *P. aeruginosa* motility as well as pyocyanin production and biofilm formation. Both extracts also showed capability in reducing LasA protease production. *T. catappa* and *A. altilis* are an interesting sources of innovative plant derived quorum quenching compound(s), thus can be used in the development of new antipathogenic drug.

ethanol extract, anti-pathogenic drugs, quorum quenching, quorum sensing inhibitor, *Pseudomonas aeruginosa*

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Poster session-PO-59:

Chemical composition, antioxidant and anti-inflammatory activity in human neutrophils of *Gaultheria procumbens* stem extracts

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Gaultheria procumbens L. (American wintergreen, Ericaceae) is an evergreen shrub native to North America and commonly used in traditional medicine as anti-inflammatory, analgesic and antipyretic drug [1]. The active wintergreen components are salicylates, but also other polyphenols, i.e. flavonoids, proanthocyanidins and monocaffeoylquinic acids [2,3]. The plant parts used for medicinal purposes are leaves, stems and fruits, among which stems are the least characterized both in terms of chemical composition and biological activity. Therefore, the present study was conducted for thorough phytochemical profiling of *G. procumbens* stem extracts and measuring their anti-inflammatory and antioxidant effects in model of human neutrophils.

The dry extracts were prepared by direct extraction of the plant material with the use of five solvents of different polarity. The first stage was the UHPLC-PDA-ESI-MS3 qualitative analysis, that led to the full or tentative structural identification of over forty phenolic constituents. The quantitative standardization was conducted by HPLC-PDA-fingerprinting and by spectrophotometric determination of total polyphenolic (183.7-347.8 mg GAE/g dw) and total proanthocyanidin (51.6-241.6 mg CyE/g dw) contents. In the next stage, the influence of the extract richest in polyphenols on pro-inflammatory and pro-oxidant functions of neutrophils stimulated with LPS and f-MLP was examined in the release tests of elastase, matrix metalloproteinase and pro-inflammatory cytokines, i.e. interleukins IL-8, IL-1 β , TNF- α , as well as in the model of oxidative burst. The results showed that the *G. procumbens* stem extracts are rich source of structurally divers polyphenols, especially proanthocyanidins and gaultherin, and exhibit significant anti-inflammatory and antioxidant activity in in vitro cellular models.

Acknowledgements : This work was financially supported by National Science Centre, Poland (Grant Project: 2015/19/N/NZ7/00959).

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Poster session-PO-60:

The Cytotoxic Activity on T47D Breast Cancer Cell of Isoflavones of tempeh -A Fermented Product of Soybean (Glycine Max)

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As fermented soybean (Glycine max), tempeh has been reported as a good source of isoflavones, which have shown anticancer activities in various cancer cells resulted from their activity as phytoestrogens. The exploration of cytotoxic effect of tempeh on T47D cells employing 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) resulted in IC₅₀ values of 196.066 ± 15.956 µg/mL (1). In this research, we investigated the cytotoxic activity of isoflavones of tempeh such as daidzein and genistein and also their glucoside forms daidzin and daidzein on T47D breast cancer cell. In vitro experiments using MTT method showed that daidzein and genistein had potent cytotoxicity activity with IC₅₀ values were 14.53 and 31.71 µM, respectively, whereas their glucoside forms had no cytotoxicity activity. (1) Yuliani S. H, Istyastono E. P, Riswanto F. D. O. The Cytotoxic Activity on T47D Breast Cancer Cell of Genistein-Standardized Ethanolic Extract of tempeh -A Fermented Product of Soybean (Glycine Max). *Orient J Chem* 2016;32(3).

Poster session-PO-61:

Disturbing the *P.aeruginosa* biofilm architecture by natural products: an original strategy to synergize infectious diseases treatments

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Antibiotic resistance constitutes a serious threat in healthcare. Across the world, antibiotic resistance has reached an unsafe level and, if the situation doesn't change, even benign infections will reveal dangerous. The increase and spread of antibiotic resistance need to be urgently solved. The WHO provides a priority list for bacteria antibiotic resistance, divided into three groups according to the urgent need for new treatments. *Pseudomonas aeruginosa* is part of the group 1: « critical » level. *P. aeruginosa*, a gram negative, opportunistic pathogenic bacteria, is a major cause of various infections because of its ability to form biofilms in several environments. In this context, the aim of this work is to discover new natural antimicrobial agents with original modes of action. The strategy we propose relies on the disruption of the biofilm architecture to improve the penetration of antimicrobial agents. Thanks to a CDC bioreactor system, we generated biofilms imitating *in vivo* conditions and characterized their architecture using electron microscopy. The effects of antimicrobial agents have been studied (i) on biofilm architecture using microfluidic cells and (ii) on biofilm formation using Nunc TSP plates. From these data, we intend to develop a new, easier and faster method to measure the disruption of biofilm architecture based on electrical potentials measurements. The use of selected mutant strains and known modulators of QS systems will allow to elucidate the mechanism and targets of discovered natural molecules active towards the biofilms.

Poster session-PO-62:

Screening of Spanish endophytic actinomycetes and investigation of *Actinomycetospora* sp. for novel molecules with skin-whitening activity

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In order to explore the potential of endophytic actinomycetes to produce secondary metabolites with applications in the cosmeceutical industry, 138 potential candidate endophytic actinomycetes originated from Spanish biodiverse arid areas were selected to be studied. In order to broadly express the production of different secondary metabolites, OSMAC approach was applied and four nutritional conditions have been used for the cultivation of the selected strains in liquid media. In total 552 extracts have been generated and cell-free and cell-based bioassays have been incorporated for the evaluation of their skin-whitening activity. In parallel cytotoxicity evaluation was performed to the selected bioactive extracts in order to exclude those that exert cytotoxic effects.

The novel actinomycete strain CA287887 was selected as the most promising and was cultivated in large scale using the optimum fermentation medium (MO16). The scaled-up fermentation broth (3lt) was extracted with acetone (3lt). The active extract was loaded on a column packed with SP207SS reversed-phase brominated resin, while the aqueous phase was concentrated until dryness (aqueous extract). The loaded column was eluted using a linear gradient from 10 to 100% acetone in water. Bioassays revealed the active fractions, while the aqueous extract demonstrated the most significant activity. Sequential preparative & semi-preparative HPLC fractionations and liquid-liquid extraction with EtOAc were performed in order to purify the bioactive metabolites from the active fractions and the aqueous extract respectively.

The full set of spectroscopic data (MS and NMR) were recorded for all isolated compounds in order to unambiguously elucidate their structure. Special attention was given to the isolated alkaloid derivatives Cyclo-(histidine-proline) (C₁₁H₁₄N₄O₂) and Cyclo(Pro-Tyr) (C₁₄H₁₆N₂O₃) due to their remarkable whitening activity and to one novel alkaloid with molecular formula C₃₀H₄₉N₇O₁₀. Therefore, this can serve as a proof of concept that microbial ingredients can have successful applications in cosmeceutical industry.

Poster session-PO-63:

Differentiation of *Vaccinium* SPP. L. by NMR Similarity Analysis For Quality Assessment and Adulteration Detection

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Traditionally, *Vaccinium* spp. plant material has been used by indigenous cultures of the Circumboreal Region for treating a variety of disease conditions.¹ Evaluation of the constituents in extracts from various species show variation in the quantities of the bioactive compounds.² The variation between species and the high hybridization of *Vaccinium* spp. accentuates the need for quality control of extracts to support safety and expected bioactivity of samples from these plants. In our earlier work,^{3,4} it was established that NMR provided a robust and reproducible technique for quality evaluation of *Vaccinium* spp. L. (Blueberries, Cranberries, and Bilberries) of the Ericaceae family. With a NMR fingerprint approach quality of material was established using a simultaneous targeted and non-targeted analysis approach for quantification of key metabolites and classification against species dependent models. Here, rather than use a traditional SIMCA outlier detection model for identification of the plant material and quantification of specific components, we explore the use of various similarity algorithms for material assessment. Correlation coefficients for similarity of the entire spectra, spectral contrast, and barcode match are assessed on various species of NMR spectra from a worldwide *Vaccinium* spp. collection⁵ that includes *V. angustifolium*, *V. boreale*, *V. corymbosum*, *V.*

macrocarpon, *V. myrtilloides*, *V. myrtillus*, *V. ovalifolium*, and *V. uliginosum*. Similarity comparisons preclude the need for the collection of a large number of samples for model development.

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Poster session-PO-64:

A general method for the detection of toxic pyrrolizidine alkaloids? Investigation of qPCR for measuring DNA adduction

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Pyrrolizidine alkaloids (PAs) are toxic natural products that present a major health concern. 1-2 unsaturated PAs can be bioactivated by CYP450 enzymes to yield pyrrole derivatives, which are involved in adverse effects, notably hepatic veno-occlusions, but also proved to be genotoxic. Even though these toxicities have been described since the 70's, regulatory agencies only recently acknowledged the scope of the problem, encouraging efforts to collect more data on pyrrolizidine alkaloids in traditional herbal medicines, homeopathic medicines, and food and dietary supplements. However, there are still no universal methods able to detect all toxic PAs with high sensitivity.

Current PAs detection methods are mostly based on either HPLC-MS/MS or GC-MS. Nonetheless, even with state-of-the-art technological refinements, it is still difficult to detect and quantify every 1-2 unsaturated PAs in herb or food samples. As those approaches seem to reach stalemate, our work describes a new qPCR-based method of detection, combining the potential adduct-mediated genotoxicity of 1-2 unsaturated PAs with the adduct-sensing capability of qPCR.

This approach consists in incubating specifically-designed synthetic double-strand oligonucleotides with chemically activated PAs, in order to yield adducted-DNA. The whole process follows a simple 4-steps plan: (i) SPE-SCX extraction of PAs from samples, (ii) chemical-metabolization to adducting moieties, (iii) incubation of oligonucleotides and alkaloid extracts and (iv) the qPCR itself.

Using retrorsine as standard, we are able to distinguish a 5 μ M solution from a suitable blank, using a 48H, 67°C incubation of ds oligonucleotides. Optimization is in progress, aiming to decrease incubation time to a practical overnight and to lower the limit of detection to 0.5 μ M in order to match HMPC/EMA skip testing requirements.

Poster session-PO-65:

New benzophenone and anti-inflammatory constituents of *Hypericum sampsonii*

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Abstract:

Hypericum sampsonii Hance, also known as Sampson St. John's wort (元寶草), is traditionally used for the treatment of backache, burns, diarrhea, snakebites, and swelling [1]. A new benzophenone, 4-geranyloxy-6-isoprenyloxy-2-hydroxybenzophenone (1), has been isolated from the aerial part of *H. sampsonii*, together with 4 known compounds 2-methoxyxanthone (2), 1-hydroxy-7-methoxyxanthone (3), sampsonione J (4), and 2,4,6-trihydroxybenzophenone 4-O-geranyl ether (5). The structure of new compound 1 was determined through spectroscopic and MS analyses. Among the isolated compounds, sampsonione J (3) inhibited NO production with IC_{50} of 15.0 μ M in LPS-stimulated RAW 264.7 cells. In addition, 2-methoxyxanthone (2), 1-hydroxy-7-methoxyxanthone (3), and sampsonione J (4) could suppress LPS-induced NO production in RAW264.7 macrophages and did not induce cytotoxicity against RAW 264.7 cells after 24-hour treatment. Our study suggests *H. sampsonii* and its isolates may deserve further investigation for the treatment or prevention of various inflammatory diseases.

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Poster session-PO-66:

Temulawak: Source to Patient natural medicine development program

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Introduction:

Curcuma xanthorrhiza, also known as Temulawak, is traditionally used in Indonesian Jamu medicine to treat a number of health conditions including hepatitis, liver disorders, rheumatism, and skin inflammation.

In addition to curcumin, Temulawak also contains bioactive oils, the most abundant is xanthorrhizol which is unique to this species. The aim of this study was to develop a range of clinically proven anti-inflammatory products based on standardized extracts of Temulawak with source to patient controlled cultivation, that aligned to the traditional usage of the plant in Jamu medicine and that could deliver medically relevant benefits to patients for the treatment and prevention of diseases.

Materials and Methods:

i) Extraction methodology trials using various solvents to determine relative yields of curcuminoids and volatile oil based compounds. ii) Formulation and bioavailability studies to examine the plasma concentrations of key bioactive compounds. iii) Human clinical trial to determine the safety profile and whole genome effects of Temulawak on mRNA expression.

Results and Conclusion:

We have optimized the cultivation and extraction method of the two key active constituents of Temulawak enabling the production of a standardized extract with characteristics suitable for a solid dose formulation. In a first of its kind study we evaluated the safety profile of the standardized extract in 32 healthy subjects and examined the full human genome mRNA expression patterns in peripheral blood. We identified a large number of pathways associated with inflammation and innate immune signaling that were significantly modified after Temulawak treatment. We also demonstrated that curcuminoid and xanthorrhizol separately showed anti-inflammatory activity in cell models of inflammation. The Temulawak formulation has been optimized to improve bioavailability of both xanthorrhizol and curcumin and preclinical studies are now underway to evaluate the efficacy on selected models of inflammatory diseases

Poster session-PO-67:

Antioxidant and Immunosuppressive activities of Extracts of Endophytic fungi isolated from *Psidium guajava* and *Newbouldia laevis*

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Generation of reactive oxygen has been implicated in the pathogenesis of cancer and other diseases

(Halliwell and Gutteridge, 1999). Four endophytic fungi PGS1, NLS2 and PGL3, NLL3 were isolated using standard methods from the stem and leaf of *Psidium guajava* and *Newbouldia laevis* respectively. The fungal secondary metabolites were extracted with ethyl acetate after solid state fermentation of the isolated fungi on rice media for 21 days at 22°C. The antioxidant activity of the extracts were evaluated in vitro using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay and their IC₅₀ were calculated using quercetin as the standard. The immunomodulatory activity of the extracts were also evaluated by cyclophosphamide induced myelosuppression. The extracts were subjected to High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) analysis for the detection bioactive components. Two of the endophytic fungi (PGS1 and NLL3) at 100 mg/ml significantly reduced the total White Blood Cells count of the mice after five days treatment of the mice with the extracts $p < 0.005$. The crude extracts of PGS1 and NLL3 scavenged the DPPH with percentage inhibition of 69.84 and 76.58 % and gave the highest observed antioxidant activity in this study with IC₅₀ of 44.1 and 41.1 µg/ml respectively. The significant antioxidant activity of these extracts explain the strong suppressive activity of the extract. There was a perfect correlation between antioxidant and immunosuppressive activities. The HPLC-DAD analysis revealed the presence of Protocatechuic acid, beauvericin, catechin and asteric acid previously reported to have antioxidant and immunosuppressant properties. Extracts of endophytic fungi isolated from these *P.guajava* and *N.laevis* possesses antioxidant and immunosuppressive activities supporting a great therapeutic potential and a wide range of clinical applications.

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Poster session-PO-68:

Protective effects of Ginkgo biloba (GK501) and Panax ginseng (G115) extracts in primary neuronal cultures exposed to excitotoxic insults.

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Introduction:

Many neurological diseases are associated with excitotoxicity and increased glutamate levels in the CNS. Several studies show that Ginkgo biloba and Panax ginseng demonstrate neuroprotective and antioxidants properties without undesirable side effects [1]. The aim of this study was to investigate the protective effect

of extracts of Ginkgo biloba (GK501) and Panax ginseng (G115) alone or in combination in rat organotypic hippocampal slice cultures and mixed cortical cells exposed to excitotoxic insults.

Materials and Methods:

G115 and GK501 alone or in combination were tested in two experimental models of primary cultures exposed to excitotoxicity. Rat organotypic hippocampal slices were exposed to either 5 μ M kainic acid or 10 μ M N-Methyl-D-aspartate (NMDA) for 24hr and cell death in the CA3 or CA1 subregions quantified using propidium iodide fluorescence [2]. Murine mixed cortical cells were exposed to 300 μ M NMDA (10mins) and at 24hr cell damage was evaluated by measuring levels of lactate dehydrogenase [3].

Results and Conclusion:

No adverse effect or injury was observed in the hippocampal slices or the mixed cortical cells when exposed to GK501 and G115 extracts alone or in combination. However, when present in the incubation medium during NMDA or kainate insult a dose-dependent neuroprotective effect was observed that reached maximal significance at 0.01mg/ml for G115, 0.017mg/ml for GK501 and 0.027mg/ml for the combination for both the hippocampal slices and mixed cortical cells. Our results suggest that Ginkgo biloba (GK501) and Panax ginseng (G115) extracts alone or in combination have neuroprotective effects that were increased when in combination. These results suggest a role of this combination as a novel potential approach for the treatment and prevention of neurodegenerative diseases.

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Poster session-PO-69:

Investigation of the in vivo oral acute toxicity and genotoxicity of Chios mastic gum in male Wistar rats.

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Chios mastic gum (CMG), the resin of Pistacia lentiscus L. var. Chia, is a product of Protected Designation of Origin (PDO) and of major financial importance in Greece. It presents increasing interest in the global

nutraceutical and cosmeceutical market, as scientific evidence about its biological effects continuously grows [1]. However, little is known concerning its potential toxicity, especially after extended or high dose consumption [2,3]. In this study the acute toxicity and genotoxicity of CMG were investigated.

CMG was orally administered by gavage to rats at 2000mg/kg b.w. (OECD 423 guideline). The bioavailability of the resin was ensured after the unambiguous detection of its characteristic triterpenic acids in plasma by UHPLC-HRMS/MS. No signs of toxicity, mortality or adverse effects in terms of body weight changes and gross organ pathology were observed. Histopathological analysis of the liver, was also carried out. Ongoing HRMS-based metabolomics analysis in plasma, urine and liver is expected to produce valuable information regarding the metabolic pathways possibly triggered or suppressed after oral administration of CMG.

The CMG genotoxic potential was investigated in vivo using the Mammalian Erythrocyte Micronucleus Test (OECD 474 guideline). CMG was orally administered to rats at 2000mg/kg b.w. for three consecutive days. The administered doses were well tolerated by the rats and no signs of toxicity were observed. The characteristic mastic triterpenic acids were unambiguously detected in bone marrow, one and two hours after administration. The analysis of the results as regards the frequency of micronucleated polychromatic erythrocytes is still in progress aiming to bridge the scientific information gap in relation to the genotoxicity of CMG, not studied before.

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Poster session-PO-70:

Respiratory protective and therapeutic effect of *Salvia plebeia* in combination with *Panax ginseng*

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Air pollutants increase an airway inflammation during the arrival at the alveolar. Here, we investigated protective effects of *Salvia plebeia* extracts in the airway inflammation and synergistic effect of *Salvia plebeia* and *Panax ginseng* (Korean red ginseng) that has been used to treat various immune diseases including asthma

To evaluate the anti-inflammatory activity of *Salvia plebeia*, we measured the inhibitory effect of *Salvia plebeia* extract on cyteinly leukotriene and reactive oxygen species (ROS) production, inflammatory

mediators expression, and immune cell infiltration in RBL2K3 mast cells and MH-S alveola macrophage cells and Ambient Particulate Matter (APM)-exposed airway inflammation mice model. The *Salvia plebeia* extract inhibited the production of ROS and expression of IL-4, IL-10, and IL-15 mRNA in APM-stimulated MH-S cells. Oral administration of *Salvia plebeia* extract suppressed the APM-induced inflammatory symptoms such as alveolar wall thickness, collagen fibers, as well as decrease of mRNA expression of chemokines (CCR9, CCL5, CCR3), inflammatory cytokines (IL-15, TNF- α), and IL-4 Th2 cytokine in lung. *Salvia plebeia* extract also inhibited chemical induced ear edema and bronchoconstriction using animal model. Finally we confirmed respiratory protective and therapeutic effect by combined treatment with *Salvia plebeia* and Korean red ginseng using animal inhalation toxicity study and human clinical trial.

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Poster session-PO-71:

Pharmacognostic evaluation and HPLC-UV analysis of gedunin content in MAMA Decoction, an herbal antimalarial preparation

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MAMA Decoction is an herbal preparation that is used ethnomedically in Nigeria and has been scientifically justified for the treatment of malaria [1]. There is the need to standardise the preparation due to the possibilities of adulteration and deterioration. Therefore, this study aimed to determine some pharmacognostical and physicochemical parameters with a view to contributing to the quality control of the preparation.

The component leaves of MAMA Decoction, *Mangifera indica* L. (Anacardiaceae), *Azadirachta indica* A. Juss (Meliaceae), *Morinda lucida* Benth (Rubiaceae) and *Alstonia boonei* De Wild (Apocynaceae), were collected, authenticated and powdered. The mixture of the leaves was extracted using the decoction method. The filtrate was freeze-dried into a powdered extract. The organoleptic evaluation of the leaves and decoction was determined. The moisture content, the extractive yield, pH and relative density of the decoction were also determined. Qualitative and quantitative assessments of gedunin in MAMA Decoction and the freeze-dried extract were determined by thin layer chromatography and a validated high pressure liquid

chromatography assay, respectively.

The moisture content was 6% - 16%, extractive yield was 18.95 ± 3.10 % , while the pH and relative density of the decoction were 5.44 ± 0.12 and 1.01 ± 0.003 g/mL , respectively. The marker, gedunin with retardation factor of 0.49, was present at 0.73 µg/mL in the Decoction and 0.22 µg/100 µg in the freeze-dried extract.

The study provided some valuable data needed for the quality control of MAMA decoction.

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Poster session-PO-72:

Active substances in coffee charcoal (*Coffea Arabica* L.) contribute to the cytokine/chemokine-inhibiting activity of the herbal preparation

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Coffee charcoal is described as the milled, roasted to blackening outer seed parts of green dried *Coffea Arabica* L. fruits and was introduced into medical practice by August G. Heisler in 1937. Within a traditional herbal medicinal product (Myrrhinil-Intest®) it is used for the treatment of intestinal disorders. Previous pharmacological studies revealed significant effects of a coffee charcoal extract on cytokine/chemokine signalling in human macrophages.

The present investigation aimed to identify potential active components in coffee charcoal and to test their effects on cytokine/chemokine release from activated THP-1 cells.

An HPLC/LC-MS method was developed and utilised to determine UV-detectable substances in an aqueous coffee charcoal extract. Their effect on cytokine (TNF α ; IL-6) and chemokine (MCP-1) release from LPS-challenged human macrophages (THP-1) was investigated using ELISA . Budesonide served as positive control and concomitant cytotoxicity testing was conducted via MTT assay.

HPLC/LC-MS analysis detected the presence of trigonelline, caffeine, chlorogenic acid (3-CQA) and its isomers cryptochlorogenic (4-CQA) and neochlorogenic acid (5-CQA) as well as feruloylquinic acid derivatives in the coffee charcoal extract.

Cryptochlorogenic acid (4-CQA) led to an inhibition of cytokine TNF α (IC_{50} =20.7µM) and IL-6 (IC_{50} =43.3µM) as well as chemokine MCP-1 (IC_{50} : 4-CQA=13.9µM) release. 3-CQA, 5-CQA and caffeine were less effective (3-CQA: IC_{50} -TNF α =51.0µM; 5-CQA: IC_{50} -TNF α =14.3µM; caffeine: IC_{50} -TNF α =14µM; no influence on IL6 or MCP-1).

The present study revealed that pharmacologically active components in coffee charcoal affect cytokine and chemokine release from activated macrophages to a varying extent and thus contribute to the anti-inflammatory activity of the herbal substance. These data reinforce the use of coffee charcoal for the treatment of inflammatory intestinal disorders and suggests the application of 4-CQA as active marker for the anti-inflammatory activity.

Poster session-PO-73:

Botanical Drug Approval in the U.S. -A 15 Year Retrospective of the US FDA Botanical Review Team

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Botanicals are important sources of new drug discovery and development. As of December 2017, FDA received over 700 botanical investigational new drug applications (IND) and pre-IND meeting requests (PIND), with increased submission rates in the last several years. To date, two botanical new drug applications have been approved in the U.S., Veregen® in 2006 and Fulyzaq® in 2012.

In this presentation, we discuss our experience with these INDs containing a wide variety of botanical raw materials (single vs. multiple herbs), having various previous human experience, and originating from broad geographic regions. The data show that the indications for these proposed products have great diversity from cancer prevention/treatment, to mitigating common warts, to pain relief. One third of the indications were for oncology products. Given the inherent chemical and biological complexity of these products, ensuring therapeutic and quality consistency is often a challenge.

With accumulated experience and knowledge on botanical drug products, FDA revised the Botanical Drug Development-Guidance for Industry in December of 2016. The guidance was expanded to address the challenging issues for later phase trial and provided further recommendations to better facilitate botanical drug development by taking a holistic “Totality-of-Evidence” approach. This talk will highlight the implementing guidelines to our regulatory review practice.

Poster session-PO-74:

Synergistic antiproliferative effects of 10- hydroxy-2-decenoic acid co-treatment with doxorubicin against MCF- 7 breast cancer cells

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Combination chemotherapy is being increasingly explored due to its potential superior effectiveness. 10-Hydroxy-2-decenoic acid (10- H2DA), a special fatty acid from royal jelly, has been reported to have antitumor activity. This study investigated antiproliferative effects and underlying mechanisms of 10-H2DA treatments in combination with doxorubicin (DXR), a potent chemotherapeutic compound, against MCF-7 breast cancer cells. By MTS tetrazolium assay, co-treatment of 10-H2DA (0.0125-125 µg/mL) with DXR (1 µM) for 24h significantly inhibited MCF-7 cell proliferation in a variable dose-dependent manner ($p < 0.05$). The highest dose of 125 µg/mL 10-H2DA co-treatment maximally decreased cell proliferation by 71%, whereas DXR treatment alone decreased by 42%, compared to the medium control. Western blot analysis revealed that DXR treatment alone moderately decreased levels of c-MYC (0.3-fold), BCL2/BAX (0.3-fold) and cyclin B1 (0.4-fold) while slightly decreasing CDK4 (0.8-fold); however, it slightly increased cyclin D1 (1.2-fold). In comparison to DXR treatment alone, 125 µg/mL 10-H2DA co-treatment moderately decreased levels of c-MYC (0.4-fold), BCL2/BAX (0.4-fold), cyclin D1 (0.4-fold), and CDK4 (0.3-fold), but extensively increased cyclin B1(3.9-fold). Our research demonstrated 125 µg/mL 10-H2DA co-treatment synergistically enhanced the antiproliferative effects of DXR against MCF-7 breast cancer cells through reduction of c-MYC, the oncoprotein responsible for stimulating cell growth; induction of cell cycle arrest, especially at G0/G1 phases through inhibition of cyclin D1 and CDK4; and induction of apoptosis through inhibition of BCL2/BAX. Further, in vivo studies are required to verify the potential of 10-H2DA for chemotherapy.

Poster session-PO-75:

Lignans from Forsythia x intermedia leaves and flowers attenuate the pro-inflammatory function of neutrophils

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Forsythia fruits, which are usually obtained from *F. suspensa* (Thumb) Vahl and *F. viridissima* Lindley, are known in Asia as diuretic, hypotensive, anti-allergic, anti-inflammatory, antipyretic, anti-infective and antidote agents. In Europe, these plants do not form fruits and, in Europe, more attention is paid to the leaves and flowers as a source of valuable compounds [1]. The present study we demonstrated that leaves and flowers of *Forsythia x intermedia* are source of lignans which were able to mediate pro-inflammatory function of neutrophils.

Using bio-guided fractionation, we isolated the active compounds and determined their biological activity on human neutrophil model. We examined: (I) cytotoxicity, (II) expression of adhesion molecules CD11a/CD18 and CD11b/CD18, (III) phosphorylation level of p38, ERK1/2 and JNK MAP kinases and (IV) pro-inflammatory cytokine release (IL-8, IL-1 β and TNF α).

Cytotoxicity of lignans was determined by a standard flow cytometric probe using propidium iodide staining. The expression of adhesion molecules CD11a and CD11b was analyzed with flow cytometry. Phosphorylation of p38, ERK1/2 and JNK MAPK was determined by immunoblotting analysis. The effect on chemokines production was measured by enzyme-linked immunosorbent assay (ELISA).

The bio-guided fractionation led to the isolation of the following lignan aglycones: (+) pinoresinol, (+)-epipinoresinol, (-)-matairesinol, (+)-phillygenin and (-)-arctigenin. Compounds significantly decreased the surface expression of CD11a and CD11b at 50 μ M. Moreover all lignans significantly inhibited TNF- α and IL-1 β production at 10 μ M, probably by attenuating the p38 and ERK kinase pathways. The lignans did not inhibit of interleukin 8 release.

Conclusion: Forsythia x intermedia is a valuable source of active lignans, which may be potential candidates for treating inflammatory diseases that are associated with the excessive production of cytokines such as TNF- α and IL-1 β .

Acknowledgments

This study was financially supported by research grant 2015/17/B/NZ7/03086 from the Polish National Science Center.

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Poster session-PO-76:

Investigating Marine Invertebrates and Microorganisms for The Discovery of Novel Skin Anti-Aging Compounds

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In the frame of TASC MAR, an H2020 EU-funded project, more than 280 invertebrates and 400 microorganisms were collected from the under-investigated mesophotic zone (between 30 and 100 meters'

depth) of the Indian ocean, the Red sea and the Mediterranean.

The invertebrates were lyophilized and extracted by Accelerated Solvent Extraction (ASE) methodology using a mixture of dichloromethane and methanol (50:50, v/v). The microorganisms were cultivated under solid and liquid conditions in Marine Broth in 1L scale, using in-situ solid phase extraction technology and they were extracted with AcOEt and MeOH . All 1370 extracts were evaluated for their anti-aging and skin-whitening activity using elastase and tyrosinase enzymatic assays.

According to the results for the invertebrates; 12.4% presented activity against elastase and 16.3% against tyrosinase and 0.5% of the microorganisms showed tyrosinase and elastase inhibitory activity.

Six invertebrates (Iotrochota sp.1, Iotrochota sp.2, Biemna sp., Terpios sp., Pseudoceratina sp., Thrinacophora sp.) and one microorganism (Cladosporium halotolerans) were selected for further investigation. HRMS techniques were employed in order to investigate the metabolites present in each extract. Thus various analytical chemistry techniques and software, such as the R package xMSannotator using a custom library from the Dictionary of Natural Product and a combining strategy of Molecular Networking and In silico MS/MS fragmentation pipeline using the Universal Natural Products Database, were employed for dereplication .

The extracts were fractionated by Medium Pressure Liquid Chromatography (MPLC), Flash Chromatography or Centrifugal Partition Chromatography (CPC) using various solvent systems. All the fractions were further investigated for their biological activity. Bio-guided selection of the most active fractions was followed by metabolic profiling, structure elucidation and isolation of the high added value marine compounds by NMR and LC-MS techniques.

Acknowledgement:

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Poster session-PO-77:

Extensive Phytochemical Investigation of Bioactive Compounds Isolated from *Jatropha pelargoniifolia* Roots Native to Saudi Arabia

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Jatropha pelargoniifolia Courb. is a shrub indigenous on the Arabian Peninsula and widely known as

“Obab” . The plant has been used in traditional medicine. Sap of the petiole is applied to ulcers, severe skin inflammation and for wound healing [1]. Thus, it was of interest to explore the active constituents and biological importance for titled plant. Previous phytochemical investigation, by our research group, for the biologically-active fractions of *J. pelargoniiifolia* root, resulted in isolation of β -sitosterol, β -sitosterol glucoside, curcusons C&D, naringenin, apigenin, hovetricoside C, cynaroside, linarin, propacin, cleomiscosins A&B and uracil (2). Further extensive chromatographic investigation into other fractions of the titled plant afforded more nine compounds. Two of them were isolated here for the first time from nature and their structures were verified as 6-hydroxy-8-methoxy coumarin-7-O- β -D-glycopyranoside (1) and (3-(2-(methylamino) ethyl)-1H-indol-2-yl) methanol (2). The rest of compounds were found to be indol derivative such as N-methyltryptamine (3), phenylethylamine derivatives, N-methyltyramine (4) hordenine (5), and their salts (6, 7), the latter five compounds have been isolated here for the first time from Euphorbiaceous plants. The remaining two compounds are identified as diterpene congeners; spruceanol (8) and jatropha diketone (9) previously identified in various *Jatropha* species. The structures of the isolated compounds were unambiguously determined using physical data and different spectroscopic techniques including, HRESIMS, 1D & 2D-NMR. The anti-inflammatory, antinociceptive, antipyretic and antioxidant activities were evaluated for some isolated compounds which are available in good yields and some showed promising activities.

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Poster session-PO-78:

Associated-extraction efficiency of various cyclodextrins on five isoflavonoids in *Puerariae Lobatae Radix*

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Puerariae Lobatae Radix (PLR), a well-known herbal medicine, is the root of *Pueraria lobata* that has been employed for the treatment and prevention of cardiovascular and cerebrovascular diseases. The purpose of this study was to compare the associated-extraction efficiency of 8 various cyclodextrins on five isoflavonoids, including puerarin, daidzein, daidzin, genistein and genistin, which were the major chemical

components with low water solubility in *Puerariae Lobatae Radix*. Eight various cyclodextrins were applied, including α -cyclodextrin (α -CD), β -cyclodextrin (β -CD), γ -cyclodextrin (γ -CD), hydroxypropyl- β -cyclodextrin (HP- β -CD), hydroxypropyl- γ -cyclodextrin (HP- γ -CD), methyl- β -cyclodextrin (Me- β -CD), carboxymethyl- β -cyclodextrin (CM- β -CD), and sulfobutyl ether β -cyclodextrin (SBE- β -CD). High performance liquid chromatography (HPLC) was established to analyze five flavonoids in various CD extracts and traditional aqueous extracts. Associated-extraction efficiency of various CDs followed the ranking: SBE- β -CD > HP- β -CD > Me- β -CD > CM- β -CD > HP- γ -CD > γ -CD > β -CD > α -CD. Obviously, SBE- β -CD presented the highest associated-extraction capability, and it was applied to extract the five isoflavonoids from three products of PLR, including raw product, stir-frying product, and simmering product with wheat bran. The results showed that SBE- β -CD could improve the extraction capability of isoflavonoids both in raw product and processed products of PLR. In conclusion, CDs, especially SBE- β -CD, has a promising application for associated extraction of isoflavonoids in PLR.

Poster session-PO-79:

Annona muricata leaf extract triggered intrinsic apoptotic pathway to attenuate cancerous features of triple-negative breast cancer MDA-MB-231 cells

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Triple-negative breast cancer (TNBC) is known as an uncommon type of breast cancer, but it seems to be the most aggressive among the subtypes that have been classified so far. Furthermore, the major obstacle in breast cancer therapeutic strategies is metastasis, which is the leading causes of patients' deaths. Over the last few decades, multiple studies revealed the anticancer activity of *Annona muricata* L., known as Graviola, against various types of cancers, however, no study has analyzed its in-depth mechanism on the TNBC model so far. The present study investigated possible effects of aqueous extract of Graviola, on breast cancer in vitro, specifically focused on searching for the candidate against TNBC. In this study, we selected the TNBC MDA-MB-231 cell line as the main test model and the ER(+) MCF-7 breast cancer cell line as the control. Cell viability significantly decreased in both cells within 48-hour treatment with Graviola leaf extract, whereas impaired cell migration and invasion could be only found in MDA-MB-231. Graviola also triggered the intrinsic apoptotic pathway in MDA-MB-231 cells, which are different from the known mechanism of ER-dependent apoptosis in MCF-7 [1].

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Poster session-PO-80:

Screening of ethnomedicines used by the indigenous communities of Bangladesh for anticancer activity

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This study documented the information of significant ethnomedicinal plants from the traditional healers of three indigenous communities of Bangladesh. The documented data were quantitatively analyzed for the first time in this area. The benefits, importance and coverage of ethnomedicine were expressed through several quantitative indices including Informant Consensus Factor, Use Value, Frequency of Citation, Relative Frequency of Citation and Relative Importance Index. The agreement of homogeneity between the present and previous studies and among the indigenous communities was evaluated using Jaccard Index. A total of 159 ethnomedicinal plant species were documented from 174 informants. Importantly, 16 species were reported with new therapeutic uses and to our knowledge, 7 species described herein have never been ethnobotanically and pharmacologically studied. The present study showed that traditional treatments using medicinal plants is still widespread in the study area. Documentation of new ethnomedicinal species with their therapeutic uses shall promote further phytochemical and pharmacological investigations and possibly, lead to the development of new drugs. Further to the identification of suitable lead species, present study investigated the anti-cancer effects of the selected species using in-vitro and in-vivo studies. MTT assays on a panel of cancer cell lines have yielded promising preliminary results. In parallel, fractionated extracts of the test species are undergoing testing for cytotoxic activity. These experiments represent the first formal investigation of the chosen species. These positive results corroborate the ethnomedicinal use of the selected plant and represent a starting point for subsequent investigations. Extracts which showed promising cytotoxicity will be investigated in more detail concerning (1) their activity towards cell lines from different types of solid cancers, (2) their active constituents by means of bioactivity-guided fractionation and isolation, and (3) their activity in in vitro and in vivo models.

Poster session-PO-81:

A Study on the Components of *Gleditsia sinensis* Lamark for the Development of *Dermanyssus gallinae* pesticide

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Dermanyssus gallinae(D.gallinae) is a small tick and is observed group, and at night, it is a vampire attached to the chicken. Recently, the poultry farm has been seriously infected with D.gallinae, which have reduced the productivity of chickens. And farmers are also using agricultural pesticides that are not approved as quasi-drugs for animals. So food safety, such as chicken meat and eggs, is also concerned.

Therefore, we want to develop the pesticide of D.gallinae using natural substances. we tested the pesticide effect on D.gallinae with 35 plant extracts. As a result, *Gleditsia sinensis* Lamark(G.sinensis) showed a high insecticidal rate of 89.6%. So we have started this study using G.sinensis to develop the pesticide of D.gallinae that are both safe and effective.

The G.sinensis was extracted with 95% EtOH and partitioned with n-hexane, CH₂Cl₂, EtOAc, n-BuOH and aqueous fractions. The purification of each fraction by column chromatography separation and HPLC analysis to yield compound 1-6. These compounds are all the known compounds that were identified by analysis of NMR and HPLC data, along with comparison with those in the literatures.

Poster session-PO-82:

Validation of The Antihypertensive Potential of Cecropia Obtusifolia

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One of the biological systems playing a major role in blood pressure regulation and in the pathology of the most important cardiovascular diseases is the renin-angiotensin system. It is well known that the deleterious effects of angiotensin II are mediated through the angiotensin II AT₁ (AT₁) receptor, which has been recognized as an effective target to develop antihypertensive drugs. Nevertheless, increasing interest is there to find drugs capable of activating protective and regenerative pathways like the activation of the angiotensin II AT₂ (AT₂) receptor (1).

A preliminary study of our group showed that extracts from the leaves from *Cecropia obtusifolia* Bertol., a plant used by Panamanian folk medicine as a remedy for hypertension, inhibited angiotensin II binding to the AT₁ receptor in more than 50% (2). In this study, we have isolated the major compounds present in the butanol extract of the stems and leaves of this plant and evaluated their

ability to modulate calcium signaling of the above-mentioned receptors. For this purpose, we used a cellular luminescence-based read-out system in which intracellular calcium signaling is measured in Chinese hamster ovary cells that express the calcium sensitive apo-aequorin protein. Our results showed that the crude extract blocked the response in the AT₁ receptor ($9.4 \pm 8.2\%$) and activated it in the AT₂ receptor ($91.8 \pm 2.5\%$). Interestingly, when the major compounds present in the active extract were tested individually they were less active ($36.6 \pm 11.4 - 51.0 \pm 31.8\%$ for AT₁ and $0.00 \pm 0.00 - 45.0 \pm 24.9\%$ for AT₂) than the mixture suggesting a synergistic effect of these compounds. These results validate the traditional use of *C. obtusifolia* as an antihypertensive plant.

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Poster session-PO-83:

Anti-Hyperglycaemic, Anti-Inflammatory and Anti-Oxidant Activities of Carica Papaya and Citrullus Lanatus Seeds

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The study investigated the anti-hyperglycaemic, anti-inflammatory and antioxidant potentials of methanolic seed extracts of *Carica papaya* and *Citrullus lanatus*. The inhibitory effects of the extracts (100, 200 and 400 mg/kg, orally) on α -amylase and α -glucosidase were assessed in glucose and sucrose-induced hyperglycaemic rats using glimepiride (2.5 mg/kg) and acarbose (50 mg/kg) as positive controls. The anti-inflammatory activities were evaluated by membrane stability, xanthine oxidase inhibition and inhibition of denaturation of albumin models. Their antioxidant potentials were determined using standard methods and their total phenolic and flavonoid contents were also estimated. The extracts gave a comparable ($p > 0.05$) hyperglycaemia lowering and α -glucosidase inhibitory activities to glimepiride and acarbose respectively. However, *C. papaya* gave a significantly ($p < 0.05$) higher α -amylase inhibitory activity than *C. lanatus* at all concentrations. The seed extract of *C. papaya* was significantly more active in red blood cell membrane stabilizing activity at all concentrations than *C. lanatus* and ibuprofen while their order of xanthine oxidase inhibitory activities was: allopurinol > *C. papaya* > *C. lanatus*. In albumin denaturation assay, *C. papaya* gave a comparable activity to the positive control at 0.25 – 1.00 μ g/ml and significantly higher effect at 0.0625-0.125 μ g/ml while in the 1, 1, diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, total antioxidant capacity (TAC)

and ferric reducing antioxidant power (FRAP) assays, the order of antioxidant activities was, ascorbic acid (positive control) > *C. papaya* > *C. lanatus*. The total phenolic and flavonoid contents of *C. papaya* expressed as gallic acid and quercetin equivalents were found to be 82.00 and 35.00 mg/g respectively; while those of *C. lanatus* were 40.00 and 20.00 mg/g, respectively. The results showed the seed extract of *C. papaya* to be a better anti-hyperglycaemic, anti-inflammatory and antioxidant agent than *C. lanatus* and suggested that their high flavonoid and phenolic contents could be responsible for these activities.

Poster session-PO-84:

Comprehensive comparison of different processing technology on *Polygonum multiflorum* by using untargeted metabolomics method

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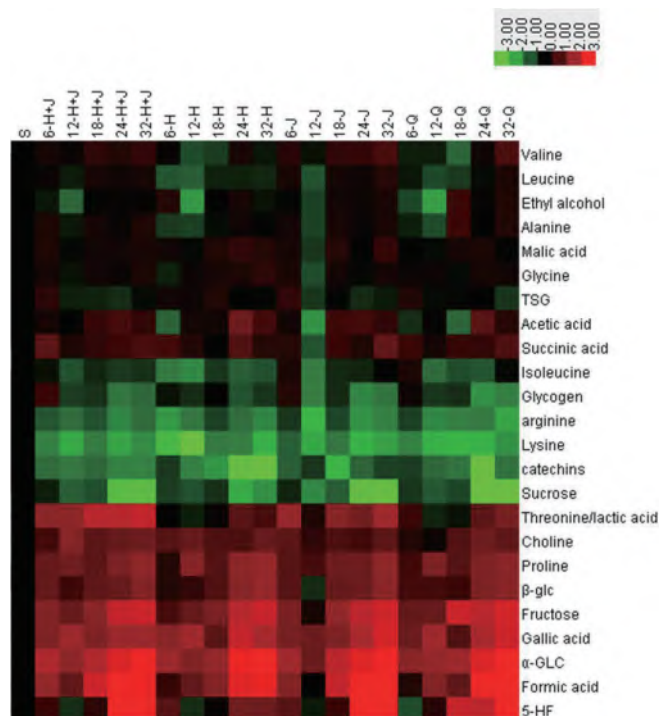
A rapid and simple method was established to compare the changes of chemical constituents in the processing of *Polygonum multiflorum* thumb (PM). Primary and secondary metabolites of PM were detected and identified by ¹H-NMR spectroscopy. Multivariate analysis method (OPLS-DA) is used for grouping and searching for potential biomarkers. Twenty-six metabolites (including fourteen potential biomarkers) were identified in samples from different processing methods. The results suggest that the content changes of fourteen potential biomarkers (including Valine, Ethyl alcohol, Threonine, Lactic acid, Alanine, Acetic acid, Proline, Succinic acid, Lysine, Choline, Sucrose, Glycine, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, β-D-glucoside, Gallic acid, 5-HF) in different processing methods of PM can influence on the pharmacological activity in vivo and to provide reference for proper use of PM.

Table ¹H NMR chemical shifts of metabolites identified in the PM samples.

No.	Metabolites	Chemical shift	Correlated NMR spectra	p-value
1	Leucine	0.97(t),3.742(m)	HSQC,J-res	
2	Isoleucine	0.95(t),1.02(d)	COSY,HSQC,J-res	
3	Valine*	1.00(d),1.05(d),2.29(m),3.63(d)	COSY,HSQC,J-res,HMBC	<0.05
4	Ethanol **	1.22(t), 3.68	J-res, COSY, HSQC, HMBC	<0.001
5	Threonine*	1.36(d),3.62(d),4.38(m)	COSY,HSQC,J-res, HMBC	<0.05
6	Lactate*	1.37(d),4.10(q)	COSY,HSQC,J-res	<0.001
7	Alanine**	1.52(d), 3.82	J-res, COSY	<0.001
8	Arginine	1.94(m), 3.22, 3.59	COSY, J-res	
9	Acetate**	1.96(s)	HSQC,J-res, HMBC	<0.001
10	Proline*	2.00(m), 2.10(m), 2.36(m),4.16(dd)	COSY,HSQC,J-res, HMBC	<0.05
11	Succinate**	2.45(s)	J-res, HSQC, HMBC	<0.001
12	Malate	2.36(dd), 2.71(dd), 4.36(dd)	COSY,HSQC, HMBC,TOCSY	
13	γ-aminobutyrate *	3.05(t), 1.92, 2.32	COSY,HSQC,J-res,HMBC	<0.001
14	α-ketoglutaric acid	3.05(t), 2.40	COSY, HSQC,J-res	
15	Malonate	3.16(s)	HSQC, HMBC, J-res	
16	Choline*	3.18(s)	J-res, HSQC, HMBC	<0.05
17	Sucrose**	3.51(t),3.60(dd),3.72(s),4.09(t),4.25(d),5.45(d)	COSY,HSQC,TOCSY,HMBC	<0.001
18	Glycine*	3.60(s)	J-res	<0.05
19	Fructose	4.07(dd)	COSY,HSQC,J-res	
20	TSG**	4.40(d),6.34(d),6.56(d),6.71(d),6.78(d),7.23(d),7.30(d),3.8(m)	COSY,HSQC,HMBC,TOCSY	<0.001
21	β-glucoside**	4.69(d),3.29(dd)	COSY,HSQC,HMBC,J-res	<0.001
22	α-glucoside	5.28(d),3.84(m)	COSY,HSQC,HMBC,J-res	
23	Gallic acid**	7.08(s)	HSQC,J-res	<0.001
24	Formic acid	8.50(s)	HSQC,J-res	

p- values are the result of T test

*(p<0.05) ** (p<0.001).



Poster session-PO-85:

Investigation of bioactive secondary metabolites from *Alkanna* species of Greece

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Studies have already shown that interlinking microbiomes with medicinal plants play a key role on plant growth processing. The plants-associated microorganisms may modulate the biosynthesis of bioactive secondary metabolites and stimulate their production. In the frame of the EU H2020 “MICROMETABOLITE” project, innovative technologies are developed by integrating microorganisms, including endophytic bacteria and fungi but also arbuscular mycorrhizal fungi (AMF), on plant materials of Boraginaceae family.

As part of our study, eight different populations of *Alkanna tinctoria* were collected so far, from different locations close to Athens. The aerial part of the plant were separated from the roots, for each collection respectively and treated similarly. On the other hand, three other species of the genus *Alkanna* (*A. graeca*, *A. hellenica* and *A. sfikasiana*) were collected from Southern Greece, while a commercial sample of *Alkanna tinctoria* from Pakistan was used as a reference material.

Part of this study was focused on investigating and comparing metabolome variations between the four species in the aerial part, from different geographical regions of Greece, and the commercial sample. Some extracts were very rich in secondary metabolites. In addition pyrrolizidine alkaloids that are characteristic compounds of this family but also well known for their toxicity, were analyzed.

The extraction process of the plant material was performed sequentially with solvents of increasing polarity (c-Hexane, EtOAc and EtOH/H₂O 50:50) to get a broad metabolic profile. The extracts were analyzed with methods based on HPTLC, HPLC-PDA-ELSD, GC-MS and UPLC-HRMS. On those extracts a dereplication process combining the Dictionary of Natural Products, Molecular Networks, in silico MS/MS dereplication using customized libraries and algorithms using R statistical language were developed that will lead to a targeted isolation and identification of promising and new compounds.

Poster session-PO-86:

Antioxidant activity and phenolic composition in leaves and flowers of *Taraxacum officinale* F.H. Wigg.

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Taraxacum officinale F.H. Wigg is native to Europe and widely distributed in the warmer temperate zones of the Northern Hemisphere. Phenolic acids and flavonoids are abundant in all parts of the plant. These compounds are characterized by antioxidant activities, which result in diverse biological effects. Dandelion leaves and flowers have antipyretic, sedative, anti-inflammatory, diuretic, spasmolytic, anti-allergic, cholagogue, choleretic effects, improve digestion, pancreas, liver and kidney function, regulate metabolism [1]. Quantitative analysis of phenolic compounds in dandelion and setting of antioxidant activity is important to assess the quality of raw materials and its pharmacological potency. The aim of this study was to determine the phenolic and antioxidant activity variation in Lithuania naturally growing common dandelion leaves and flowers during the growing season. Radical scavenging and reducing activities were evaluated using ABTS and CuPRAC methods, respectively. Phenolic composition was determined using HPLC-PDA. The content of phenolics in leaves and flowers and anti-oxidant activity of extracts vary significantly during their growing season ($p < 0.05$). Greatest radical scavenging and reducing activities were determined in the leaf extracts, $449,36 \pm 7,49 \mu\text{mol/g}$ and $1003,92 \pm 4,37 \mu\text{mol/g}$, respectively. Identified phenolic compounds complex in leaves and flowers of dandelion was comprised of cichoric, chlorogenic, caftaric acids, and luteolin-7-O-glucoside. Cichoric acid was determined as the predominant compound in all plant parts: in the leaves— $82,82 \text{ mg/g}$ and in the flowers— $19,24 \text{ mg/g}$. In the middle of June dandelion leaves contain the greatest amounts of phenolic compounds and antioxidant activity.

References:

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Taraxacum officinale, cichoric acid, HPLC, ABTS, CuPRAC.

Poster session-PO-87:

Insight into Healing Herbs used in Post-famine Ireland

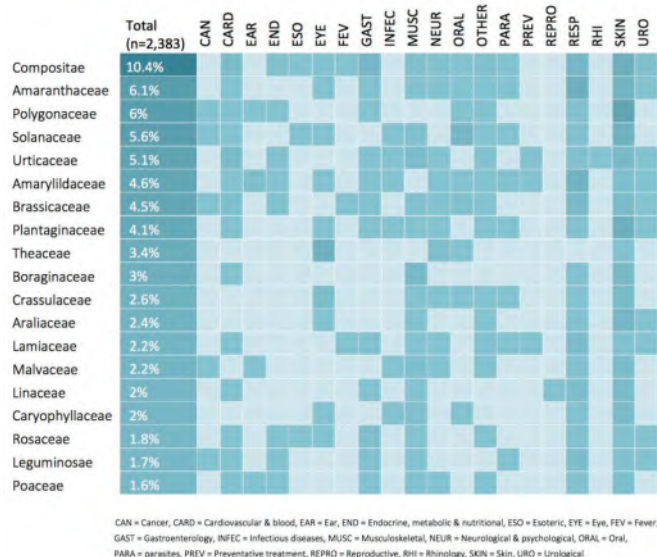
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The National Folklore Collection in University College Dublin holds one of the largest collections of ethnographic material in the world. The value of this material was recognised in 2017, when it was inscribed into UNESCO Memory of the World Register. The Schools' Manuscript Collection (SMC) is one body of information stored within this archive, that contains over 700,000 pages of regional Irish traditional knowledge (TM), collected by school children in the 1930s. The SMC is unique in content and in terms of how, when and why it was collected, with no comparative body of ethnographic information existing globally. In a recent study we identified and interviewed original participants to this scheme, documented their recollections and compared them with their original archival entries [1]. In this study we analyse and categorise ethnomedical material stored in the archive, obtained from two geographically and socio-economically different regions in the country, Counties Roscommon and Wexford, providing us with an insight into the healing herbs used and associated medicinal beliefs of Irish people at that time. Our analysis, comprised of 5,224 enthomedicinal data entries extracted from 190 different schools, involves two key steps (i) generation of a disease classification system, enabling identification of the most prominent conditions treated, (ii) identification of the main treatment categories; plant, animal, natural substances, religious, ritual and other. Subsequently, we analysed the entries citing use of a 'plant' (41.8%) and have identified the key genera and plant species used in the two regions (Figure 1). This has enabled us to draw comparisons, identify relationships and interpret the key medicinal treatments used in 1930s Ireland.

Most Frequently Cited Plant Families & Conditions Treated



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Poster session-PO-88:

Investigation Of Plant-Microbe Interactions For The Production Of Bioactive Secondary Metabolites In Boraginaceae Plants

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Natural products derived from plant sources - known also as plant secondary metabolites (SMs) - are of great interest for pharmaceutical, cosmeceutical and food supplements industries. SM biosynthesis in plants is subject to the influence of multiple factors and stimuli such as plant hormones, herbivore and pathogen-derived elicitors, as well as the abiotic environment. Plant-associated microbiota (such as endophytic bacteria

and fungi) are tightly interacting with their hosts and they may induce host biosynthesis pathways. Moreover, endophytic bacteria and fungi themselves are known as producers of various SM compounds.

In the frame of the EU H2020 project "MICROMETABOLITE", microbial communities of Boraginaceae plants, specifically from *Alkanna tinctoria* and *Lithospermum erythrorhizon* roots, are studied, in order to further investigate those interactions.

In more details, this study is focused on exploring the participation of microorganisms in the enhancement of plant SMs production, especially for the enantiomeric naphthoquinones, alkannin and shikonin, well known for their wound healing, anti-microbial, and anti-inflammatory activities. Eco-friendly and innovative technologies, like semi-hydroponic and in-vitro cultivation systems, integrating microorganisms in plant cultivation are developed, to determine the optimal cultivation conditions for producing the desired naphthoquinones. Furthermore, plants both from the wild collections and cultivations are sampled at several vegetation stages and subjected to detailed analysis of microbial communities and naphthoquinones. The ultimate goal is to establish a link between microorganisms and naphthoquinone production as well as to isolate microorganisms involved in naphthoquinone production. Finally this study will determine also the optimal cultivation conditions that should be used for *Alkanna* spp. in order to produce the desired naphthoquinones for industrial use.

Acknowledgments:

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Poster session-PO-89:

Best practice for the evaluation of Traditional Herbal Medicines: *Houttuynia cordata* Thunb (鱼腥草 Yu Xing Cao) Saururaceae - A Case Study

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Houttuynia cordata Thunb (Yu Xing Cao) 鱼腥草 (Saururaceae) commonly known as Chinese lizard tail. This medicinal use of this herb is well known in Asia, with references from the 1st century. In Traditional Chinese Medicine (TCM) *H. cordata* has many applications including its use in the treatment of cancer, inflammation and fever. The plant is harvested during the growing season and is administered as the Traditional Medicinal decoction (TM). *H. cordata* contains flavonoids, essential oil, alkaloids and fatty acids [1]. Many studies document the bioactivity of organic extracts of *H. cordata*, as opposed to the TM decoction. Studies using

decoctions frequently fail to present chemical fingerprints [2]. Many factors influence the qualitative and quantitative metabolomic profile of a TM, hence different extracts potentially represent different medicines [3]. In this study we present the results on the chemical fingerprinting of thirteen different samples of *H. cordata*. Eleven of these samples were plant material and two samples in the study are in the form of modernised granules. Microscopic features were determined and compared with the monograph of *H. cordata* thumb from the Hong-Kong Pharmacopeia. All plant samples were extracted as decoctions and aqueous extracts of the modernised granules were prepared. All samples were lyophilised. Chemical profiles of the extracts were generated and analyzed by HPLC, LC-MS and NMR (Figure 1). Chromatograms and spectra show distinct variation between some samples. In our ongoing studies the bio-activity of decoctions will be evaluated in THP-1 and Caco-2 cells, to establish the activity and to correlate these activities with the metabolomic fingerprints using multivariate analysis.

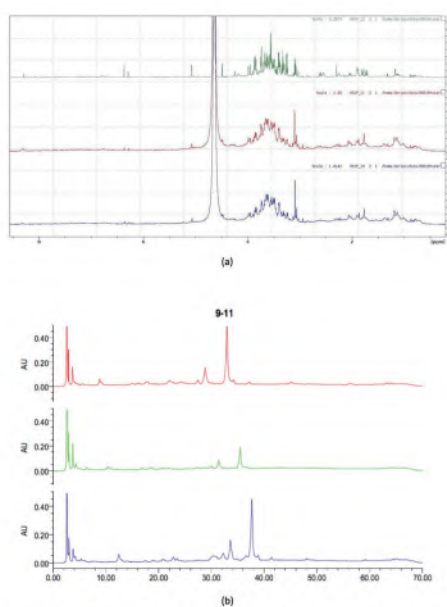


Figure 1: 600 MHz spectra (a) and HPLC chromatograms (b) of some *Houttuynia cordata* samples

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[2] Lee J et al. J Ethnopharmacol 2008; 17(1):34-40.

[3] Sheridan H et al. Journal of Ethnopharmacology 2012; 140, 482-491

Poster session-PO-90:

Does fermentation change the alkaloid profile of *Sceletium tortuosum* – a “mind and mood” plant used in South Africa?

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Sceletium tortuosum is an indigenous South African succulent. The KhoiSan of Southern Africa used fermented *S. tortuosum* preparations to induce psychoactive effects over the past 300 years. *Sceletium* contains alkaloids such as mesembrine, mesembrenol, mesembranol and mesembrenone responsible for the psychoactive properties, of which mesembrine is regarded as the most potent. It is claimed that the traditional fermentation process changes the alkaloid composition, thereby increasing the potency of the psychoactive response. In this study, ultra performance liquid chromatography-mass spectrometry was used to quantify the content of alkaloids prior to and after fermentation of samples of *Sceletium tortuosum*. The results revealed that although the mesembrenol and mesembranol content changed marginally during fermentation, the mesembrine content increased significantly from not detected - 1.6 µg/mL to 7.40 - 20.8 µg/mL. A corresponding decrease in the mesembrenone content was observed after fermentation from 8.00 - 33.0 µg/mL to 1.30 - 32.7 µg/mL. The total alkaloid content increased as a result of fermentation. This study confirmed that the fermented method increased the content of the psychoactive molecule, mesembrine during the traditional method of preparation [1].

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Poster session-PO-91:

Food products vs ACE inhibitors for hypertension: Results from randomized control trials with “kinkeliba” and “bissap” in Senegal

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Bissap, the Senegalese name of *Hibiscus sabdariffa* (HS) is well-known for its effect on blood pressure [1,2] and kinkeliba” the health Tea” namely the *Combretum micranthum* (CM) plant has started being assessed with promising results. Since both are popular drinks in West Africa, we compared them in moderately hypertensive patients (systolic blood pressure 140 -179 mm Hg and/or diastolic 90-109 mm Hg) in tablet form of dried plants, with the traditional tea recipe. This was done in an equivalence 5-arm multi-centric randomized control trial, with captopril as the standard control and a 6-months follow-up. In this study, herbal

drugs were constituted from flowers (calyx) of HS and leaves of CM, in the dried form for teas and as powder in tablets. The study protocol was submitted and accepted by the local ethical committee.

Results from one site of the study, Toubá, are already available. With 85 included patients (a final follow-up for 72), 39% of patients in the plant products group had normalized their systolic pressure, and 80% their diastolic pressure versus 31% for systolic pressure, and 50% for diastolic pressure in the captopril group. Food products were well tolerated, with occasional epigastric pain for bissap. One patient (under captopril) was excluded at day 21 because of hypertensive crisis; 6 patients moved from single to combined (2 plant) treatment in order to achieve sufficient blood pressure control and 5 to captopril.

These preliminary results suggest that the tested traditional African food products may have an effectiveness similar to the standard captopril treatment for mild/moderate hypertension.

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Poster session-PO-92:

Comparative study of the cytoprotective effects of *Baccharis trimera* and *Baccharis dracunculifolia* in vitro against oxidative insults

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Baccharis trimera (BT) and *Baccharis dracunculifolia* (BD), two species occurring in tropical areas of South America, are widely used in folk medicine as digestive aid, anti-inflammatory and anti-diabetic agents. This study aims the comparison between these two species regarding their cytoprotective effects against the insults t-BHP (0,5 mM) and paraquat (1 mM), using two cell lines: human hepatocellular carcinoma (HepG2) and the mouse microglia BV2, providing first insights regarding the protective underlying mechanism.

BT and BD extracts, showing no significant toxicity at 100 µg/ml, were evaluated for HepG2 cytoprotection against the insults in pre-incubation assays. The extracts were pre-incubated for 2 and 6h before the application of t-BHP and paraquat respectively and then co-incubated with the insults (4h). Cellular viability (MTT assay) was assessed immediately or 16 hours later, after t-BHP or paraquat insults, respectively. Both BT and BD showed significant cytoprotection in HepG2 against t-BHP with 0,5- and 1-fold increase in

cellular viability. However, only BD showed a significant protective effect against t-BHP in BV2 cells with 1-fold increase. Also, only BD showed significant cytoprotection against paraquat in HepG2 with 0,7-fold increase in cellular viability. Higher cellular viability has been confirmed through flow cytometry with a 0,37 fold-decrease in apoptotic cells following incubation with BD. To understand the protective mechanism against t-BHP toxicity, cellular oxidative stress has been quantified in pre-incubation assay (with t-BHP insult) with a 0,6-fold significant decrease in ROS production for both extracts. Moreover, 0,2- and 0,5-fold decreases in NO have been determined following 2h-pre-incubation of BV2 with the BT and BD, respectively and 16h-co-incubation with LPS 1 mg/ml. Further studies including the induction of antioxidant enzyme activities, are warranted. This work is financed by INTERACT - ISAC project, no. NORTE-01-0145-FEDER-000017, co-financed by the European Regional Development Fund (ERDF) through NORTE 2020 (North Regional Operational Program 2014/2020).

Poster session-PO-93:

Improvement effect of peripheral circulatory disturbance by Japanese Angelica Root and its ingredients.

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In recent years, the cultivation of medicinal plants in Japan has become an active. However, the natural medicinal plants are known to change the content rate of components depending on the cultivation area and cultivation conditions. That is, there is a need to confirm the content of active ingredients in medicinal parts of crude drug. But, as in Japanese Angelica Root, it's not always true that the principal ingredient is an active ingredient.

Therefore, for the purpose of searching active ingredients of Japanese Angelica Root, we tried the comprehensive analysis of Japanese Angelica Root extract using high performance liquid chromatography (HPLC) and the identification of the compound of the main peak. In addition, using in vivo assay method for monitoring the blood flow decrease in tail vein microcirculation of mice subjected to sensitization with hen-egg white lysozyme (HEL), we investigated the improvement of peripheral circulatory disturbance of Japanese Angelica Root and the ingredient.

Peripheral blood flow in the mouse tail was monitored using a laser doppler blood flow meter as previously described. The normal blood flow was measured for 10 min at 1 day before the experiment. The blood flow of each mouse was measured for 10 min without anesthesia. The results were expressed as the mean \pm S.E. of the percent of the normal blood flow of each mouse.

We identified the compounds in Japanese Angelica Root of the main peaks on HPLC pattern as ligustilide, xanthotoxin and adenosine, respectively. In addition, adenosine is known as a therapeutic agent for ischemic

disease, and it significantly improved the peripheral circulatory disturbance, using in vivo assay method for monitoring the blood flow in this study. We suggesting that adenosine may be involved in the improvement of blood flow in Japanese Angelica Root.

Poster session-PO-94:

Vasorelaxant effect of *Serjania triquetra* Radlk. (Sapindaceae)

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Serjania triquetra Radlk. (Sapindaceae) is a liana-like climbing plant formed by brown bark vines, commonly known as "Palo de tres costillas". In Mexican traditional medicine it is used to treat kidney diseases, urinary infection, inflammation, uterine hemorrhages and hepatitis [1,2]. Previous phytochemical studies of the aerial parts resulted in the isolation and characterization of stigmasterol, oleanolic acid, morolic acid and hederagenin, with a wide range of biological properties reported for these compounds [3]. In the current work a *S. triquetra* collected in Santiago Ixcuintla, Nayarit, México in november of 2017 was studied, preliminary phytochemical study allowed us to isolated ursolic acid as a major compound, and it was confirmed by UPLC-MS (Fig.1A).

To explore pharmacological activity, the vasorelaxnt effect of an ethanol extract from *S. triquetra* it was evaluated on aortic rat rings pre-contracted with noradrenaline 0.1µM. It was found to induce significant relaxant effect in a concentration-dependent manner, showing Emax: 81.6%± 4.6%, EC₅₀ 0.26± 0.04 µg/mL with endothelium; and Emax: 4.3 ± 1.1, EC₅₀ > 100 µg/mL without endothelium respectively as shown in Fig.1-B. When aortic rings were denuded of endothelium, the relaxant effect was greatly reduced, these results endothelium-dependent suggest the participation of relaxing factors such as nitric oxide (NO), Endothelium -Derived Hyperpolarizing (EDHF), and prostacyclin (PGI₂), as mediators. For the determination of the possible vasorelaxant mechanism exerted by *S. triquetra* its underlying functional mechanism of action should be explore. To our knowledge this is the first pharmacological report of the vasorelaxant effect of this plant.

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Poster session-PO-95:

Acute oral toxicity of *Guazuma ulmifolia* Lam.

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Guazuma ulmifolia is a native tree of America and widely distributed in Mexico, is commonly known as "Guacima". The fruit is used in traditional Mexican medicine to treat kidney diseases, dysentery and diabetes, despite of it is widely used there is not information of its phytochemical composition [1]. Likewise, this plant is used by the population, there is a lack of evidence of it safety, to contribute to that knowledge the oral acute toxicity of an ethanolic extract of *G. ulmifolia* (EEGu) was conducted. The pharmacological evaluation or the acute oral toxicity (AOT) test was carried out using a modified protocol from 423 guide according to the criteria established by the OECD [2]. Preliminary phytochemical study allowed us to identify the presence of terpenoid like compounds. EEGu was subjected to UPLC-MS Figure 1A [3]. As shown in the chromatogram a mass of 455.10 with retention time of 4.320 was found; the chromatogram was compared with and ruling out the presence of ursolic and oleanolic acid, the compound found in elucidation process.

For AOT mice were administered with doses of 50, 300 and 2,000 mg/kg b.w. intragastrically and observed daily for 14 days to record mortality and weight variation (Fig.1B). and visible toxic effects in the pattern of behavior. At the end of the experiment, the animals were euthanized and a necropsy was performed. Animals showed signs of toxicity characterized by decreased activity, respiratory changes, intermittent unstable movements and erratic behavior without changes in weight or macroscopic changes in organs. According this results the extract is classified in category 5 "not classified" (LD₅₀5,000 mg/kg).

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Poster session-PO-96:

Molecular Identification and Chemical Analysis of Aconiti Kusnezoffii Tuber on the domestic market

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Aconiti Kusnezoffii Tuber has been traditionally used to treat the symptoms of rheumatoid arthritis and joint pain. The main constituents are diterpenoid alkaloids such as benzoylmesaconine, benzoylaconine, mesaconitine, aconitine, and hypaconitine. In Korea, Aconiti Kusnezoffii Tuber is officially defined as the tubers of Aconitum kusnezoffii Reichb., A. ciliare Decasine, and A. triphyllum Nakai. On the other hand, only the tuber of A. kusnezoffii is to be used in China. In order to identify the botanical origin of Aconiti Kusnezoffii Tuber circulated in Korea, we analyzed 24 samples of Aconiti Kusnezoffii Tuber obtained from local markets for comparative DNA analysis. The sequence analysis of nrRNA ITS 1 was useful to distinguish Aconitum species and revealed that the roots of A. karakolicum were circulated in Korean markets without discretion. HPLC quantitative analysis showed that aconitine was detected at the highest amount in A. karakolicum. Authentic diterpenoid alkaloids were coinjected for quantification of aconitine-type ingredients. All data were statistically grouped by Principal Component Analysis (PCA). This study suggests that both molecular and chemical analyses should be utilized for the standardization and the quality control for Aconiti Kusnezoffii Tuber.

Poster session-PO-97:

Recovery effects of fermented extract from Ginger (Zingiber officinale) on pancreatic islets recovery of diabetic zebrafish

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Diabetes mellitus is a chronic metabolic disease characterized by high levels of blood glucose resulting from a lack of insulin secretion or insulin resistance [1]. Insulin is produced in pancreatic islets (PIs) by β -cells, which comprise 70-80% of the PI mass, diabetes develops as a result of a decreased number of

pancreatic β -cells and/or pancreatic β -cell dysfunction. In this regard, type 1 diabetes is characterized by autoimmune destruction of pancreatic beta (β) cells, resulting in severe insulin deficiency, unlike type 2 diabetes, characterized by deficient insulin action caused by insulin resistance [1]. Ginger is one of the most famous medicinal herbs in traditional Chinese and Indian Medicine, reporting efficacy in several diseases including Diabetes mellitus, diabetic complications and metabolic syndrome, being the most active ingredients gingerols and shogaol [2]. Thus, we aim to enhance the antidiabetic activity of ginger extract by using a bioconversion approach in order to increase bioactive components concentration. First, we confirmed diabetic condition in zebrafish model by observing pancreatic islet damaged either by diabetogenic agent Alloxan or insulin resistance, using 2-NBDG which is widely used to assess glucose uptake in the cells. Next, we established a model for obesity by overfeeding zebrafish since obesity is a risk for developing metabolic disorders such as diabetes [3]. Finally, our results suggest that fermented extract enhanced efficacy of ginger on diabetic zebrafish.

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Poster session-PO-98:

An ethnobotanical survey and antifungal activity of *Piper guineense* used for the treatment of fungal infections in West-African traditional medicine

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Piper guineense is a medicinal plant used for the treatment of fungal infections in African traditional medicine [1]; [2]. Due to frequent and common occurrence of fungal diseases in Africa, this study was performed in Imo state, south-eastern Nigeria, where *P. guineense* is predominantly used for the treatment of tropical fungal diseases.

In our study, a house to house ethnobotanical survey was conducted on the traditional uses of *P. guineense* extracts for the treatment of fungal infections. The investigation focused on how traditional healers recognize and diagnose fungal infections, how *P. guineense* is collected, on the various parts used for treatment, methods of preparation, administration and treatments. From our ethnobotanical results, a total of 12

fractions of *P. guineense*, in addition to piperine and piperlongumine, were selected for the screening against four pathogenic strains of yeast and *Cryptococcus neoformans*, a yeast-like basidiomycete.

A total of twenty traditional medical practitioners (TMP) explained their methods of administration of *P. guineense* extracts. According to these TMPs, the leaves and fruits are the most often used plant parts. The oral intake of extracts in ethanol (Kai-kai) is the most common method of administration. For the dosage, the TMPs use a small glass tumbler, which measures about 100mL, administered 3 to 4 times daily. The TMPs sometimes send their patients to the government hospitals if the symptoms persist as a result of their failed treatment. The TMPs claimed that their methods were more effective than conventional antibiotics for the treatment of fungal infections. In our antifungal screenings, the extracts were active against the tested fungal strains with MIC values ranging from 79 µg/mL-2500 µg/mL.

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Poster session-PO-99:

Effects of Ginger extract and Processed Ginger extract on stress-induced cold hypersensitivity in mice

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The rhizome of ginger has been used in the Kampo medicine for several hundred years. In the Japanese Pharmacopoeia (17th ed.), two crude drugs derived from the rhizome of *Zingiber officinale* are listed as "Ginger" and "Processed Ginger". The latter is dried after being passed through hot water or being steamed and is known to have stronger thermogenic actions. We established the mice model to evaluate the thermogenic effects of crude drugs and the thermogenic effects of Ginger extract and Processed Ginger extract were evaluated. When normal mice were forced to swim in water at 25 °C for 15 min, their core body temperature dropped by ca. 6 °C, and then quickly recovered to normal temperature after the mice were transferred to a dry cage at room temperature (25 °C). A 1-h immobilization before swimming caused the core body temperature to drop by ca. 11 °C (5 °C lower than normal mice), and the recovery time of core body temperature doubled. We considered this delay in recovery from hypothermia to be a sign of stress-induced cold hypersensitivity [1]. In this study, we showed that recovery from the stress-induced hypothermia were remarkably faster in mice administered Processed Ginger extract than those administered Ginger extract. The contents of two major thermogenic compounds, 6-shogaol and 6-gingerol [2], were also quantified and their contribution in thermogenic actions were discussed.

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Poster session-PO-100:

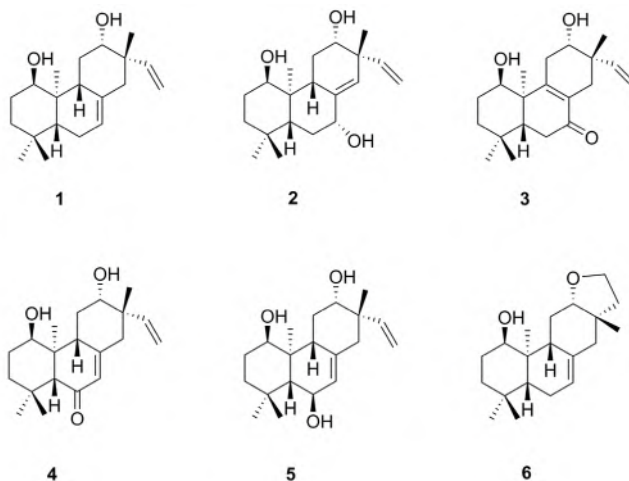
New pimarane diterpenoids from *Hymenocrater elegans*

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Lamiaceae Family, with 236 genera and more than 7,000 species is a rich source of structurally diverse terpenoids. The genus *hymenocrater* represents 9 species in Iran possessing anti-inflammatory, anti-spasm, sedative and anti-emphysema activities in traditional and folk medicine [1]. Except for the essential oil compositions a few phytochemical investigations of this genus plants have been reported so far [2,3]. As part of a project aimed at the discovery of structurally new bioactive metabolites from Iranian *hymenocrater*, we studied *hymenocrater elegans*.

Fractionation of the n-hexane extract by a combination of open column chromatography on silica gel and preparative TLC afforded six new pimarane diterpenoids (1-6). Their structures were established on the basis of the extensive spectroscopic analysis, including HRMS, 1D and 2D NMR.



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Poster session-PO-101:

Separation and Cytotoxicity of Enzymatic Transformed Prosaikogenins from *Bupleurum falcatum* L. by Countercurrent Chromatography and Preparative High Performance Liquid Chromatography

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Saikosaponins are bioactive compounds from the roots of *Bupleurum falcatum* L. Despite of various pharmacological benefits, the application of those compounds is restrained due to their lower bioavailability and the lack of large-scale separation method. To prepare lipophilic prosaikogenins, saikosaponins were enzymatically transformed in vitro. The separation method for these metabolites was developed using countercurrent chromatography (CCC) and preparative HPLC. Glucose at C-3 position of saikosaponins was eliminated by the enzymatic transformation of saponin-enriched fraction with cellulase. The converted fraction was then successfully separated by CCC. The optimum solvent system, dichloromethane/methanol/water (4:3:2, v/v/v), was selected and the relationship between rotation speed and retention of target compounds was investigated. Prosaikogenin G (PSG G) and prosaikogenin F (PSG F) were preparatively separated from the deglycosylated fraction. The residue in stationary phase on CCC separation (fraction S) was further bio-transformed by α -L-rhamnosidase (fraction SR) and cellulase (fraction SRC) in series. Finally, four prosaikogenins (PSG E1, E3, F, and G) were isolated by enzymatic transformation of major saikosaponins of roots of *Bupleurum falcatum* L. Through the investigation on the cytotoxicity of the separated compounds against six cancer cell lines and two normal human cell lines (MDA-MB-468, A-549, HepG2, AGS, PANC-1, HCT116, MCF-10A, and NKNT-3), PSG G, most cytotoxic compound, significantly inhibited the viability of cancer cells, while exerting less of an effect on their normal counterparts.

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Poster session-PO-102:

A potential anti-psoriatic sesquiterpenoid from *Tussilago farfara* as an Nrf2 activator and inhibitors of NF- κ B and STAT3 in keratinocytes

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The therapeutic potentials of 14-acetoxy-7 β -(3'-ethyl cis-crotonoyloxy)-1 α -(2'-methylbutyryloxy)-notonipetranone (AECN), a sesquiterpenoid separated from *Tussilago farfara*, for the psoriasis were investigated in the immortalized human keratinocyte cell line (HaCaT cells). Treatment of AECN upregulated the ARE-luciferase activity dose-dependently, showing 21.6-fold increase at 2.5 μ M. Also, it induced the protein expression of Nrf2 and its downstream target, heme oxygenase-1. In addition, AECN treatment increased the nuclear translocation of Nrf2, suggesting that AECN activates Nrf2 pathway. To identify whether AECN could inhibit the activation of STAT3 and NF- κ B, crucial transcription factors involved in the pathogenesis of psoriasis, we conducted western blot analysis and luciferase assay. AECN inhibited IL-6-induced STAT3 phosphorylation and STAT3-dependent transcriptional luciferase activity, and also suppressed TNF- α -induced NF- κ B activation. Moreover, TNF- α -induced mRNA levels of IL-17, IL-17A, IL-23, and TNF- α were reduced, suggesting the potency of AECN to decrease inflammatory mediators regulated by NF- κ B activation. Proliferation assay revealed that 2.5 μ M AECN inhibited IL-6-induced hyperproliferation of HaCaT cell and the mRNA expression of cyclin d1, one of the markers for proliferation. Taken together, these results indicate that AECN, as an effective Nrf2 activator, can be a promising therapeutic candidate for treatment psoriasis through inhibition of STAT3 and NF- κ B activation.

Poster session-PO-103:

Photoprotective and anti-wrinkle effects of Compound A isolated from *Aralia elata* against UVA-induced oxidative stress in human dermal fibroblast cells

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There are various factors that cause skin wrinkles such as UV irradiation, toxic substances, and genetic factors. Among them, UV irradiation is one of the most contributive elements causing wrinkles of the skin.

It has been known that plants belonging to the Araliaceae family have various kinds of biological activities, therefore, they have long been used for many diseases. In this study, we investigated that Compound A isolated from the n-butanol fraction of *Aralia elata* has the protective role against the UVA-induced photo-oxidative stress in human dermal fibroblast cells. These cells are located in the dermis of the skin and have an important role in producing collagen, which contributes to maintaining the skin elasticity. However, the collagen can be degraded by the high concentration of the matrix metalloproteinase 1 (MMP1) in the cells caused by UV-induced reactive oxygen species. Therefore, it is important to find a substance that reduces MMP1 while promoting the collagen production in anti-wrinkle experiments. Our results revealed that the treatment with Compound A can increase the concentration of procollagen (precursor molecules of collagens) in both UV-irradiated and non-UV-irradiated cells compared to the control cells. Also the increase of MMP1 concentration via UV irradiation was significantly suppressed by Compound A. Furthermore, Compound A activated the nuclear factor-erythroid-2-related factor 2 (Nrf2), resulting in the expression of the antioxidant proteins. Taken together, these results indicate that Compound A is capable of protecting skin cells and preventing wrinkles induced by UV irradiation.

Poster session-PO-104:

Phytosomal preparation containing *Centella asiatica* L. and *Curcuma longa* L. impairs oxidative stress and inflammation in the central nervous system.

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Oxidative stress and inflammation are processes strictly connected each other. An increase number of evidence shows that their alterations are involved in the development of several diseases, such as major depressive disorders [1]. Moreover, unbalanced redox state and increased inflammatory conditions are associated with aging [2].

Centella asiatica (*Centella*) and *Curcuma longa* (*Turmeric*) may be effective since they both display anti-inflammatory and antioxidant properties. In addition, *Centella asiatica* administration improved microcirculation and vascular protection.

In the present study, we investigate the effect of oral administration of a phytosomal preparation containing both *Centella asiatica* and *Curcuma longa* on redox and inflammatory processes in the central nervous system in comparison with *Centella asiatica* alone. Moreover, we investigated if the effects depend on the period of life by treating adult and aged animals.

Male Sprague Dawley rats 4-month or 18-month old were treated per os with *Centella asiatica* and *Curcuma longa* (250 mg/kg; total curcuminoids 29,1%; asiaticoside 3,8%), *Centella asiatica* (100 mg/kg, asiaticoside

6,8%), or vehicle for 5 days and sacrificed 48 hours after the last administration.

Male Sprague Dawley rats 4-month or 18-month old were treated per os with Centella asiatica and Curcuma longa (250 mg/kg; total curcuminoids 29,1%; asiaticoside 3,8%), Centella asiatica (100 mg/kg, asiaticoside 6,8%), or vehicle for 5 days and sacrificed 48 hours after the last administration.

The molecular analyses of key players of redox and inflammatory mechanisms (including Nox2 and Nrf2) were carried out in the dorsal hippocampus and in the prefrontal cortex. Gene expression was measured through real time RT-PCR.

Sub-chronic administration of Centella alone or in combination with Turmeric significantly decreased the mRNA levels of Nox2 in the dorsal hippocampus of adult rats, while Nrf2 specifically increased after treatment with Centella in the prefrontal cortex of aged rats.

Our results confirm the ability of phytosome from Centella and Turmeric to target genes involved in oxidative stress as well as inflammatory processes, thus supporting their potential in the treatment of diseases characterized by alteration of these mechanisms.

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Poster session-PO-105:

Anti-inflammatory effects of Cannabis sativa L. in human keratinocytes

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Dermatitis and psoriasis are inflammatory diseases in which keratinocytes, the most abundant cells in the epidermis, play a key role in the release of numerous pro-inflammatory mediators (e.g. IL-8, MMP9, and VEGF) [1,2]. IL-8 is involved in neutrophil recruitment and VEGF regulates the angiogenesis process, while MMP9 contributes to extracellular matrix degradation. These pro-inflammatory mediators are regulated by different transcription factors, including NF-κB. Cannabidiol (CBD) [3] is the second major cannabinoid occurring in Cannabis sativa L. (C. sativa) and its anti-inflammatory activity on skin has been demonstrated in mice [4]; however, no studies on human keratinocytes inflammation have been reported so far.

The aim of this work was to evaluate the anti-inflammatory activity of two C. sativa extracts, standardized in CBD and cannabidiolic acid (CBDA), in human keratinocytes. The extracts, containing 1% or 5% CBD+CBDA, were prepared by LINNEA SA (Riazzino, Switzerland). IL-8, MMP-9 and VEGF release were analyzed by ELISA assays, NF-κB driven transcription by a reporter plasmid, while gene expression by real-time PCR.

Both the extracts inhibited IL-8, MMP9 and VEGF release and NF-κB driven transcription induced by TNFα, with IC₅₀s below 50 µg/mL, while CBD was active only on NF-κB driven transcription (IC₅₀: 2.85 µM), suggesting that other compounds are involved in the biological activity.

The extract containing 5% CBD+CBDA (25 µg/mL) and the corresponding concentration of cannabidiol (4 µM) were tested on the expression of 84 genes related to inflammation. The extract decreased the mRNA levels of several pro-inflammatory genes and for some of them CBD was responsible, at least in part, for the activity.

These results suggest that *C. sativa* extracts may counteract the cutaneous inflammatory processes.

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	NF-κB driven transcription IC ₅₀ (µg/mL)	IL-8 release IC ₅₀ (µg/mL)	VEGF release IC ₅₀ (µg/mL)	MMP9 release IC ₅₀ (µg/mL)
<i>Cannabis sativa</i> L. extract containing 1% CBD+CBDA	38.1	33.9	21.4	36.4
<i>Cannabis sativa</i> L. extract containing 5% CBD+CBDA	21.4	43.5	26.8	18.0

Poster session-PO-106:

Quantitative analysis of 3(2H)-furanones in infected *Allium cepa*

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Onions (*Allium cepa* L.) are one of the most popular food ingredients in the world. The production of dried onions is more than 88 million tons every year. [1] The extensive usage of onions for industrial food production requires good storage properties. However, onions are prone to several microbial infections reducing their yield and quality. [2] In bulbs, which were infected by fungi or bacteria, two highly volatile compounds, namely 2-hexyl-5-methyl-3(2H)-furanone and 2-octyl-5-methyl-3(2H)-furanone, could be identified and structure has been elucidated by NMR and MS. For quantitative determination of both compounds, dimethyl dihydrofuranone DMDHF has been established as internal as well as external standard. The results showed that 3(2H)-furanones occur in concentrations between 1 and 30 ppm, related to the fresh mass of onions. The exact concentration seems to depend on the grade of infection.

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Poster session-PO-107:

Building a prediction model to distinguish Saint John's wort samples based on their ¹H-NMR profile

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2 Applied, Industrial & Clinical MR Market Division, Bruker BioSpin GmbH,, Rheinstetten, Germany*

Saint John's wort (SJW, *Hypericum perforatum* L.) is a widely spread and well-known medicinal plant. Previous studies conducted in our group (Booker et al. 2018; Scotti et al. in preparation), using a combination of NMR metabolomics and HPTLC, have shown some notable differences exist between materia prima samples' of different origin, some of which are ascribable to geographical provenance,. These "variations" are not represented in SJW European Pharmacopoeia (EP) standard, nor mentioned in its monograph's HPTLC description.

80 samples of SJW crude drug material belonging to our previously acquired collection (Scotti et al. in preparation), were analysed by ¹H-NMR at Bruker (Rheinstetten, Germany). The NMR results were used to build a statistical model using PCA/LDA combined with Monte-Carlo-crossing for assessing the possibility of predicting samples belonging to distinguishable chemical entities, based uniquely on their ¹H-NMR spectra. The trials initially looked at possible distinction based on geographical origin with special consideration for samples from China, known to have a unique fingerprint (Booker et al. 2018, Scotti et al. in preparation). Further trials aimed at finding a predictive model for pharmacopoeial compliance. The resulting models had limited ability to predict geographical provenance but had stronger ability indicating which samples are of Chinese origin (92% correct estimate). A significantly increased capability to determine compliance to the EP standard (up to 99%) was obtained.

One limitation of this study was the circumscribed number of samples belonging to each geographically distinct area, which derived in the necessity to cluster adjacent areas as single groups with larger number of samples, which might not necessarily share the same chemical profile. Therefore, we hope to implement the sample collection to provide stronger bases and more significant geographical boundaries for a more comprehensive predictive model.

References:

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Scotti et al. MS in preparation

Poster session-PO-108:

In vitro approach to study the bioequivalence of Ginkgo biloba L. leaves extracts in human endothelial cells

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Ginkgo biloba L. leaves extract is a remedy traditionally used against age-related cardiovascular dysfunctions and cognitive impairment. Healthy effects of Ginkgo are sustained by biological studies concerning the anti-inflammatory and anti-oxidant effect at vascular level. In some countries the use of ethanol extract in food supplement is preferred to acetone, although the latter is widely cited in the scientific literature. The presence of flavone glycosides and terpene lactones is important for the efficacy of the extract, thus implying that an adequate standardization is mandatory.

This work aims to assess the equivalence between acetone (G24) and ethanol extracts (G4E) from Ginkgo biloba L. leaves in terms of either biological activity in human endothelial cells (HUVEC) challenged with TNF α (20 ng/mL) and chemical profile. Both the extracts were prepared and titrated by LINNEA SA (Riazzino, Switzerland).

The inhibitory effect of G24 and G4E on inflammatory adhesion molecules was not significantly different (IC_{50} reported in Table 1). The activity was due, at least in part, to the impairment of NF- κ B nuclear translocation with comparable IC_{50} s (77.5 and 70.4 μ g/ml, respectively). Notably, the extracts inhibited the release of soluble adhesion molecules (sVCAM-1, sICAM-1 and sE-SEL) with lower IC_{50} values than CAMs exposed on cell surface, thus suggesting the possible involvement of further mechanisms of action.

Moreover, Ginkgo biloba L. has a well-established role in the reduction of oxidative stress during endothelial inflammation. Intracellular ROS formation induced by H_2O_2 (500 μ M) was reduced by 30% and 40% respectively for G24 and G4E (500 μ g/mL), while TNF α (20-50 ng/mL) was unable to induce oxidative stress.

The present study demonstrates for the first time that ethanol and acetone extracts show comparable biological activities in human endothelial cells, providing new insights on the usage of ethanol extracts in those countries where restrictions in amount of acetone are present.

IC ₅₀ (μg/ml)	sICAM-1	sVCAM-1	sE-SELECTIN
G4E	209	1.1	170
G24	129	2.0	137
IC ₅₀ (μg/ml)	ICAM-1	VCAM-1	E-SELECTIN
G4E	25% <u>inhibition at</u> 1000 μg/ml	475.7	840.4
G24	40% <u>inhibition at</u> 1000 μg/ml	372.5	696.1

Tab. 1: IC₅₀ of the extracts on the adhesion molecules

Poster session-PO-109:

A bio-guided approach for the development of a chestnut-based nutraceutical with potential anti-gastritis properties

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Gastritis is a widely spread inflammatory disease, mostly caused by *Helicobacter pylori* infection. Release of IL-8 by the stomach epithelium is a hallmark of gastritis and contributes to the amplification of the inflammatory state. Pharmacological modulation of IL-8 release is a strategy to relieve gastric inflammation and prevent more severe clinical outcomes. In search of nutraceuticals with potential anti-gastritis properties we used a bio-guided approach based on IL-8 secretion by gastric cells to characterize extracts from the fruits of different chestnut varieties. Chestnuts from five varieties (Venégon, Paié, Russirö, Verdésa and Piliscé) of *Castanea sativa* Mill. were collected by the farmer consortium and aqueous and hydro-alcoholic extracts were prepared. Three parts of the fruits, endosperm (kernel) and the outer parts episperm (which directly covers the kernel) and pericarp (the woody part), were separated and extracted with the same solvent.

We found that the ability to inhibit IL-8 secretion correlated with the amount of proanthocyanidins and was associated to the not edible parts of chestnut in all the tested varieties. We also found that the anti-inflammatory activity is preserved upon mild thermal treatment and after in vitro simulated gastric digestion. By combining a robust bio-guided approach with a comprehensive analysis of the tannin fraction of chestnut extracts, we provide evidence for the potential use of chestnut-based nutraceuticals in human gastritis. The bioactive components of chestnut fruits inhibit IL-8 secretion by impairing NF-κB signaling and by other mechanisms, thus opening new applications of proanthocyanidins for inflammation-based diseases.

Phenolic Compounds and Antioxidant Activity Determination of *Tripleurospermum decipiens* (Fisch. & Mey.) Bornm. from Turkey

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Genus *Tripleurospermum* (Asteraceae) is represented in Turkey by 31 taxa, 15 of which are endemic [1]. In traditional Turkish folk medicine some species of the genus used for the treatment of cold and cough [2].

In the current study phenolic compounds of *Tripleurospermum decipiens*(Fisch. & Mey.) Bornm. (Asteraceae) were investigated by LC-MS/MS. Plant material was collected from Adana: Saimbeyli-Yeşilkent (2014) and voucher specimen was deposited in the herbarium of Faculty of Pharmacy, Istanbul University (ISTE 115054). Methanol extracts of the root and aerial parts of *T. decipiens* were investigated by LC-MS/MS. Experiments were performed with a Shimadzu 20A HPLC system coupled with an Applied Biosystems 3200 Q-Trap MS/MS detector. Separations were performed on a GL Science Intersil ODS 250 x 4,6 mm, i.d., 5 µm particle size column operating at 40° C at a flow rate of 0.7 mL/min. Detection was also carried out with a PDA detector. Elution was carried out using a binary gradient of the solvent mixture acetonitrile:water:formic acid (10:89:1, v/v/v) (solvent A) and acetonitrile:water:formic acid (89:10:1, v/v/v) (solvent B). The composition of B was increased from 10% to 100% in 40 min. Analyst 1.6 software was used for the data acquisition and analysis. DPPH and ABTS radical scavenging activity tests were performed for antioxidant activity.

According to the LC-MS/MS analysis, 5-Caffeoylquinic acid and 3,5-Dicaffeoylquinic acid were determined as the main components of both extracts. Luteolin, isorhamnetin and their glucosides were also identified in the aerial parts extract . IC₅₀ value of DPPH radical scavenging was determined 0.18 mg/ml for aerial part extract and 0.17 mg/ml for the root extract. ABTS radical scavenging activity was determined 0,51 mM Trolox Equivalent for aerial part extract and 0,41 mM Trolox Equivalent for root extract.

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Poster session-PO-111:

The sensitive LC/MS method for silver birch (*Betula bendula*) main triterpenoid

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3, Joensuu, Finland

We studied the quantification of silver birch (*Betula bendula*) triterpenoid, papyriferic acid with high performance liquid chromatography and mass spectrometry (LC/MS) using atmospheric pressure electrospray ionization (API-ES), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) techniques, in positive and negative ion modes. The results show that in positive ion mode API-ES exhibited the highest sensitivity compared with the other ionization systems. Ionization process of API-ES under the experimental conditions is soft and only few and small fragment ions were observed. The simple electrospray spectrum of papyriferic acid consisted mainly of $[M+Na]^+$ ions, while also $[M+K]^+$ ions were found. The detection limit for positive ions of papyriferic acid was 100 pg, 400 pg and 3 ng for API-ES, APCI and APPI, respectively. At negative mode, the detection sensitivity of papyriferic acid was fairly low (for API-ES 200 pg and APCI 20 ng) or it was not detected at all (APPI).

Poster session-PO-112:

Understanding the complexity of metabolic pathways via mutant chemotyping: Two phenol biosynthetic genes involved in oat saponin biosynthesis

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Through systematic analysis of a comprehensive suite of oat mutants defective in the biosynthesis of the UV-fluorescent triterpene glycoside avenacin A-1, we characterized two genes overlapping with the avenacin pathway, and participate in the biosynthesis of avenacins. SAD4 is a broad-substrate phenol glucosyltransferase and PAL2 is a phenylalanine ammonia-lyase catalysing the first committed step in phenol biosynthesis. They are involved in avenacin biosynthesis by synthesizing the donors for its glycosylation and acylation.

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Poster session-PO-113:

Using Modern 2D High Performance Thin Layer Chromatography coupled with MALDI-TOF-MS for a first screening approach of plant extracts

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2D development on modern thin layer chromatography in combination with the mass spectrometric method MALDI-TOF-MS (matrix-assisted laser desorption/ionization time of flight mass spectrometry) combines the advantage of a multi development high performance chromatographic separation with a high resolution mass spectrometric method. [1] The combination of these two powerful methods is very helpful for fast and, nearly complete characterization. Plant extracts are difficult to separate, due to their high number of single compounds.[2,3]. Different solvent gradients (stepwise) with different polarities in the two-dimensional chromatography lead to better separation results compared to one-dimensional chromatography and gain various helpful information for the design of following clean-up processes. Nevertheless, not fully separated clusters of substances can be analyzed by using MALDI-TOF-MS which makes it possible to separate substances within the mass spectrometric measurement.

An intuitive visualization of the results makes HPTLC- MALDI-MS coupling a useful method for the analysis of complex plant extract raw materials and new products.

The technique of coupling 2D thin layer chromatography with MALDI-TOF-MS is demonstrated by the identification of Flavonoids and other contents in *Walteria Indica* and *Eugenia uniflora* extracts.

Poster session-PO-114:

Mulberroside A improves the gut-barrier damage by activating NLRP6 inflammasome, preventing hippocampal neuroinflammation in fructose-fed mice

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Recent studies suggest that high-fructose diet induces neuroinflammation in mice¹, yet the underlying

mechanisms are unclear. Here, we explored the possible causal role of the gut microbiota in high-fructose diet-induced neuroinflammation. To this end, mice were fed a control or high-fructose diet with or without broad-spectrum antibiotics for 30 d. Our data showed that high-fructose diet-induced neuroinflammation depended on the presence of the gut microbiota, because neuroinflammation was completely eliminated by antibiotics, although high-fructose diet-induced intestinal barrier impairment was sustained in these mice. Short-chain fatty acids (SCFAs) are products of dietary components fermented by the gut microbiota. We found that high-fructose diet reduced the number of intestinal SCFAs-producing microbiota and fecal SCFAs. The activation of nucleotide-binding oligomerization domain protein-like receptors, pyrin-domain containing (NLRP)-6 inflammasome, a recently described essential mechanism of maintaining intestinal barrier and healthy gut microbiota², was also inhibited in mice fed with fructose. Treatment of high-fructose diet-mice with SCFAs could activate NLRP6 inflammasome, maintain intestinal barrier integrity and reduce neuroinflammation. Mulberroside A is a natural polyhydroxylated stilbene compound isolated from the roots and twigs of *Morus alba* L. It is known for its antibacterial, anti-inflammatory, antioxidant and neuroprotective effects. We found that mulberroside A could reverse gut microbiota dysbiosis, increase the concentration of fecal SCFAs, activate NLRP6 inflammasome, maintain intestinal barrier integrity and reduce neuroinflammation. These results not only suggest that gut microbiota dysbiosis may provide a new pathogenic mechanism for fructose-induced neuroinflammation, but also indicate that mulberroside A may be a new therapeutic approach to prevent gut microbiota dysbiosis and neuroinflammation induced by high-fructose diet.

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[2] Levy M, Thaïs CA, Zeevi D, et al. *Cell* 2015; 163:1428-43.

Poster session-PO-115:

Chemical profiling of Katangan *Vitex* species used in traditional human and veterinary medicine (DR Congo)

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Leaves, root bark, stems and fruits of some Katangan *Vitex* species (Lamiaceae), are widely used in traditional medicine to treat diabetes, diarrhea, and parasitic diseases, notably malaria. The observed morphological variability of *Vitex* species may imply a variability in their complex chemical compositions; their chemical profiles are then likely to vary according to morphology as well as to collection site. High

performance thin-layer chromatography (HPTLC), thanks to the holistic fingerprints produced, is often recommended for the quality control of plant material.

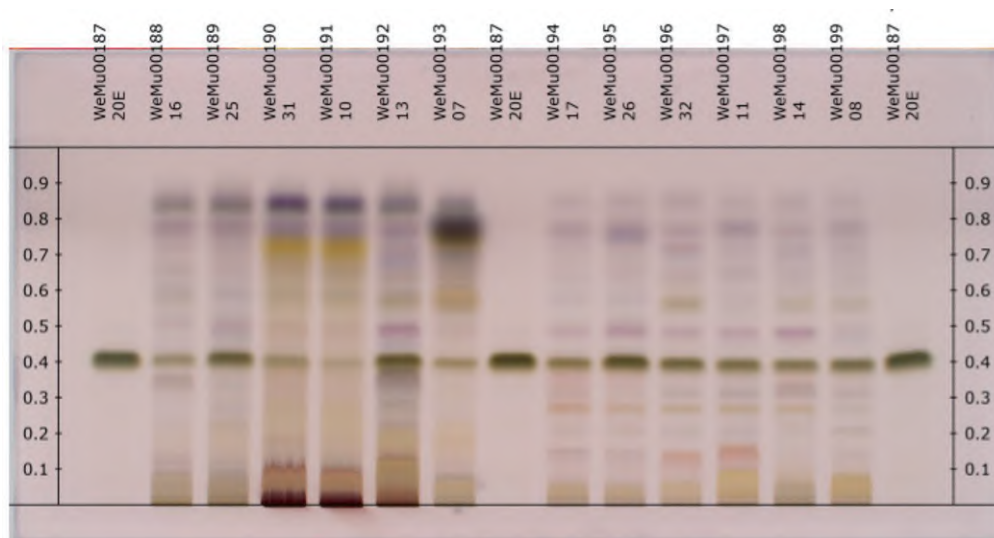
This study aims to evaluate the chemical variability of five *Vitex* species and their morphotypes, by establishing chemical profiling methods. Leaves, root bark, stems and fruits samples were collected from various parts of Katanga and methanolic extracts were used to profile flavonoids, iridoids, diterpenes and triterpenes. Using Camag application, development and imaging instruments, HPTLC methods were developed and optimized, images being captured under UV (254 nm and 366 nm) and white light, before and after derivatization.

In methanolic extracts, organ-specific bands could be detected; we could identify some compounds, i.e. 20-hydroxyecdysone, aucubin, casticin and rotundifuran. Iridoids profiling yielded a very intense band common to all the samples investigated.

Although the chemical profiling of studied species showed strong similarities between them, there are certain specific bands that allow to differentiate them. The different morphotypes can be recognized by intensity differences in some major bands and by the presence or absence of certain bands.

Figure 1

Identification of 20-hydroxy-ecdysone in methanolic extracts of various *Vitex* samples. Mobile phase : AcOEt-MeOH (80:20, v/v); detection : vanillin + sulfuric acid, 105°C, 5 min



Poster session-PO-116:

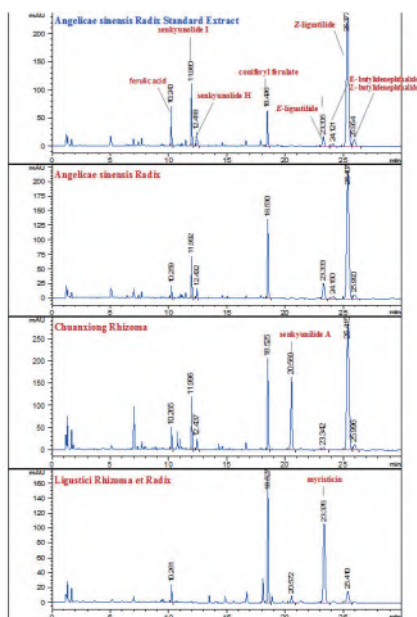
Characteristic chromatogram for *Angelicae Sinensis Radix* and its adulterates based on simultaneous determination of eight components with single reference standard

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Abstract: A characteristic chromatogram combined with single standard to determine multi-components (SSDMC) method was established by high-performance liquid chromatography (HPLC) to evaluate the quality of *Angelicae sinensis Radix* (ASR) through a simultaneous determination of eight components, including ferulic acid (FA), senkyunolide I (SI), senkyunolide (SH), coniferyl ferulate (CF), E -ligustilide (EL), E-butylidenephthalide (EB), Z -ligustilide (ZL) and Z-butylidenephthalide (ZB). Since most of the eight components were unstable and difficult to obtain, either FA or ZL can be selected as the single marker, and the contents of the other seven compounds were determined by their respective conversion factors (0.46-1.15). The chromatogram obtained could be used to characterize ASR and its adulterates, including *Chuanxiong Rhizoma* (CR) and *Ligustici Rhizoma et Radix* (LR) (Fig. 1). The method was validated for accuracy, precision, specificity, linearity, range, stability, ruggedness, robustness, and then successfully applied to evaluate 44 batches of ASR from four origins of China, including Gansu, Sichuan, Hubei and Yunnan. Results showed this SSDMC method could obtain true value compared with external standard method, and the eight components assayed had no significant difference among the four origins. The contents of total ferulic acids and total butylidenephthalides can both be used to evaluate the quality of ASR with acceptance criteria set as 0.15% and 0.70% respectively. This is a simple, rapid, low-cost and reliable method established for quantitative analysis and quality control of *Angelicae sinensis Radix*, as well as *Chuanxiong Rhizoma* and *Ligustici Rhizoma et Radix*.

single standard to determine multi-components, conversion factor, quality control, *Angelicae Sinensis Radix*



Poster session-PO-117:

New pterocarpan and derrisisoflavones from the root of *Millettia aboensis*

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Pterocarpan and derrisisoflavones have been associated with a broad range of biological activities, including insecticidal, antimicrobial, cytotoxic, and antioxidant activities. These phytochemicals have been isolated from the genus *Derris* belonging to the Leguminosae family [1]. In the present study, some pterocarpan and derrisisoflavones were isolated from the root bark of *Millettia aboensis* using a combination of different chromatographic techniques including VLC, Sephadex LH-20 separation and semi-preparative HPLC. The structures of the compounds were elucidated by a combination of MS and NMR spectroscopy. The crude methanol root extract was screened for its cytotoxic activity on mouse lymphoma cell line (L5178Y) and the isolated compounds were tested for their antioxidant activity using 2, 2-diphenylhydrazyl (DPPH) radical scavenging model. The crude methanol root extract exhibited a growth inhibition of 87.5% on mouse lymphoma cell line (L5178Y). The ethyl acetate fraction of the methanol root extract of *M. aboensis* yielded three new compounds; one pterocarpan, 9-hydro-3,8-dimethoxyl pterocarpan (1); two derrisisoflavones, derrisisoflavone L (3) and derrisisoflavone M (4), which were identified on the basis of spectroscopic techniques. Two known compounds maackiain (2) and derrisisoflavone G (5) were also isolated. The isolated compounds showed promising antioxidant activity with compound 2 having the highest activity with an IC₅₀ of 83 µg/ml. This is the first report on the isolation of pterocarpan and derrisisoflavones from the root of *M. aboensis*. These compounds hold great potential for development into novel therapeutic molecules

Reference

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Poster session-PO-118:

Chemical constituents from *Siegesbeckia pubescens*

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Lee, Bang Yeon Hwang

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Siegesbeckia pubescens Makino (Compositae) is an annual herb widely distributed in Korea, China, and Japan. The aerial parts of this plant have been used as traditional medicine to treat rheumatic arthritis, asthma, hypertension, and malaria.^[1] Previous phytochemical investigations of the genus *Siegesbeckia* have revealed the presence of sesquiterpenoids, ent-kaurane and ent-pimarane type diterpenoids.^[2] In the course of ongoing research for the discovery of inhibitors of nitric oxide (NO) production, the methanolic extracts of *S. pubescens* were partitioned with n-hexane, CH₂Cl₂, EtOAc, and water, successively. The CH₂Cl₂ fraction and ethyl acetate fraction were chromatographed on silica gel, Sephadex LH-20, RP-18, and preparative HPLC. As a result, two new germacrane sesquiterpenoids (1-2) and a new ent-pimarane diterpenoid (3) along with eighteen known compounds were isolated. The structure of these compounds was determined by 1D-(¹H, ¹³C), 2D-(HSQC and HMBC) NMR, and HR-ESI-MS spectrum. Highly oxygenated germacrane type sesquiterpenoids (1-2 and 13-14) showed significant inhibitory effects with IC₅₀ values ranging from 3.9 to 16.8 μM.

[1] Li H, Kim JY, Hyeon J, Lee HJ, Ryu JH. *Phytotherapy research* 2011; 25: 1323-1327

[2] Wang JP, Zhou YM, Ye YJ, Shan XM, Cai YL, Xiong CM, Hu HX. *Journal of ethnopharmacology* 2011; 137: 1089-1094

Poster session-PO-119:

Secondary Metabolites of The Roots of *Artemisia Argyi*

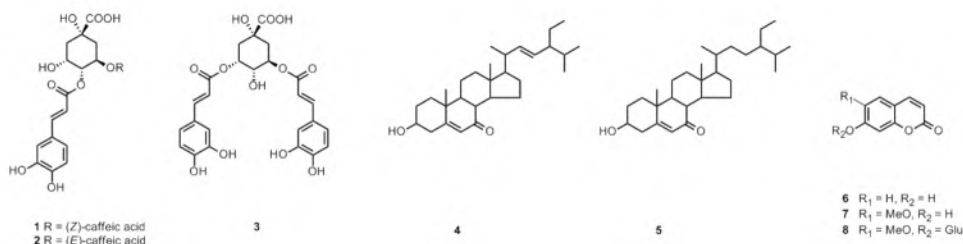
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The leaves of *Artemisia argyi* Levl. et Vant have been used as a hemostatic and sedative agent in traditional Chinese medicine [1]. However, there was no research on the secondary metabolites of the roots of *A. argyi* up to date. Repeated chromatography of EtOAc-soluble fraction from 70% EtOH extract of the roots of *A. argyi* led to the isolation of a new caffeoylquinic acid, (E,Z)-4,5-di-O-caffeoylquinic acid (1), along with two dicaffeoylquinic acids, (E,E)-4,5-di-O-caffeoylquinic acid (2) and (E,E)-3,5-di-O-caffeoylquinic acid (3), two sterols, 7-ketostigmasterol (4) and 7-ketositosterol (5), and three coumarins, umbeliferone (6), scopoletin (7), and scopolin (8). The structure of the new compound 1 was elucidated by physical and spectroscopic data interpretation.

[1] Yoshikawa M, Shimada H, Matsuda H, Yamahara J and Murakami N. Chemical and pharmaceutical bulletin 1996; 44(9): 1656-1662;



Poster session-PO-120:

Studies on Phytochemical Constituents from *Petasites Japonicus*

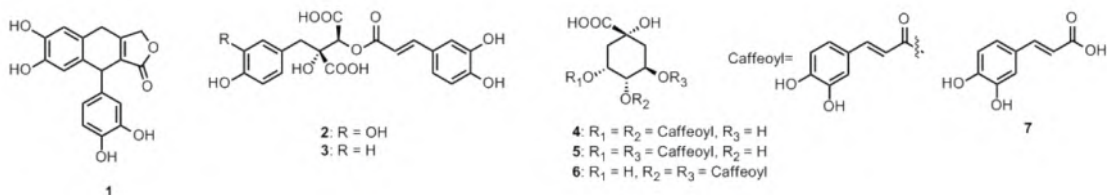
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Petasites japonicus Maxim is a perennial herb that belongs to the Compositae. The leaves or stalks of *P. japonicus* commonly consumed as vegetable in Korea and Japan. Moreover, the aerial parts of *P. japonicus* has been used as a Japanese folk medicine for antitussive, antipyretic, or wound healing agent. [1] Phytochemical study on a hot water extract from the leaves of *P. japonicus* resulted in the isolation and characterization of a new lignan (1), along with two phenolic compounds (2 and 3), three dicaffeoylquinic acid (4-6), and one phenylpropanoid (7) having previously known chemical structures. The structure of the new compound 1 was determined by interpretation of spectroscopic data, particularly by 1D- and 2D-NMR studies.

[1] Mizuno M., Tanaka T.. Nihon Yakuso Zensyo. Shin-Nihon-kouki: Tokyo; 1995: 557-559



Poster session-PO-121:

Beneficial influence of an extract from *Aronia melanocarpa* L. berries against low-level exposure to cadmium-induced disturbances in the liver glutathione homeostasis

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Recently the growing interest has been focused on the possibility of using natural antioxidants, including polyphenols, in order to protect against the unfavourable outcomes of exposure to cadmium (Cd) [1]. Oxidative stress is recognized as one of the mechanisms of this metal hepatotoxicity. The aim of this study was to examine whether the consumption of a polyphenol-rich extract from *Aronia melanocarpa* berries (AE) may prevent Cd-induced disturbances in the concentration of reduced and oxidized glutathione (GSH and GSSG) and their ratio (GSH/GSSG), as well as the activity of glutathione reductase (GR) in the liver. Wistar rats were administrated a 0.1% aqueous AE as a drinking fluid and/or Cd in a diet (1 and 5 mg/kg) for up to 24 months. The concentrations of GSH and GSSG were measured in the liver homogenates by using Glutathione Assay Kit by Cayman Chemical Company, and their ratio was calculated. The activity of GR was assayed using BIOXYTECH® GR-340™ kit by Percipio Biosciences. The exposure to Cd increased the concentration of GSSG, decreased the concentration of GSH and the ratio of GSH/GSSG, and influenced the activity of GR in the liver, whereas the administration of AE during this metal intoxication increased the concentration of GSH and the ratio of GSH/GSSG, decreased the concentration of GSSG, and had modifying effect on the activity of GR in this organ. The results allow the conclusion that consumption of *Aronia melanocarpa* berries may prevent Cd-induced disturbances in the liver homeostasis of glutathione.

[1] Brzóska MM, Rogalska J, Roszczenko A, Galazyn-Sidorczuk M, Tomczyk M. *Planta Med* 2016;82:621-631

Acknowledgements: This study was financially supported by a grant (No. 136/KNOW/15) from KNOW (Poland)

Poster session-PO-122:

Ethanol in medicines for children: Food more relevant than phyto-medicines

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Introduction:

Liquid dosage forms of phytomedicines are very suitable for children, as they allow the adaption of the dose to the age group. However, as they contain ethanol in many cases, they have been repeatedly triggering critical questions.

Materials and Methods:

Therefore, data from non-interventional studies on herbal medicinal products containing ethanol were evaluated. In addition, analytical data for ethanol in food items usual for children were generated by gas chromatography and a scenario for the exposure of a 6 year-old child to ethanol through food intake was generated.

Results and Discussion:

When using common herbal medicinal products, an estimation of the ethanol intake for a 6 year-old child results in a dose of between 70 and 180 mg per single dose. With an intake 3 times daily and related to a body weight [b.w.] of 20 kg, this is 10.1 – 27.0 mg/kg per day [1].

An evaluation of the side effects of these herbal medicinal products was based on non-interventional studies in more than 50.000 children and on spontaneous reports from the use in approximately 3 million children, and did not reveal any ethanol related side effects.

For evaluating the uptake of ethanol with food items commonly used in children, ethanol contents in food items were measured [3]. Based on these data, a scenario for the mean ethanol exposure was developed, using data on nutritional habits from USA and Germany. The resulting scenarios lead to an uptake of up to 12.5 – 23.3 mg/kg b.w..

Conclusions: According to these data, the ethanol uptake with herbal medicinal products in children is in an order of magnitude comparable to everyday exposure with usual food items, and therefore there is no cause for toxicological concerns.

References

[1] Kelber O et al. *PharmInd* 2008, 70:1124–1127

[2] Kelber O et al. *Wien Med Wochenschr* 2016, 167:183-188

[3] Gorgus E et al. *J Analyt Toxicol* 2016, doi:10.1093/jat/bkw046

Poster session-PO-123:

Mode of action of a herbal combination used in painful complaints in degenerative and inflammatory rheumatic diseases

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Herbal medicinal products such as STW 1, a combination product containing extracts of trembling poplar leaves and bark, golden rod herb and common ash bark, are used in painful complaints caused by degenerative and inflammatory rheumatic diseases. This use is based on a large number of clinical studies [1].

Therefore, the question is which mechanisms of action contribute to this therapeutic effect?

Accordingly, a review was conducted with the aim to identify data on the pharmacology of this product.

As could be shown, the action of the combination product as well as those of its components included antiinflammatory, antioedematous, antioxidative and analgesic properties. These modes of action are even broader than those of synthetic antirheumatics.

Accordingly, these data can explain the action and efficacy of STW 1 in randomised, placebo- or verum-controlled double-blind trials, as well as in non-interventional studies, which have been performed in different subtypes of rheumatic diseases and even documented as a successful combination therapy with non-steroidal antiinflammatory drugs (NSAIDs).

Accordingly, STW 1 complements NSAIDs and cyclooxygenase 2 (COX-2) inhibitors in particular in these indications.

Reference: [1] Gundermann KJ and Müller J. *Wien Med Wochenschr.* 2007;157(13-14):343-7

Poster session-PO-124:

Action of aqueous willow bark extract in rheumatic complaints, fever and headache - more than just tradition

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Willow bark extracts are authorized for rheumatic complaints and headache. As they contain a large number of polar constituents, the salicylates being the basis of their standardization, relevance of these for the mode

of action is a matter of interest, especially with regard to the use of aqueous willow bark extracts as STW 33-I in therapy.

Therefore a literature review was conducted, focussed on the mode of action of willow bark extracts and fractions thereof.

This review revealed a considerable number of pharmacological studies [1], including in vitro- and in vivo studies in different pharmacological models [2], supporting the assumption that especially the polar fractions of the extract contribute to the anti-inflammatory action. Salicylic acid derivatives and their metabolites [3], together with different other polyphenols, were identified as relevant for the mechanisms of action, as was also confirmed by gene expression analyses [4].

Accordingly, by studies on the mode of action of an aqueous willow bark extract, STW 33-I, it could be shown, that the action is based on more than just tradition, given on the broad available evidence on willow bark and the salicylates, and can be well explained on a molecular level.

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[2] Khayyal MT et al., *Arzneimittelforschung.* 2005;55(11):677-87.

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Poster session-PO-125:

Health care research in phytomedicine: PhytoVIS, a NIS in 20,870 users of herbal medicinal products

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³ *CRI – Clinical Research International Ltd., Köln, Germany*

Information on the use of herbal medicinal products is at present available only from product specific studies, sales statistics and pharmacovigilance data. The data base PhytoVIS was therefore created as a tool to retrospectively document user's experience with these products.

The survey was conducted in pharmacies and doctors' practices in Germany by students of pharmacy or medicine. The only inclusion criterium was the use of herbal medicinal products within the last 8 weeks before the survey. Based on the therapeutic indication, the efficacy and tolerability of the products were endpoints. Furthermore, information on the point of sale and the recommendation of the use of the product was requested.

20,870 patients were included and their experience was documented in 24,056 questionnaires. In 78% of the participants, treatment was conducted for acute complaints, with 47% suffering from cold, flu and fever, 11% from bowel pain and digestive complaints and 9% from stomach and biliary complaints. Overall, 1433 different products were used. In 45% of the participants, the effect of the products was described as very good and in 39% as moderate. Products were mainly recommended by the pharmacists (35%) and physicians (27%). The products were bought in the pharmacy in 84% of the cases.

PhytoVIS turned out to be a suitable tool to study tolerability, safety and efficacy based on the rating of the patients. By the high number of users included, special patient groups as toddlers, pregnant women and elderly were also covered. The data base is an excellent source for medical information on the full range of herbal medicinal products in use.

Acknowledgement: The study is supported by Kooperation Phytopharmaka, Bonn, Germany

Poster session-PO-126:

Modern bioautography - a fast analytical tool to discover active compounds in plant extracts used for cosmetics

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Today's high-performance thin-layer chromatography (HPTLC) combines the advantage of a robust chromatographic separation with high sample throughput and the possibility of simple coupling to additional analytical techniques, like mass spectrometry (MS) or bioassays [1]. This increases the amount of information for a fast and efficient activity-based screening and the identification of raw materials for new cosmetic products.

Lengthy, time-consuming clean-up processes for first activity tests are avoided by adapting the activity test directly on the HPTLC plate. Assays for special target activities can be performed for many samples in parallel after chromatography. The transfer of discovered active substance zones to the mass spectrometer allows the direct characterization of the target substance without additional clean-up steps. Examples for such an "activity based" screening of plant extracts will be presented.

Poster session-PO-127:

Chemical Composition and Biological Activity of the Essential Oil from *Jatropha*

pelargoniifolia Root Indigenous to the Arabian peninsula

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The essential oil of *Jatropha pelargoniifolia* roots, obtained by hydrodistillation, was characterized in terms of its chemical composition by GC-FID. The analysis revealed the presence of 80 compounds, representing 99.99% of the total oil, with 77.31% of sesquiterpenes, 14.62% fatty acids, 7.21% of other components (i.e. phenolics hydrocarbons/ cyclic) and 0.85% of monoterpenes. The major compounds of the oil were γ -eudesmol (35.31%), 5-guaien-11-ol (14.43%), epi-cedrol (8.19%), oleic acid (5.23%), bulnesol (4.45%), α -linoleic acid (4.20%), 3,4-dimethoxycinnamic acid (3.83%), palmitic acid (2.69%), isolongifolanone (2.68%), eicosane (1.41%) and cedrol (1.14%). Oxygenated sesquiterpenes was found to be the major group and represents more than half percentage of the oil content. It consists almost entirely of γ -eudesmol (35.31%), 5-guaien-11-ol (14.43%) and epi-cedrol (8.19%).

Moreover, the essential oil was evaluated for its potency as anti-inflammatory, antioxidant, antipyretic and antinociceptive agents by using in vivo and in vitro models. The anti-inflammatory activity was screened by using the carrageenan-induced paw edema and cotton pellet granuloma effect in rats. The yeast-induced hyperthermia in mice method was used for evaluation of its antipyretic potency. Antinociceptive effect was assessed via hot plate method, acetic acid-induced writhing and tail flick in mice methods. Additionally, the antioxidant potential for the oil was evaluated using various in vitro antioxidant tests, including DPPH•, ABTS•+ and FRAP. Finally, results for essential oil biological investigations were satisfactory, especially at dose 240 μ l/kg, for the anti-inflammatory, antipyretic and antinociceptive activities and showed significant ($p < 0.001$) effect compared with standard drug. As well, the antioxidant activity for root oil was superior to ascorbic acid. These findings demonstrated that the investigated essential oil of *Jatropha pelargoniifolia* root could be used as a natural source for their anti-inflammatory, antinociceptive, antipyretic and antioxidant effects.

Poster session-PO-128:

Studies on the Chemical Composition of Essential Oils of *Jatropha pelargoniifolia* and *Jatropha gluca* endogenous on the Arabian peninsula

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Many of the *Jatropha* species have found applications in folk medicine. This study aimed to evaluate the biological activity of *Jatropha pelargonifolia* and *Jatropha glauca* total alcoholic extracts for root and aerial parts Euphorbiaceae family indigenous on the Arabian peninsula. *J. glauca* and *J. pelargonifolia*, Euphorbiaceae, were collected in September 2015 from Wadi

Shogare and Wadi Mojasas, respectively in Jazan south area of Kingdom of Saudi Arabia. In vivo and in vitro studies for their possible effects as hepatoprotective, antinociceptive, anti-inflammatory and antidiabetic/hypoglycemic medicinal herbal drug in comparison with standard clinical drugs. Hepatoprotective was assessed via serum biochemical parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and total bilirubin. Tissue parameters such as malonaldehyde (MDA), non-protein sulfhydryl groups (NP-SH) and total protein (TP) were also measured. Antinociceptive activity was explored by using hot plate and writhing methods. In addition, the anti-inflammatory effect was evaluated by using carrageenan-induced paw edema. Finally, hypoglycemic and antidiabetic activities in alloxan induced diabetic mice were evaluated for both *Jatropha* plants root and aerial parts. In conclusion: The root of *J. glauca* showed significant hepatoprotective and antinociceptive activities. The *J. pelargonifolia* root revealed higher anti-inflammatory effect and hypoglycemic activity. Finally, *J. glauca* aerial part achieved significant antidiabetic activity in alloxan induced diabetes compared with glibenclamide.

Poster session-PO-129:

Secondary metabolites from the whole plants of *Gynura procumbens* (Lour.) Merr

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procumbens (Lour.) Merr. is a medicinal plant commonly found in tropical Asia countries. Traditionally, it is widely used in many different countries for the treatment of a wide variety of health ailments such as kidney discomfort, rheumatism, diabetes mellitus, constipation, and hypertension [1]. Modern pharmacological research on *G. procumbens* has shown that it has multiple biological activities, such as antihypertensive, cardioprotective, antihyperglycemic, fertility enhancement, anticancer, antimicrobial, antioxidant, organ protective, and anti-inflammatory activity [2]. However, few studies have focused on the chemical constituents from *G. procumbens*. In the current work, phytochemical investigation of whole plants of *G. procumbens* led to the isolation and purification of ten compounds. The structures of these compounds were determined on the basis of their ^1H NMR, ^{13}C NMR, and ESI-MS spectroscopic data, and by comparison with those previously reported in the literature, as compounds (1–10): hexacosanoic acid (1), β -sitosterol (2),

daucosterol (3), β -stigmasterol (4), 5 α -stigmastna-3-one (5) methyl linoleate (6), 1-methoxyhenicosan-1-ol (7), homoorientin (8), kaempferol (9), and eriocitrin (10). It is worth noting that all the ten compounds (1~10) were isolated here for the first time from *G. procumbens*, and compounds 1, 5-8, and 10 have never previously been reported from *Gynura* genus.

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Poster session-PO-130:

Immunomodulatory effects of polysaccharides isolated from *Cinamomum camphora* fruits

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Natural polysaccharides are an important class of macromolecules that can profoundly affect the immune system and, therefore, show the potential for use as immunomodulators with broad clinical applications [1]. *Cinamomum camphora* has been shown to possess a lot of biological activities, including antifungal, antioxidant, antibacterial, anti-allergic, and anti-inflammatory activities [2-3]. However, little research has been reported on the *C. camphora* fruit polysaccharides (CCFP). Therefore the objectives of this study were to isolate and characterize the polysaccharides from *C. camphora* and to evaluate its immunomodulatory activities on RAW264.7 macrophages in vivo. The results indicated that CCFP has an average molecular weight of 2.53×10^5 Da and is mainly composed of rhamnose, arabinose and galactose in a molar ratio of 1:3.75:4.56. CCFP significantly increased the concentrations of intracellular nitric oxide (NO) and cytokines, such as prostaglandin E₂ (PGE₂) and tumor necrosis factor- α (TNF- α) in RAW 264.7 cells. The result of RT-PCR analysis indicated that CCFP also enhanced inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and TNF- α expression. Further analyses demonstrated that CCFP rapidly activated the extracellular signal-regulated kinase (ERK) and the transcriptional activities of activator protein-1 (AP-1) and nuclear factor (NF)- κ B via toll-like receptor 4 (TLR4). Taken together, these results suggest that CCFP can improve immunity, and could be explored as a potential immunomodulatory agent.

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This work was funded by the Science Foundation for Young Doctors of Jiangxi academy of science (2016-YYB-07), the Science and Technology Major Project Foundation of Jiangxi Academy of Sciences (2018-YZD2-18) . Foundation of Key Project of Research and Development Program of Jiangxi Province (No. 20171BBH80017)

Poster session-PO-131:

The effect of galactans from jackfruit *Artocarpus heterophyllus* Lam. on human colon carcinoma cells cultured in vitro

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The phytochemical studies revealed that jackfruit (*Artocarpus heterophyllus* Lam.) was rich in bioactive compounds including mainly carotenoids, flavonoids, volatile acids, tannins, and lectins, which are responsible for several biological activities. The purpose of the present study was to analyse biological activity of (1 → 4)-β-D-galactans isolated from jackfruit in the field of immunomodulatory, cytotoxic and antioxidant effects connected with human colon carcinoma cells viability and proliferation. The biological activity of galactans from *Artocarpus heterophyllus* revealed no toxic influences on viability of human colon tumor cells (HT29 and SW620) analyzed by Neutral Red (NR) uptake assay. After 24 h and 48 h of incubation cellular viability was no lower than 94%. Similarly, metabolic activity of cells (MTT test) at the highest compound concentration (250 μg/mL) applied was higher than 92% in comparison to control. At the same concentration, galactans had no significant influence on morphology of cells as visualized by May-Grünwald-Giemsa (MGG) staining. Nitric oxide production by tumor cells after their incubation with galactans showed changes in the radical level dependently on time of incubation and previous cells' 2 h stimulation with endotoxin (LPS). Tested galactans stimulated significant amounts of IL-1β

production by HT29 and SW620 cells. The compound at the concentration of 200 µg/mL stimulated almost 2-times higher (5.7 pg/mL) amounts of the cytokine as compared to the level released by HT29 cells. IL-6 level increased but IL-10 decreased in a galactans concentration dependent manner after incubation with HT29 cell culture. The effect was not detected in SW620. Tested galactans had strong concentration dependent reducing activity of DPPH and Fe³⁺ ions. The highest galactans concentration applied (250 µg/mL) reduced DPPH at the level corresponding to 16.2 µg/mL of Trolox, while in the case of FRAP method it was equivalent to 48.4 µg/mL of ascorbic acid.

Poster session-PO-132:

Metabolization of Silymarin constituents by human fecal bacteria in vitro

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Silymarin is a special fruit extract from milk thistle (*Silybum marianum* (L.) Gaertn., Asteraceae) predominantly consisting of stereo- and regioisomeric flavonolignans. It is mainly used for the supportive treatment of liver diseases. Since the oral bioavailability of *S. marianum* flavonolignans is very low [1], they presumably reach the colon and may be metabolized by gut microbes.

In order to assess gut microbial metabolization of the main constituents of Silymarin, it was incubated (0.21 mg/ml) with 0.1% human fecal suspension under physiological conditions (anoxic, 37°C). Samples were taken after 0.5 h, 4 h and 24 h of incubation and analyzed by LC-MS metabolomics for metabolic profile changes.

After 4 h of incubation, dihydroquercetin, the main Silymarin flavonoid, was almost completely metabolized, while the flavonolignan levels did not yet significantly change. 3-(3-Hydroxyphenyl)- and 3-(4-hydroxyphenyl) propionic acid were identified as putative dihydroquercetin metabolites.

After 24 h of incubation, also the levels of all main *Silybum* flavonolignans were significantly reduced, and three different series of main putative flavonolignan metabolites could be detected on the basis of HRMS data: (a) Four compounds with a molecular weight of 484.1364 were tentatively assigned as metabolites formed by cleavage of the dioxane ring; (b) two compounds with molecular weight 468.1050 were tentatively assigned as demethylation products; (c) five compounds with molecular weight 470.1207 were tentatively assigned as metabolites formed by demethylation plus dioxan ring cleavage.

These results indicate that human gut bacteria may indeed be capable to metabolize *S. marianum* flavonolignans. Semipreparative isolation and NMR-based structure elucidation of the flavonolignan

metabolites are in progress.

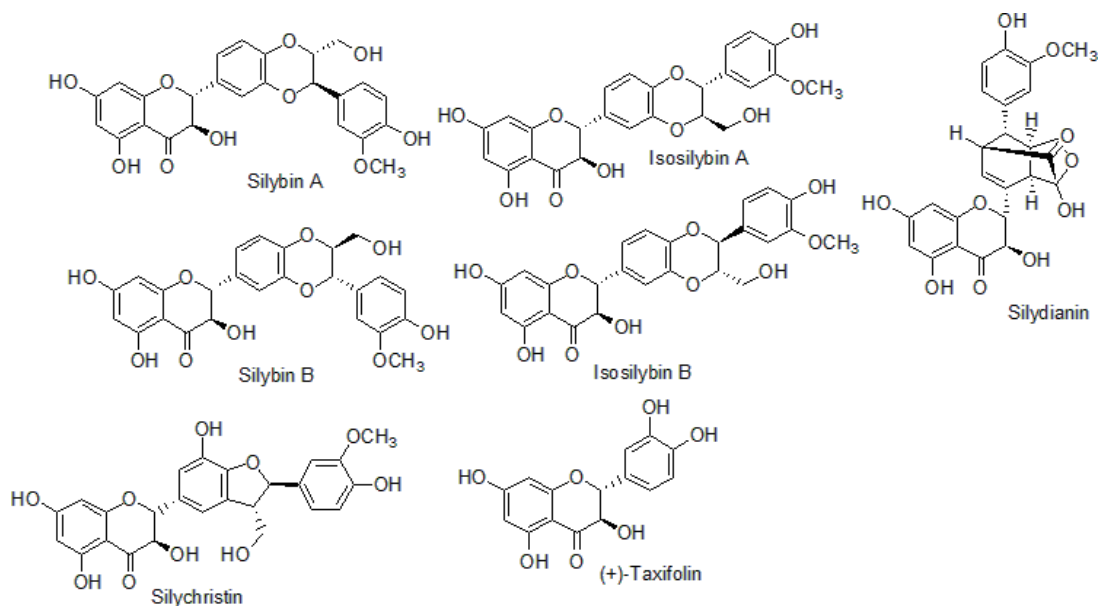


Figure: Main constituents of Silymarin

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Poster session-PO-133:

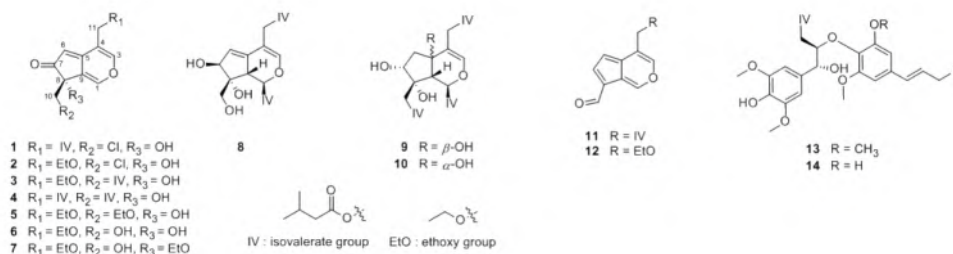
Studies on Phytochemical Constituents from *Petasites Japonicus*

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Patrinia scabra Bunge is a perennial herb that belongs to the Valerianaceae . The roots of *P. scabra* have been used as a traditional Chinese medicine to treat malaria, dysentery, leukemia, gastric cancer , typhoid fever, injuries from falls, and leucorrhoea.[1] Repeated chromatography of a 70% aqueous EtOH extract of the roots of *P. scabra* led to the isolation and characterization of ten new iridoids (1-10), along with two iridoids (11 and 12) and two lignans (13 and 14) having previously known chemical structures. The structures of the new compounds were determined by interpretation of spectroscopic data, particularly by 1D- and 2D-NMR studies.

[1] Editorial Board of Chinese Herbal, State Administration of Traditional Chinese Medicine, Chinese herbal:



Poster session-PO-134:

Secondary metabolites of the sapwood of *Juglans sigillata* Dode

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Juglans sigillata Dode (Juglandaceae) is a fast growing deciduous tree, which is grown native in Tibet, Yunnan, Sichuan, and Guizhou provinces of China [1] . The plant extracts of *Juglans* species are widely used as folk remedies to cure or prevent eczema, cancer, and rheumatic diseases [2] . Fresh husks of some species in *Juglans* genus , officially listed in Compendium of Materia Medica as " Qing Long Yi" , have been medicinally used in Eastern Asian countries for thousands of years due to their antitumor, antiinflammatory , antioxidant, and antinociceptive effects [3] . Previous phytochemical study of *J. sigillata* stem barks and fruit husks resulted in the purification of flavonoids, tannins, α -tetralones, diarylheptanoids, naphthalene glucosides , and their derivatives [4] . However, up to now , no investigation has ever been performed to investigate the secondary metabolites in the sapwood of *J. sigillata* . In this work , we reported the isolation and structural elucidation of a new cis - caffeoyl isoflavone glycoside , 3'-methoxy-5'-hydroxy-isoflavone-7-O-(4"- cis - caffeoyl)- β -D-glucopyranoside (1), and two known flavonol glucosides, quercetin -3-O - β -D-glucopyranoside (2) and kaempferol -3-O - β -D-glucopyranoside (3), from the sap wood of *J. sigillata* for the first time.

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Acknowledgements:

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Poster session-PO-135:

Inhibition activities of selected extracts and flavonoids from *Bidens tripartita* on serine proteases

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Proteases play a key role in a variety of pathologies, including cancer, pancreatitis and thrombosis, which can be treated with low molecular inhibitors. Analyses of trypsin, thrombin and urokinase inhibition activity for selected extracts (BH4, diethyl ether; BH5, ethyl acetate, BH6, n-buthanolic) and flavonoids luteolin, cynaroside, (luteolin 7-O-glucoside), favanomarein from *Bidens tripartia* herb were determined. Quercetin was used as a positive control in tests on serine proteases (trypsin, thrombin and urokinase). BH5 showed a highly selective effect with a value of percentage inhibition activity (IA) = 91.68% for thrombin. Interesting results were observed in IA = 90.57% for BH6 for urokinase. The highest value of IA = 97.75% on plasmin was observed for BH4. Among the tested flavonoid compounds, the highest percentage inhibition activity with respect to the tested enzymes was shown by cynaroside indicating IA = 83.77-97.88%. The obtained results suggest that both extracts and the tested compounds are potential therapeutic resources for the development of serine proteases inhibitors.

Poster session-PO-136:

Eupatoriopicrin inhibit the pro-inflammatory functions of human neutrophils via suppression p38 and ERK 1/2 MAP kinases pathways

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The p38 and ERK MAPK pathways are activated in human neutrophils by chemo attractants, pro-inflammatory cytokines, lipopolysaccharide (LPS), and Fcγ receptor ligation. Pharmacologic inhibition of p38 MAPK activation attenuates neutrophil respiratory burst activity, exocytosis, chemotaxis, adhesion, IL-8 synthesis and stress-induced apoptosis. Pharmacologic inhibition of ERK activity enhances neutrophil apoptosis, while the role of ERK in respiratory burst activity remains controversial [1]. Inhibition of both kinases is a promising therapeutic strategy to treat chronic inflammatory diseases.

The present study tested the hypothesis that eupatoriopicrin (sesquiterpene lactone, isolated from *E.cannabinum* L.) mediates multiple p38 and ERK1/2 MAPK-dependent responses in human neutrophils.

To verify the hypothesis we tested activity of eupatoriopicrin and positive controls (quercetin and clarithromycin) on LPS and f-MLP-stimulated neutrophils. We examined: (I) phosphorylation level of p38, ERK1/2 and JNK MAP kinases, (II) degranulation; respiratory burst activity and elastase release, (III) pro-inflammatory cytokine release (IL-8, IL-1β and TNFα) and (IV) apoptosis.

Phosphorylation of p38, ERK1/2 and JNK MAPK was determined by immunoblotting analysis. The inhibition of ROS production was determined using luminol dependent chemiluminescence method. Neutrophil elastase release was established spectrophotometrically. The effect on chemokines production was measured by enzyme-linked immunosorbent assay (ELISA). The apoptosis of neutrophils was analyzed with flow cytometry.

Inhibition of p38 kinase activity by eupatoriopicrin significantly attenuated degranulation and pro-inflammatory cytokine release. Respiratory burst and elastase activity were significantly inhibited by eupatoriopicrin at 2.5μM, compared with quercetin and clarithromycin tested at 50 μM . Lipopolysaccharide-induced IL-8 and TNFα production was significantly inhibited by eupatoriopicrin at 0.25 μM ($IC_{50} < 1 \mu M$). Inhibition of p38 and ERK1/2 activity by eupatoriopicrin at 0.25 μM resulted in an abolition of LPS-delay of neutrophils apoptosis. These data suggest that eupatoriopicrin mediates both p38- and ERK MAPK-dependent neutrophil responses leading to potential beneficial health effects.

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Poster session-PO-137:

Lignans from *Forsythia x intermedia* leaves and flowers attenuate the pro-inflammatory function of neutrophils

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Forsythia fruits, which are usually obtained from *F. suspensa* (Thumb) Vahl and *F. viridissima* Lindley, are

known in Asia as diuretic, hypotensive, anti-allergic, anti-inflammatory, antipyretic, anti-infective and antidote agents. Moreover, the plant material is listed by the Chinese, Japanese and Korean Pharmacopoeias [1]. In Europe, *F. x intermedia*, *F. suspensa* and *F. viridissima* are naturalized and cultivated as decorative shrubs. In temperate climates, these plants do not form fruits and, in Europe, more attention is paid to the leaves and flowers as a source of valuable compounds [2]. The present study we demonstrated that leaves and flowers of *Forsythia x intermedia* are source of lignans which were able to mediate pro-inflammatory function of neutrophils.

Using bio-guided fractionation, we isolated the active compounds and determined their biological activity on human neutrophil model. We examined: (I) cytotoxicity, (II) expression of adhesion molecules CD11a/CD18 and CD11b/CD18, (III) phosphorylation level of p38, ERK1/2 and JNK MAP kinases and (IV) pro-inflammatory cytokine release (IL-8, IL-1 β and TNF α).

Cytotoxicity of lignans was determined by a standard flow cytometric probe using propidium iodide staining. The expression of adhesion molecules CD11a and CD11b was analyzed with flow cytometry. Phosphorylation of p38, ERK1/2 and JNK MAPK was determined by immunoblotting analysis. The effect on chemokines production was measured by enzyme-linked immunosorbent assay (ELISA).

The bio-guided fractionation led to the isolation of the following lignan aglycones: (+) pinoresinol, (+)-epipinoresinol, (-)-matairesinol, (+)-phillygenin and (-)-arctigenin. Compounds significantly decreased the surface expression of CD11a and CD11b at 50 μ M. Moreover all lignans significantly inhibited TNF- α and IL-1 β production at 10 μ M, probably by attenuating the p38 and ERK kinase pathways. The lignans did not inhibit of interleukin 8 release.

Conclusion: *Forsythia x intermedia* is a valuable source of active lignans, which may be potential candidates for treating inflammatory diseases that are associated with the excessive production of cytokines such as TNF- α and IL-1 β .

Acknowledgments

This study was financially supported by research grant 2015/17/B/NZ7/03086 from the Polish National Science Center.

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Poster session-PO-138:

Specific and holistic quality assessment of Colla corii asini based on peptidomic approach using nanoLC-Orbitrap HRMS combined with LC-MS/MS

Jing-Xian Zhang, Qing Hu, Hong-Shuang Dong, Jian Sun, Rui Feng, Su Zhang, Hong Yu, Xiu-Hong Mao, Shen Ji

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Colla corii asini (CCA) is a very famous traditional Chinese medicine (TCM) for nourishing blood, replenishing yin, and moistening dryness [1]. Unfortunately the counterfeit gelatins made from other animal skins rather than donkey have emerged, which could lead to adverse drug or allergic reactions in patients. A specific method, taking one unique peptide for each animal species as the detection marker, was developed by Li, et al [2], which would control the counterfeit phenomenon effectively. However, the identification of authentic CCA [1] by taking one peptide as the marker lack specificity and it is obvious there is a potential risk that this peptide would be added in counterfeits directly. Therefore, it is still imperative to develop more effective and comprehensive identification method. In the study, peptidomic approach was applied by using nanoLC-Orbitrap HRMS to analyze the in-house-made donkey, mule and horse gelatins, which have highly consistent amino acid sequences for their collagen. The peptides identification was carried out by Proteome Discoverer software. As a result, 1 peptide was defined to be common for CCA and mule gelatins and 9 were simultaneously detected in CCA, mule and horse gelatins. Subsequently, a characteristic profile was developed by taking the 10 peptides as the quality markers (Q-marker) using LC-MS/MS with MRM mode. This method was verified to be specific for the 10 markers were not detected in the gelatins made from cattle, sheep, and pig skins. The quantification results suggested that the total content ranged from 1.04%% to 2.11% with little difference for all the samples. Principal component analysis based on the content showed that the three kinds of gelatins could be differentiated apparently. The proposed method, involving both the qualitative and quantitative aspects, should play a significant role in standardizing and providing quality control to CCA market.

Key words: Colla corii asini; peptidomic analysis; Q-marker; nanoLC-Orbitrap HRMS; LC-MS/MS

Rerefence

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Poster session-PO-139:

New protostane-type triterpenoids from *Alisma plantago-aquatica* with anti-inflammatory activity in Caco-2 cells

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Alisma plantago-aquatica Linn. Thunb. (Alismaceae) is widely distributed in the marshes in Fujian province of China. It has long been used in traditional medicine for the treatment with oliguria, edema, diarrhea, nephropathy, dizziness and hyperlipidemia, diabetes, atherosclerotic, and inflammation. Previous phytochemical studies have reported the isolation of protostane-type triterpenoids, phenylpropanoid amides, and sesquiterpenes. Among them, protostane-type triterpenoids are considered to be the main bioactive components for purgative diarrhea. Herein, we report the isolation and structural elucidation of twenty undescribed protostane-type triterpenoids (1-15), which can be divided into different structural types: 17-spirost protostane-type, nor-protostanes-type, carbonylation derivatives of alisol A and alisol B type, and dehydroxylation derivatives of alisol A and alisol B type. The structures of new compounds are elucidated by 1D-, and 2D-NMR techniques including HSQC, HMBC, 1H-1H COSY and NOESY spectra. Based on these, we summarize characteristic NMR signals of those compounds for the rapid identification of protostane-type. All isolates were evaluated for their inhibitory effects on LPS-induced NO production in Caco-2 cells.

Poster session-PO-140:

Multi-residue analysis of 508 pesticides in *Panax notoginseng* using UHPLC-Q-Orbitrap and UHPLC-MS/MS

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Concerns about the pesticide residues in medicinal herbs have markedly increased in recent years. To ensure the safety of the herbal products, it is important to regulate the uses of pesticides and to monitor their levels for compliance. The current analytical methods applied in many routine laboratories on pesticide residue control generally consist of 100-200 compounds, which may be insufficient with the perspective of over 800 pesticides present on the market.

In this study, a semiautomated qualitative method for target screening of 508 pesticide residues in *Panax*

notoginseng was developed using ultra-high performance liquid chromatography coupled to quadrupole-Orbitrap high resolution mass spectrometry (UHPLC-Q-Orbitrap). Full MS/dd-MS2 (data dependent acquisition) was utilized for sample data acquisition, and the screening detection limits (SDLs) ≤ 0.05 mg/kg were achieved for 95% of the investigated pesticides. Meanwhile, a reliable quantitative method for simultaneous determination of 508 pesticide residues in Panax notoginseng was also developed and validated using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Satisfactory linearity with correlative coefficient (R^2) > 0.98 was achieved for all analytes. The limits of quantitation (LOQs) were 0.01 mg/kg for all analytes. Recoveries of 412 analytes at four levels (0.01, 0.05, 0.10 and 0.20 mg/kg) were between 70% and 120% with relative standard deviation $< 15\%$. The combination of the above two analytical systems can simplify the routine workflow for multi-residue analysis of large scale pesticides with less workload and better performance.

Poster session-PO-141:

A Plasma pharmacology strategy for discovering multi-components interactions of Xian-Ling-Gu-Bao capsule on exerting anti-osteoporosis activity

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Objectives:

The present study aims to explore the in vivo effective ingredients of XLGB, and to establish a method for evaluating the osteogenic and anti-resorption activity of XLGB-containing plasma in vitro.

Methods:

Nine-week-old Sprague-Dawley rats were induced for postmenopausal osteoporosis (PMOP) by bilateral ovariectomy and divided into four groups as follows: sham-operated group (Sham), ovariectomized (OVX) control group, OVX treated with Estradiol (E2) and OVX treated with XLGB (XLGB). Treatments were given orally for three months. Three-dimensional bone structure of the fourth lumbar was scanned by Micro-CT and then analyzed. In addition, the effects of standardized XLGB-containing plasma were evaluated on MC3T3-E1 for the differentiation and mineralization of osteoblast cell. The osteoclast bone resorption assay of XLGB-containing plasma was also performed on RANKL-induced RAW 264.7 cells using a commercially available bone resorption kit.

Results:

Three-dimensional images of the 4th lumbar showed that the trabecular bone in Sham, E2 and XLGB group was obviously more than that in OVX group. Similarly, BMD in Sham, E2 and XLGB group was significantly higher than that in the OVX group ($p < 0.05$). The in vitro bioassay results demonstrated that XLGB-containing plasma not only effectively promoted proliferation, differentiation and mineralization of osteoblast, but also significantly inhibited the progress of bone resorption of osteoclast.

Conclusion:

XLGB possessed potent anti-osteoporotic activity in OVX rats which could be an effective treatment for postmenopausal osteoporosis. In our present study, the concept of standard TCM containing plasma was firstly proposed, and an in vitro bioassay platform for evaluating the osteogenic and anti-resorption activity of TCM containing plasma was also developed. It provided a novel plasma-pharmacology strategy to investigate the medical materials and their action mechanism among absorbed multiple components in herbal medicine.

Acknowledgement:

This work was supported by National Natural Science Foundation of China (81220108028) and the project of the Science Foundation for Distinguished Young Scholars of Guangdong Province (Grant No. 2014A030306043).

Poster session-PO-142:

Four phenolic glycoside isomers from the *Idesia polycarpa* Maxim. Leaves

Lei Huang^{1, 2, 3}, Tong Peng^{2, 3}, Yu Li^{1, 2, 3}, Yunyun He^{2, 3}, Li Wang^{2, 3}, Shiyang Zhang^{2, 3}, Fang Chen^{1, 2, 3}, Lin Tang^{1, 2, 3}

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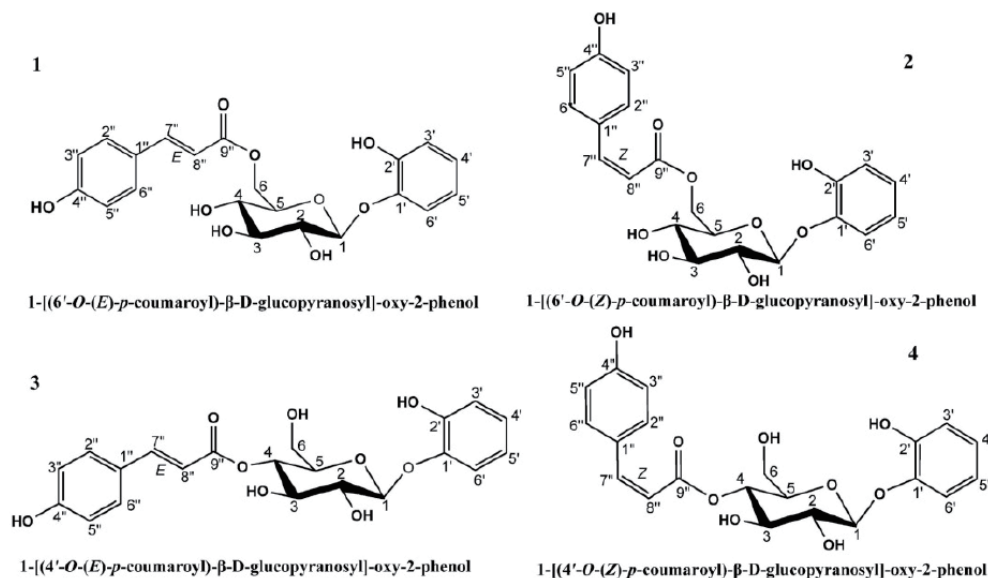
² *National and Local Joint Engineering Laboratory for Energy Plant Bio-Oil Production and Application, Sichuan University, Chengdu, China*

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Abstracts :

Phytochemical investigation on the ethyl acetate extract of *Idesia polycarpa* Maxim. leaves led to the isolation of four phenolic glycoside isomers (1-4). Compound 2 appeared to be new reported phenolic glycoside, while compound 1 was the first time isolated from the titled species. Their structures were established by IR, UV, HRESI-MS and 1D and 2D NMR spectroscopies analysis and comparison of spectral data with previously reported data. The compounds 3 and 4 showed stronger activity of scavenging the DPPH free radical than the other two compounds, while the compounds 1 and 2 showed a significant activity of scavenging the

ABTS free radical. Compounds 2 and 4 exhibited stronger cytotoxicity against HepG2 cell lines compared to compounds 1 and 3. Moreover, compound 3 presented the highest cytotoxicity against MCF cell lines with IC_{50} value of $37.17 \pm 0.26 \mu\text{g/mL}$ than compounds 1, 2 and 4.



Keywords :

Idesia polycarpa Maxim.; phenolic glycoside isomers; cytotoxicity ; antioxidant

Poster session-PO-143:

Cytotoxicity study of *Equisetum diffusum* D. Don., *Glochidion eriocarpum* Champ. ex Benth., and *Clerodendrum cyrtophyllum* Turcz. collected in Northern Vietnam

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Introduction: Three plants, *Equisetum diffusum* D. Don. (KT03 – whole plant), *Glochidion eriocarpum* Champ. ex Benth. (KT05 – whole plant), *Clerodendrum cyrtophyllum* Turcz. (KT07 – branches and leaves), grown in Northern Vietnam, are used for the treatment of stomachache, diarrhea, dysentery, gastralgia, pharyngitis and healing of insect wounds in traditional Vietnamese medicine. The purpose of this study is to examine the cytotoxicity of extracts of these three medicinal plants via MTT assay. This study is part of a project aiming at (i) evaluating the safety of Vietnamese traditional medicines; and (ii) their eventual interest in drug discovery.

Methods: These medicinal plants were collected in autumn in the Northern provinces of Vietnam then air-dried and powdered. The samples were extracted at room temperature, sequentially with solvents of

increasing polarities: n-hexane, ethyl acetate and finally methanol. The cytotoxicity of the 12 obtained extracts was tested using MTT assay on two cell lines: non-cancerous epithelial intestinal cells (FHs74 Int) and epithelioid cervix carcinoma cells (Hela).

Results: The ethyl acetate extract of branches of *Clerodendrum cyrtophyllum* Turcz. (KT07) showed the highest toxicity towards FHs74Int (IC₅₀ = 0.39 mg/mL) and Hela (IC₅₀ = 0.27 mg/mL) cell lines. The methanolic extract of *Glochidion eriocarpum* Champ. ex Benth. (KT05) showed cytotoxicity against non-cancerous epithelial intestinal cells (IC₅₀ = 0.48 mg/mL) and Hela cells (IC₅₀ = 0.56 mg/mL). We also found that the ethyl acetate extract of KT07 leaves is more cytotoxic towards intestinal cells FHs74 Int (IC₅₀ = 0.60 mg/mL) than Hela cells (IC₅₀ = 1.89 mg/mL).

Conclusions and perspectives: The ethyl acetate extract of KT07 branches has the potential to be considered for anti-cancer research. In the next steps, this extract will be studied for its cytotoxic activity on further cell lines and the active compounds will be identified.

Poster session-PO-144:

Studies on chemical constituents of *Isodon henryi*

Li-Ping Dai, Xue Jiang, Ling-Xia Zhang, Sui-Qing Chen

College of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, China

[Abstract] The chemical constituents of the water extraction of the aerial parts of *Isodon henryi* were investigated by various chromatographic methods including D-101 macroporous adsorptive resins, silica gel, Sephadex LH-20, and semi-preparative HPLC. As a result, ten compounds were separated and purified. By analyses of the UV, IR, MS, NMR spectra, their structures were determined as rabdosinate (1), lasiokaurin (2), enmenol-1- β -glucoside (3), epinodosinol (4), rabdosichuanin C (5), epinodosin (6), hebeirubescensins (k) (7), rubescensin C (8), enmenol (9), oridonin (10). Compounds 1-9 were isolated from *Isodon henryi* for the first time, as well as cytotoxicity of ten compounds. [Keywords] *Isodon henryi*; structural identification; diterpenes; cytotoxic

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Poster session-PO-145:

Advances in pharmacogenomic studies of docetaxel

ke pei

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Shandong qianfoshan hospital, jinan, China

Presently, docetaxel is used in the treatment of breast, non-small cell lung, prostate and other cancers. However, there exists a significant difference in individual drug sensitivity and toxicity. In this paper, genetic variation of drug metabolism related genes is reviewed. We study correlations between the genetic polymorphisms of CYP450, ABC, SLCO1B3, and GSTs as well as the clinical efficacy and adverse drug reactions of docetaxel in order to provide a theoretical basis for clinically individualized medication.

Poster session-PO-146:

Design, synthesis of “Liang Guanxi”Jun-Shi compound Bornyl caffeate and its metabolic investigation in vivovvv

Pu Jia, Tian Gao, Lingjian Yang, Baimei Shi, Yajun Bai, Xiaohui Zheng

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Salvia miltiorrhiza (Danshen) is the most frequently used medicinal herbs for treatment of cardio- and cerebrovascular disease. As one of its main component, caffeic acid was be proved to have antibacterial, anti-oxidant and anti-age dementia effects. In the previous study, the author's team found that guide herb borneol in Compound Danshen Formula can improve the bioavailability of the main components of minister herb Danshen significantly, slow down its metabolic rate, and promote the permeability of the blood-brain barrier for components in Danshen. Based on this, we chose herb pairs Danshen-Borneol which were in one group of the "Liang Guanxi" composition in the Compound Danshen Formula, and then synthesized the "Liang Guanxi" Jun-Shi compound-bornyl caffeiate utilizing the modern medicinal chemistry technique, and found that compared with caffeic acid, bornyl caffeiate could more quickly exert the effect in vivo, maintain

the effective concentration for more longer time, and show more better tissue selectivity to some extent, thus it was be concerned will be helpful to taking its therapeutic effects. In the metabolic study, HPLC-Q-TOF-MS technique was utilized to comprehensively characterizing the metabolic profiles of plasma, feces and urine in rats administered with bornyl Caffeiate. As a result, there are 31 drug prototypes and metabolites were detected and intentatively identified by reference substance, literature and database retrieve. Phase I including hydrolysis, oxidation and reduction products and phase II metabolism includes methylation, sulfation, glucuronidation, sulfonation and glycine mainly involved in the metabolism of bornyl caffeinate. Glucuronidation, sulfation, O-methylation and reduction were the main metabolic pathways of bornyl caffeate in rat. In short, we designed and synthesized the compound bornyl caffeate which belonged to 'Liang Guanxi' Jun-Shi compound, and then finished its pharmacokinetic and metabolic study thereby providing valuable information for the rational of its druggability and new idea of innovative drug discovery from Compound Danshen fomula.

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Wednesday, 29th August, 2018 - Bibo BallroomAB - 09:00 - 10:40

Invited & Short Lecture - Session 1-3

Session 1-3-IL-01:

Symbiont-derived drug-like molecules

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The impact of natural products on drug discovery pipelines keep kindling our interest in structurally unpredictable low-molecular-weight biomolecules as a promising source of pharmaceutical leads. Meanwhile,

scientists are frequently frustrated by re-isolations of known compounds from new organism collections since countless natural products have been identified since Serturmer's characterization of morphine in 1806. To address this frustration, the affordability and chemical space expansion of minor new natural products become a great concern, and chemical synthesis has been performed to produce organism-originated complex molecules and natural product-like compounds with privileged scaffolds. To add more skills to the existing arsenal of searching for highly-valued new chemicals like drug leads, this talk will present the discovery and biosynthesis of bioactive secondary metabolites from symbionts, some of which are evidenced to be evolutionally advanced owing to their non-stop interaction with multicellular hosts such as plants, insects and fishes.

Session 1-3-IL-02:

Biosynthetic Inspired Research of Complex and Bioactive Acylphloroglucinol Derivatives

Ling-yi Kong, Jianguang Luo, Jun Luo, Minghua Yang, Wenjun Xu, Yuanzheng Xia

Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, China

Inspired by the fascinating structures and intriguing biological activities (Fig. 1), we have investigated the constituents of 5 plants of genus *Hypericum*, 3 plants of family Myrtaceae, and 1 plant of genus *Melicope*, which led to the isolation of 258 acylphloroglucinol-terpene derivatives, including 211 new ones and 9 types of novel carbon skeletons. Among them, 6 compounds were chosen as “hot off the press”. These research works of complex absolute configurations, biomimetic syntheses, and biological activities have potential academic value and scientific significance for the research and development of new drugs.

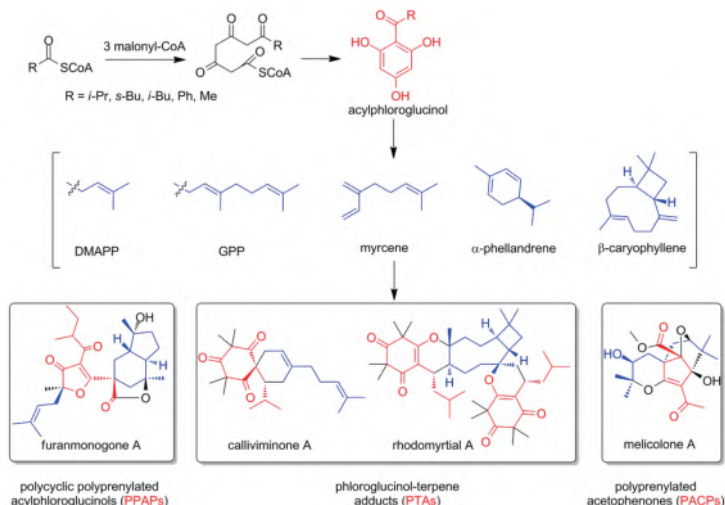


Fig. 1. The biosynthetic pathway of diverse acylphloroglucinol derivatives

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Session 1-3-IL-03:

Metabolic profiling approach for the indication of multifunctional alkaloids from *Psychotria nemorosa*

Luiz Klein-Júnior ^{1,2,3}, Sylvian Cretton ⁴, Johan Viaene ³, Emmy Tuenter ⁵, André Gasper ⁶, Sandra Apers ⁵, Luc Pieters ⁵, Philippe Christen ⁴, Yvan Vander Heyden ³, Amélia Henriques ¹

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² *Chemical-Pharmaceutical Investigation Center, Universidade do Vale do Itajaí, Itajaí, Brazil*

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⁴ *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

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⁶ *Department of Natural Sciences, Universidade Regional de Blumenau, Blumenau, Brazil*

The alkaloid fraction (AF) from *Psychotria nemorosa* leaves was found able to inhibit both monoamine oxidase-A (MAO-A) and butyrylcholinesterase (BChE). However, the alkaloids responsible for the enzymatic modulation had not been identified. Our study aims indicating the multifunctional compounds using a metabolic profiling approach. As a first step, the alkaloids extraction was optimized. Leaves of *P. nemorosa* were extracted with methanol using an ultrasonic bath. Factors were screened by a Fractional Factorial Design. The Euclidean distances between the UPLC-DAD fingerprints and the blank injection were used as response, evidencing highly diverse extraction outcomes. Coupled to the plotting of effects per time point (effect fingerprints), thermolabile peaks could be indicated. Consequently, time and temperature were selected for further optimization, using a Central Composite Design. Finally, plant:solvent ratio was set at 1:50 (m/v), number of extractions at one, particle size at $\leq 180\ \mu\text{m}$, extraction time at 65 min and temperature at 45 °C, thus avoiding degradation. The fractionation step was performed using a solid-phase extraction method on silica cartridges, optimized by a Box-Behnken Design. Finally, sample concentration was set at 150 mg mL⁻¹, 40% acetonitrile in dichloromethane was used as eluting solvent, and the eluting volume was 30 mL. Using the optimized method, 43 AFs were analyzed by UPLC-DAD and assayed for their BChE and MAO-A inhibitory potencies. To correlate chromatographic fingerprints and pharmacological activities, Orthogonal Projections to Latent Structure (O-PLS) modelling was employed and the regression

coefficients of the model were analyzed and compared to the original fingerprints. Four peaks were indicated as multifunctional compounds. All compounds were purified and their structures were determined as azepino-indole derivatives, described for the first time in *Psychotria* species.

Session 1-3-SL-04:

Pharmacological effects of novel cyclopropylacetylshikonin derivatives on melanoma cells

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³ *Division of Hematology, Medical University of Graz, Graz, Austria*

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According to the WHO, cancer is still a leading cause of morbidity and the second leading cause of death worldwide. The incidence of melanoma is rising faster than any other solid tumor type and melanoma are responsible for 79% of all skin cancer deaths. Especially at an advanced stage, melanoma is still one of the most aggressive and incurable types of cancer. Today, about 79% of all approved anti-cancer drugs are natural products or derived from them demonstrating their important role in medicine [1]. In a previous study, shikonin derivatives isolated from the roots of *Onosma paniculata* Bur. & Franch. (Boraginaceae) have emerged as interesting candidates for finding new drug leads against cancer [2]. We now prepared several novel semisynthetic shikonin derivatives and analyzed their cytotoxic potential against different melanoma cell lines. The most active compound was (R)-1-(1,4-dihydro-5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl cyclopropylacetate (CP). It was especially more active against two melanoma cell lines derived from metastatic lesions compared to the most active isolated derivative b - b -dimethylacrylshikonin (DMAS). IC₅₀ values of CP and DMAS were 4.9 µM (WM164) and 3.2 µM (MUG-Mel-2), and 8.3 µM and 7.2 µM, respectively. Further investigations revealed that CP induced apoptosis, but did not lead to cell cycle arrest. Moreover, the ApoToxGlo® assay together with the LDH assay revealed that CP did not significantly damage the cell membrane up to 48h and 5.0 µM.

Acknowledgement:

The Austrian Science Fund (FWF) is acknowledged for financial support (Project P27505).

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Session 1-3-SL-05:

Hyal-1 Inhibitors from roots of *Ononis spinosa* L.

John Addotey ¹, Mattias Lechtenberg ¹, Frank Petereit ¹, Isabelle Lengers ², Joachim Jose ², Andreas Hensel¹

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Hyaluronidases play very important roles in a number of physiological and pathological processes such as embryogenesis, angiogenesis, inflammation, disease progression and wound healing [1]. Human hyaluronidase-1 (Hyal-1) is an enzyme strongly involved in the regulation of extracellular matrix by balancing the deposition and potential degradation of hyaluronic acid (HA) in the tissue [2]. The inhibition of Hyal-1 by specific inhibitors might be a promising target for improved wound healing, tissue regeneration, and looking at renal function also for induction of diuresis. By using surface-displayed human Hyal-1 on *Escherichia coli* F470 cells, HA as substrate and stains-all method for quantification of undegraded, high molecular polymer, the enzyme activity can be determined easily [3]. From a selection of herbal materials, traditionally used for wound healing and for urinary tract infections IC₅₀ values were determined within a screening. Based on these results, *Ononis spinosa* L. roots were chosen for bioactivity guided fractionation. Two non-polar fractions of *Ononis spinosa* L. roots (ODB and ODG) were found to be most active following bioassay-guided fractionation with 86±3 % and 96±13 % inhibition respectively for 1mg/ml concentration. Further separation of ODB revealed 3 main components which were determined to be onogenin, sativanone and medicarpin. Relative inhibitions for 250µM concentrations were 25.3±18, 61.20 ± 20.6 and 22.4±16 respectively. The IC₅₀ of sativanone was determined to be 140.4 µM. Rationalization of the diuretic properties of roots of *Ononis spinosa* L. is possible based on the results.

References

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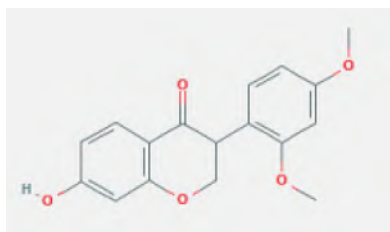


Figure 1: Sativanone

Session 1-3-SL-06:

Inhibitors of Neuroblastoma from *Juniperus Oblonga* M. BIEB.

Yi-lin Qiao ¹, Micah Glasgow ², Dana-Lynn Koomoa-Lange ², Ingo Lange ², Daniel Atha ³, Manana Khutsishvili ⁴, Vugar Kerimov ⁵, Robert Borris ¹

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Neuroblastoma (NB), the most common extracranial solid tumor of childhood, derives from neural crest cells of the peripheral sympathetic nervous system. Despite advances in cancer chemotherapy, as much as 50% of NB patients diagnosed with the high-risk form of the disease will be refractory to treatment or experience relapse after treatment [1]. In preliminary studies, three dichloromethane-soluble extracts of *Juniperus oblonga* M. Bieb. (Cupressaceae) not only inhibited proliferation of NB cells in culture, but also increased intracellular calcium in them [2]. The compounds responsible for the inhibition of NB in cell culture in *Juniperus* species have not been previously reported.

In the present study, a bioactivity guided fractionation was performed on the three active extracts, followed by structure determination based largely on LC-MS and NMR data. Twenty-six compounds have been identified, including lignans, coumarins, biflavones and diterpenes. Some of these compounds inhibit NB cell viability (with or without MYCN amplification) by altering cell cycle progression and inducing apoptosis. Additionally, these compounds show similar activity in drug resistant and drug sensitive NB. This may be clinically significant as there are currently no effective treatments for drug resistant NB. Therefore, some of the juniper compounds may represent leads toward an effective treatment for NB, particularly high risk, MYCN amplified, and drug resistant forms.

In light of the limited nature of this sample, additional *Juniperus* species from the Chinese flora have been obtained and evaluated against NB in cell culture leading to the discovery of additional compounds with chemically distinct properties.

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Wednesday, 29th August, 2018 - The Chapel - 09:00 - 10:50

Invited & Short Lecture - Session 2-2

Session 2-2-IL-01:

Protection against glomerular podocyte injury in metabolic syndrome with Chinese herbal medicine

Ling-Dong Kong

State Key Laboratory of Pharmaceutical Biotechnology, Nanjing, China

Metabolic syndrome, a cluster status of multiple metabolic disorders, has been one of the most common public health problems in the world. Insulin resistance is acknowledged as an important causative factor in the pathogenesis of metabolic syndrome, which increases the risk of developing hypertension, cardiovascular disease, type 2 diabetes, non-alcoholic fatty liver, and chronic kidney disease. Although some current drugs against metabolic syndrome or its components are available, they are still far from optimal. Novel drugs are urgently needed. Chinese herbal medicine, a promising source for the development of alternative and complementary medicines, shows unique characteristics and advantages in the prevention and treatment of metabolic syndrome. Fructose is a monosaccharide found in fruits, vegetables and honeys. High dietary fructose or extensive commercial use of high-fructose corn syrup (HFSC) can induce insulin resistance and metabolic syndrome, triggering kidney impairment with glomerular podocyte injury and proteinuria. Here, we explored and discussed the biologic mechanism in the pathogenesis of high fructose-induced podocyte injury, in which special attention is paid to oxidative stress, insulin resistance and inflammation. Furthermore, we provided the new experimental evidence regarding the effects of Chinese herbal medicine on fructose-associated podocyte injury in metabolic syndrome.

Session 2-2-SL-02:

Norway spruce balm for wound healing: Identification of constituents that enhance re-epithelialization in vitro

Thomas Goels ¹, Elke Heiss ¹, Ammar Tahir ¹, Ernst Urban ², Johannes Saukel ¹, Sabine Glasl ¹

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The balm of Norway spruce (*Picea abies* (L.) H. Karst., Pinaceae) has been used in European folk medicine as a wound healing agent since centuries. Several clinical trials already examined its wound healing and antimicrobial properties confirming its empirical use. However, scientific data concerning the active constituents, their mode of action and the quantitative composition of this natural product are scarce. This study therefore aimed at isolating and identifying constituents and examining potential positive effects on re-epithelialization. For isolation and structural elucidation of constituents preparative TLC, flash chromatography and HPLC-UV-DAD on normal phase and reversed phase material, as well as LC-MS, 1D- and 2D-NMR were employed. In vitro bioactivity was characterized in human keratinocytes (HaCaT): the resazurin conversion assay served to detect cytotoxicity of the compounds/extract; monitoring the closure of a gap in a confluent keratinocyte monolayer (scratch assay) revealed an influence of fractions/compounds on migration and proliferation and thus re-epithelialization. Pure compounds from Norway spruce balm were isolated from an extract enriched in carboxylic acids and comprised several diterpenoid resin acids of the abietic-type and the pimaric-type, phenolic acids like coumaric acid, caffeic acid and ferulic acid, as well as a labdane type diterpene (abienol) and a lignan (pinoresinol). The average non-toxic concentration range was determined between 0,1-10 µg/mL. The resin acids, abienol and pinoresinol were able to induce a significantly faster closure of the cell free area in the scratch assay compared to the vehicle control. The constituents showed an activity at concentrations of 0,3-3 µM (resin acids) and 3-30 µM (labdane diterpene and lignan). Overall, this study provides first insight into the constituents of Norway spruce balm and their potential influence on re-epithelialization during the wound healing process.

Session 2-2-SL-03:

Synergy – a key to the multi-target action of natural products

Olaf Kelber ¹, Karen Nieber ²

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² *Institute of Pharmacy, University of Leipzig, Leipzig, Germany*

Introduction

Natural products are typically characterized by their multitude of constituents addressing multiple targets in the organism. Their action has therefore been often postulated to be based on a multi drug/multi target action (1). This has been successfully proven for an herbal medicinal extract combination used in functional gastrointestinal diseases, STW 5 (2), as its action is based on very different effects in different regions of the stomach and the intestine (3). With a multiplicity of targets being the basis of its proven therapeutic efficacy and safety, synergy is assumed to be a key for its action.

Methods

As a model, rat and guinea pig small intestinal smooth muscle preparations, stimulated with ACh (4), or incubated with TNBS for inducing an inflammation (5), were used. STW 5 and its components, alone or in combinations, were tested. A Box-Behnken-Design and the isobologram method were used for analysis.

Results

The smooth muscle-relaxing effects of STW 5 were supra-additive in the model of ACh-induced contraction in comparison to the single components. In the TNBS-model, synergistic, additive as well as antagonistic effects were identified, depending from the combinations of extracts tested.

Discussion

Our results support the concept of a multi-target therapy, with synergy as a key factor. Further evidence for this concept is presently generated by modern gene expression profiling methods (6) giving a rationale for the good clinical efficacy and safety of natural products used in modern phytotherapy, and proven in modern clinical trials.

References

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Session 2-2-SL-04:

Ellagitannins from *Combretum aculeatum* as possible effective prodrugs in TB treatment

ElHadji Assane Diop^{1,3}, Emerson Ferreira Queiroz¹, Laurence Marcourt¹, Sebastien Kicka², Serge Rudaz¹, Tahir A. Diop³, Thierry Soldati²

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Tuberculosis (TB), an infectious bacterial disease caused by *Mycobacterium tuberculosis* with extensive resistance against antibiotic. Using a stringent cell-based assay with *Acanthamoeba castellanii* as host for the pathogen *M. marinum*, the extracts from an ethnopharmacologic survey were screened for

antimycobacterial activity [Kicka & al PlosOne 2014] . Among the plants evaluated, the extract of *Combretum aculeatum* exhibited a relevant antimycobacterial activity [Diop E.A & al JEP 2017] . In order to identify the active principles, a bioassay-guided fractionation was undertaken using flash chromatography. The higher activities were linked to the fractions containing the anomers (α - and β) of the ellagitannin punicalagin (PNG) that were further purified. PNG have been reported to be responsible for various pharmacological activities, such as inhibition of carcinogenesis, host-mediated antitumor activities and antiviral activities. These PNG are however known to be extensively metabolized when ingested orally. Ellagitannins hydrolysis yield ellagic acid, which is subsequently transformed by the human colon microflora into urolithins derivatives [Cerdeira & al J. Agr. Food. Chem 2003] .

To better document the possible anti-TB efficacy of *C. aculeatum*, the metabolites urolithin A, B and D were thus acquired and their antimycobacterial activity was assessed. Ellagic acid inhibited the growth of *M. marinum* in broth with an IC_{50} of 48.3 $\mu\text{g/ml}$. Urolithin D exhibited relevant anti-infective activities , with IC_{50} of 89.91 $\mu\text{g/ml}$. A quantification of PNG in the *C. aculeatum* preparation showed that its consumption at the usual doses may yield plasmatic levels of active metabolites in a concentration range similar to the IC_{50} determined in the in vitro assays. These results provide a rationale for the use of this decoction for TB management.

Session 2-2-SL-05:

Identification of α -Glucosidase Inhibitors from *Cyclocarya paliurus* Leaves Tea by using Centrifugal Ultrafiltration coupled with UPLC-ESI-QTOF-MS Method

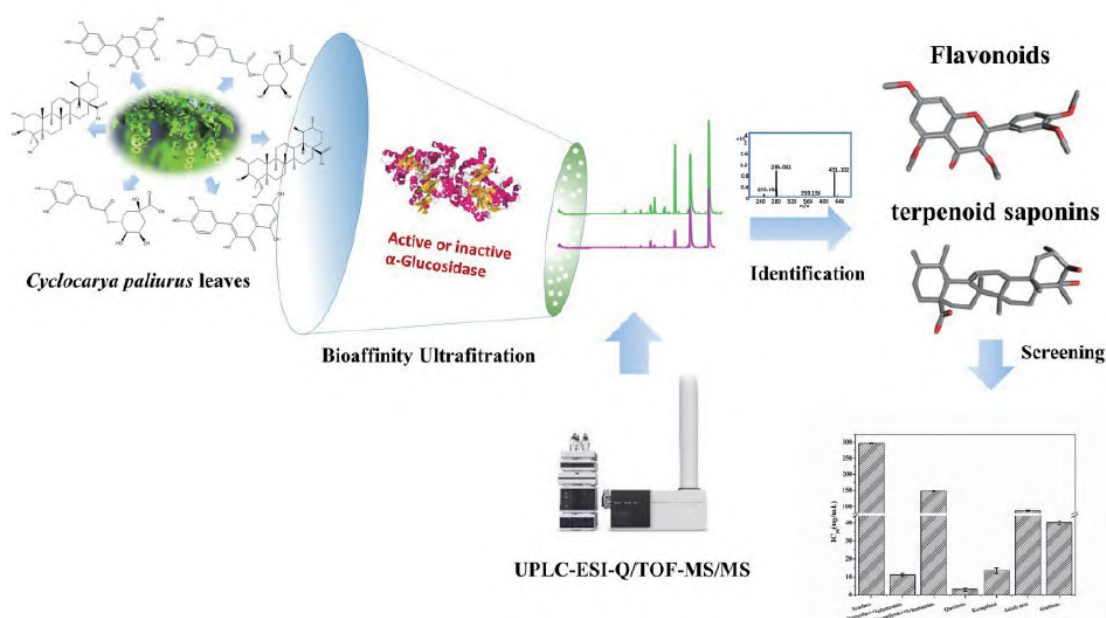
Zi-wan Ning ¹, Lixiang Zhai ¹, Tao Huang ¹, Bo Wen ^{1,2}, Chenghui Liao ², Chengyuan Lin ¹, Ling Zhao ¹, Zhaoxiang Bian ^{1,2}, Haitao Xiao ^{1,2}

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² Shenzhen Research Institute and Continuing Education, Shenzhen, China

Leaf of *Cyclocarya paliurus* (Batalin) Iljinsk. (CP) (Juglandaceae) is a famous sweet tea traditionally used in obese and diabetic population in China. It has been reported with hypoglycemic effect in mice^{1,2} but the active composition responsible for its beneficial properties remain unclear. In this study, we found that the water extract of CP tea leaves (CP extract) exhibited inhibitory activity with an IC_{50} at $31.5 \pm 1.05 \mu\text{g/mL}$, which was higher than acarbose at $296.6 \pm 1.06 \mu\text{g/mL}$, and then we employed a rapid method combining centrifugal ultrafiltration separation subsequent ultra-performance liquid chromatography with quadrupole time of flight tandem mass spectrometry (UPLC-Q/TOF-MS/MS) determination to screen potential α -glucosidase inhibitors from CP extract. When CP extract was incubated with α -glucosidase, potential inhibitors of CP extract can bound to this enzyme and unbound small molecules can be separated from the ligand- α -glucosidase complexes or α -glucosidase by the ultrafiltration membrane to identify the potential

α -glucosidase inhibitors (ligands) quickly. Binding with α -glucosidase, 12 binding ligands from CP extract were characterized and 11 potential α -glucosidase inhibitors were identified: quercetin-3-glucoside(1), quercetin-3-O-glucuronide(2), quercetin 3-O-galactoside(3), quercetin-3-O-rhamnoside(4), kaempferol-3-O-rhamnoside(5), quercetin(6), kaempferol(7), quadranoside IV(8), kaempferol-3-rhamnoside-7-rhamnoside(9), asiatic acid(11), genistein(12). By using an in vitro model for studying α -glucosidase inhibitors, we found quercetin-3-O-glucuronide, kaempferol-3-O-rhamnoside, quercetin, kaempferol, asiatic acid and genistein exhibited potent α -glucosidase inhibitory activities with IC_{50} of 11.11 ± 0.82 , 148.40 ± 1.12 , 2.92 ± 1.08 , 13.43 ± 1.67 , 88.45 ± 1.24 μ g/ml and 40.09 ± 0.94 , respectively. It would benefit us mining natural α -glucosidase inhibitors and understanding the mechanism of CP hypoglycemic activity.(Figure 1)



References:

1. Xiao, H., Wen, B., et al. Sci. Rep 2017;7, 9155.
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Session 2-2-SL-06:

Microbiota-assisted isolation of flavonoids from an infusion of *Filipendula ulmaria* (L.) Maxim.

Sebastian Granica, Dominik Popowski, Karolina Pawłowska, Jakub Piwowarski

Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

Water infusions from *Filipendula ulmaria* (L.) Maxim. flowers are used in the treatment of fever, rheumatism

and traditionally in the urinary tract diseases. Active constituents of meadowsweet water extracts belong to groups of phenolic glycosides, ellagitannins and flavonoid derivatives. The presented research is focused on flavonoid-rich fraction, responsible for the diuretic activity.

Gut microbiota metabolism of xenobiotics, including natural products, is paid a lot of attention lately. For instance, researchers described gut microbial biotransformation of ellagitannins to urolithins, which are linked to health beneficial activity of ellagitannin-rich products.

Preliminary studies, based on UPLC-DAD/MSn meadowsweet infusion analysis, showed that among other flavonoid glycosides, the infusion contains two galloyl derivatives of quercetin and one galloyl derivative of kaempferol glycoside. Incubation of an infusion with human gut microbiota cultures in a small scale and chromatographic analysis were performed to examine changes over the time in a post-growth medium. Differences in dynamics of biodecomposition of mentioned galloyl derivatives in the comparison to other flavonoid metabolism were noticed. Scaled up incubation was performed in order to isolate galloyl derivatives. The process was controlled using chromatographic analysis of test samples, collected in several time points and the incubation was terminated when post-growth medium composition was proper for isolation. The medium was extracted using diethyl ether and ethyl acetate. Galloyl derivatives were isolated from ethyl acetate fraction using preparative HPLC in average yield of 10 mg and characterized using 1D and 2D NMR techniques.

The obtained results showed for the first time that galloyl derivatives of flavonoids are transformed more slowly than simple glycosides. This feature was used for the gut microbiota assisted isolation of three compounds – namely quercetin 3-O- β -(2'-O-galloyl)-D-galactopyranoside, quercetin 3-O- β -(2'-O-galloyl)-D-glucopyranoside and kaempferol 3-O- β -(2'-O-galloyl)-D-glucopyranoside. The presence of all three compounds was confirmed for the first time in flowers of *Filipendula ulmaria*.

Session 2-2-SL-07:

Modulating activity of latex from *Euphorbia Mauritanica* L. on human skin cells' immune response.

Florian Guenther, Matthias F. Melzig

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In recent years, literature shows that proteases have the potential to induce inflammation through activation of protease-activated receptors (PAR) [1]. Besides secondary plant constituents like phenols, di-, and polyterpenes, proteases are abundant in latices from plants of Euphorbiaceae Juss. [2,3]. It is known, that these latices induce strong skin inflammation. Therefore, we investigated the plant latex from *Euphorbia mauritanica* L. (Euphorbiaceae). Human skin is the first barrier of the body in defense from environmental

influences and the inflammatory response is an important tool against pathogens. In our study, we are focused on the combination of diterpenes with proteases regarding its inflammatory potential on human skin. Therefore, Mauritanicain, a serine protease from *E. mauritanica*, was isolated by ion exchange and size exclusion chromatography and characterized by MALDI-TOF-MS/MS. We used HaCaT cell line and primary human dermal fibroblasts as monocultures, as well as human skin model. The skin model was composed by primary keratinocytes and fibroblasts. Mauritanicain and PMA were incubated in different concentrations, depending on the cell system. 48 hours after removing the stimulating substances, medium was removed and investigated regarding IL1- β and IL-8 by FACS and ELISA. When the experiment was finished, HE-staining of the skin model was performed. The occurrence of PAR2 was visualized by immunofluorescence staining and PCR. The results showed that Mauritanicain and the combination with PMA is able to induce skin inflammatory response.

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- [3] Vasas A, Hohmann J. *Euphorbia* diterpenes: isolation, structure, biological activity, and synthesis (2008-2012). Chem Rev 2014; 114: 8579-8612

Wednesday, 29th August, 2018 - Bibo BallroomC+Room1 - 09:00 - 10:45

Short Lecture - Session 5-1

Session 5-1-IL-01:

Herbgenomics of *Panax ginseng*

Shi-lin Chen

Institute of Chinese Materia Medica China Academy of Chinese Medical Science, Shanghai, China

A new discipline, herbgenomics, has emerged, which provides an effective platform to support the chemical and biological analyses of traditional medicines. Here, we report the whole genome sequence of *Panax ginseng*-the king of herbs-as well as its transcriptomes, metabolites and rhizosphere metagenomics in order to help the analysis of ginsenosides biosynthesis and help the cultivation improvement. The 3.5 Gb nucleotide sequence contains more than 60% repeats and encodes 42 006 predicted genes, encompassing 488 cytochrome P450s, 2556 transcription factors, and 3745 transporter genes. Twenty-two transcriptome

datasets and mass spectrometry images of ginseng roots were adopted to precisely quantify the functional genes. Thirty-one genes were identified to be involved in the mevalonic acid pathway. A total of 225 UDP-glycosyltransferase (UGTs) were identified, and these UGTs accounted for one of the largest gene families in ginseng. Tandem repeats contributed to the duplication and divergence of UGTs. Molecular modeling of UGTs in the 71, 74, and 94 families revealed a regiospecific conserved motif located at the N-terminus. Molecular docking predicted that this motif captured ginsenoside precursors. Metagenomics analysis revealed that bacterial diversity decreased, whereas fungal diversity increased, in the rhizosphere soils of adult ginseng plants at the root growth stage under different ages. Few microbial community, such as Luteolibacter, Cytophagaceae, Luteibacter, Sphingomonas, Sphingomonadaceae, and Zygomycota, were observed; the relative abundance of microorganisms, namely, Brevundimonas, Enterobacteriaceae, Pandoraea, Cantharellales, Dendryphon, Fusarium, and Chytridiomycota, increased in the soils of adult ginseng plants compared with those in the soils of 2-year-old seedlings. These work are expected to contribute to ginseng breeding, cultivation, and synthesis biology.

Session 5-1-IL-02:

Insights into the anti-influenza activity of the wild syringa tree

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⁴ Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria

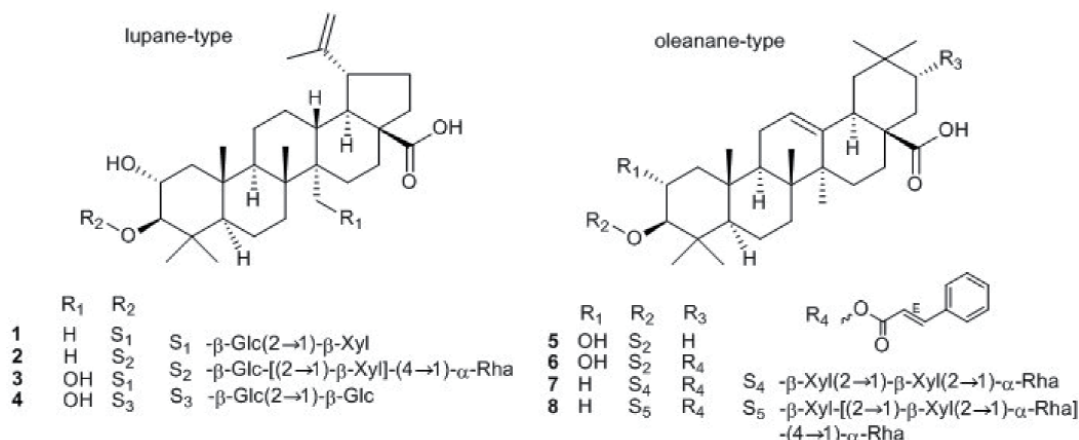
⁵ Institute of Medical Microbiology, Section Experimental Virology, Jena University Hospital, Jena, Germany

In an in vitro screening for anti-viral natural products, the bark extract of the wild syringa tree (*Burkea africana* Hook.) was found to be a promising candidate. In a cytopathic effect (CPE) inhibition assay using Madin Darby canine kidney (MDCK) cells, this extract exhibited an IC₅₀ value of 5.5 µg/mL against the H3N2 influenza virus A/Hong Kong/68 (HK/68) [1]. Moreover, it was not toxic to MDCK cells.

Therefore, the wild syringa tree bark extract was selected for a bio-guided phytochemical work-up using various chromatographic techniques including flash CC, supercritical fluid chromatography (SFC), and UPLC. In sum, eight new triterpene saponins (1-8) of the lupane and oleanane types with four so far undescribed aglycone structures were isolated and characterized via HRESIMS, 1D and 2D NMR spectroscopy. The absolute configuration of the sugar moieties was determined via GC-MS experiments after hydrolysis. This is the first report of triterpene saponins as constituents of the wild syringa tree.

The anti-influenza virus activity of the isolates on the H3N2 strain HK/68 and the 2009 pandemic H1N1 strain A/Jena/8178/09, revealed the most potent effects by compounds 7 and 8 with IC₅₀ values in the nanomolar

range between 50 and 270 nM [2].



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This work was supported by the Austrian Science Fund (FWF: P24587) and the European Social Fund (ESF & TMWAT Project 2011 FGR 0137).

Session 5-1-IL-03:

Pharmacokinetic markers of Chinese herbal medicines

Chuan Li

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

Chinese herbal medicines are often complex in chemical composition and contain multiple bioactive constituents; the effectiveness and safety of an herbal medicine normally is governed by human body exposure to its bioactive constituents and/or their bioactive metabolites. Current pharmacokinetic research on Chinese herbal medicines reveals pharmacokinetic characteristics of their bioactive constituents, including the systemic exposure and metabolism, after dosing the medicines. Such findings should be applied to guide rational clinical use of Chinese herbal medicines and to support clinical research on drug therapies including Chinese herbal medicines. To this end, a class of xenobiotic markers was proposed for use and research of complex Chinese herbal medicines; because the markers' identification results from pharmacokinetic research, they are referred to as pharmacokinetic markers (Lu et al., 2008; Hu et al., 2013; Li, 2017). Pharmacokinetic markers of a Chinese herbal medicine comprise herbal compounds, unchanged

and/or metabolized, that are measurable by contemporary techniques and that can reflect human body exposure to the herbal compounds responsible for or potentially related to the medicine's therapeutic action and the associated influencing factors. The usefulness of such markers identified from pharmacokinetic investigations could be expanded, e.g., pharmacokinetic markers are proposed to be potentially useful for reflecting abnormal cellular processes in patients receiving Chinese herbal medicine-included treatment and for predicting the prognosis in the patients (Zhang et al., 2018).

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Session 5-1-SL-04:

Exploring Bioactive Polyynes from Bacterial Sources Using Integrated Omics Analysis

Yu-Liang Yang, Ying-Ning Ho, Lin-Jie Shu, Han-Jung Lee, Pi-Yu Chen, Chia-Chi Peng, Chih Lin

Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan

Polyynes are well-known unstable secondary metabolites with various biological functions and mainly identified from plant, marine organism and fungal sources [1]. Only a few of polyynes have been identified from bacteria. The biosynthetic gene cluster (BGC) of bacterial polyne was first revealed in 2014 [2], providing us an opportunity to explore the bacterial polyynes using genome mining approach. Through the genome mining analysis, we have found only six genus of bacteria are potential to produce polyynes. The phylogenetic analysis of bacterial polyne BGCs demonstrated that *Massilia* sp. YMA4 is a unique source to produce novel polyne structures. Since the production of polyynes from *Massilia* sp. YMA4 was unstable,

here we employed an integrated omics approach, including RNA-seq transcriptomics, in situ metabolomics, together with gene inactivation to discover massilicins, the silent and unstable antifungal agents, from dual-culture of *Massilia* sp. YMA4 and *Candida albicans*. The click reaction was then employed to trap polyynes from *Massilia* sp. YMA4 extract for further isolation and structure elucidation of massilicins.

[1] Shun ALKS, Tykwinski RR. *Angew Chem Int Ed* 2006; 45: 1034-1057;

[2] Ross C, Scherlach K, Kloss F, Hertweck C. *Angew Chem Int Ed* 2014; 53: 7794-7798

Session 5-1-SL-05:

Toward engineering of cannabinoid biosynthesis: In-planta characterization of enzymes involved in the synthesis of THCA and derivatives

Heribert Warzecha ¹, Marcus Geissler ¹, Oliver Kayser ², Felix Stehle ²

¹ *Plant Biotechnology and Metabolic Engineering, Technische Universität Darmstadt, Darmstadt, Germany*

² *Laboratory of Technical Biochemistry, Department of Biochemical and Chemical Engineering, TU Dortmund University, Dortmund, Germany*

Numerous metabolites from *Cannabis sativa* L. are interesting drug targets or lead compounds for the development of semisynthetic derivatives. To uncouple targeted cannabinoid production from a THC-producing plant, a biotechnological production system with defined products would be advantageous ¹.

Here we describe the engineering of late biosynthetic genes into *Nicotiana benthamiana* Domin to evaluate biosynthetic capacity in a transient expression system. With THCA synthase, we were able to show that active enzyme can be obtained after ER/apoplast-targeting ². Moreover, THCAS seems to be glycosylated in *N. benthamiana*, suggesting that this modification has an influence on the stability of the protein. Activity assays with cannabigerolic acid as substrate showed that the recombinant enzyme not only produces THCA (123 ± 12 fkat g⁻¹ FW activity towards THCA production) but also cannabichromenic acid (CBCA; 31 ± 2.6 fkat g⁻¹ FW activity towards CBCA production). Moreover, we achieved the specific prenylation of olivetolic acid to form cannabigerolic acid by the introduction of an orthologous microbial gene. This preliminary work shows that *N. benthamiana* could be a suitable host for cannabinoid production, but towards whole pathway integration careful analysis of subcellular localization is necessary.

1 Schachtsiek, J., Warzecha, H., Kayser, O., Stehle, F. (2018) Current Perspectives on Biotechnological Cannabinoid Production in Plants. *Planta Med.* 84, 214-220.

2 Geissler, M., Volk, J., Stehle, F., Kayser, O., Warzecha, H. (2018) Subcellular localization defines modification and production of Δ^9 -tetrahydrocannabinolic acid synthase in transiently transformed *Nicotiana benthamiana*. *Biotech. Lett.* (in press)

Wednesday, 29th August, 2018 - Bibo BallroomAB - 11:10 - 12:30

Invited Lecture - Session 1-4

Session 1-4-IL-01:

The Use of Isolated Natural Products as Scaffolds for the Generation of Chemically Diverse Screening Libraries for Drug Discovery

Rohan Davis

Griffith Institute for Drug Discovery, Griffith University, Nathan, Australia

The Davis Research Group focuses on the semi-synthesis of discovery libraries based on unique natural product scaffolds that have been isolated from Australian fungi, plants and marine invertebrates [1-4]. The ultimate goal of such libraries is to assist in the identification of bioactive compounds that can impact drug discovery or chemical biology research. This approach builds upon the chemical diversity and uniqueness found in natural products and results in the generation of small screening libraries (typically 10–20 analogues per scaffold) that allows for the rapid elucidation of both structure–activity relationships and in vitro metabolic stability, whilst circumventing the initial need for total synthesis. All analogues made during this program are added to the Davis Open-Access Natural Product Library (currently 512 pure compounds), which is curated by Compounds Australia (CA) – a national compound management facility that is housed at Griffith University and which enables dispatch of compounds to collaborators for screening against various disease and chemical biology targets [5]. This presentation will describe a number of recent semi-synthetic projects that have resulted in the discovery of new compounds that perturb various biological systems.

References

- [1] E. C. Barnes, R. Kumar, R. A. Davis. *Nat. Prod. Rep.* 2016, 33, 372.
- [2] F. A. Egbewande, N. Nilsson, J. White, M. Coster, R. A. Davis. *Bioorg. Med. Chem. Lett.* 2017, 27, 3185.
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Session 1-4-IL-02:

Investigating the marine mesophotic zone for the discovery of bioactive small molecules with anti-aging activity

Nikolas FOKIALAKIS ¹, Nikolaos TSAFANTAKIS ¹, Eirini BAIRA ¹, Ioannis P. TROUGAKOS ¹, Aimilia SKLIROU ¹, Pinelopi VLACHOU ¹, Eleni-Dimitra PAPANAGNOU ¹, Christina CHEIMONIDI ¹, Pedro ÁLVAREZ ², Suchana CHAVANICH ³, Anne BIALECKI ⁴, Nicole DE VOOGD ⁵, Yehuda BENAYAHU ⁶, Michael SCHAEFFER ⁷, Géraldine LE GOFF ⁸, Jamal OUZZANI ⁸

¹ *National and Kapodistrian University of Athens, Athens, Greece, Athens, Greece*

² *iMare Natural, Granada, Spain, Granada, Spain*

³ *Chulalongkorn University, Bangkok, Thailand, Bangkok, Thailand*

⁴ *Université de la Réunion, Ile de la Réunion, France, Ile de la Réunion, France*

⁵ *Naturalis Biodiversity Center, Leiden, Netherlands, Leiden, Netherlands*

⁶ *Tel Aviv University, Tel Aviv, Israel, Tel Aviv, Israel*

⁷ *Crelux, Martinsried, Germany, Martinsried, Germany*

⁸ *ICSN-CNRS, Gif sur Yvette, France, Gif sur Yvette, France*

In the frame of TASCAR more than 180 existing collection of invertebrates (MACLIB library) and 179 targeted marine invertebrates species (TARMAC library) were collected from the under- investigated mesophotic zone (between 30 and 100 meters depth) of the Indian ocean, the Red sea and the Mediterranean. Furthermore, more than 300 (MICLIB library) and 312 (TARMIC library) associated microorganisms of MACLIB and TARMAC libraries respectively, were collected.

The samples were extracted and libraries of extracts were sent for biological evaluation. According to the results for MACLIB library, 5.30% of the extracts showed elastase and tyrosinase inhibitory activity, 7.94% inhibition to Fyn kinase, 6.35% to proteasome and 4.76% to CDK7 kinase. For TARMAC library 16.3% of the extracts showed tyrosinase inhibitory activity, 12.4% elastase inhibitory activity, 5.03% to FYN kinase, 15.64% to CDK7 kinase and 20.67% to proteasome.

All active extracts were investigated for their chemical profiling employing UHPLC-HRMS techniques and the metabolites present in each extract were identified using molecular networks, in silico fragmentation and classical dereplication techniques based on databases. Selected extracts were fractionated and a library of fractions has been forwarded for bio-evaluation. From the active fractions compounds of interest have been isolated and identified by NMR and LC-MS.

Furthermore, the biological activity of the associated microorganisms was examined. For MICLIB library, the microorganisms showed 0.5% inhibition activity to tyrosinase and elastase, 0.9% to Fyn kinase, 6.67% to CDK7 kinase and 1.67% to proteasome. For TARMIC extracts, ~80.1% were found to inhibit tyrosinase activity, 22.8% showed elastase inhibitory activity, 14.19% to FYN kinase, 7.67% to proteasome and 7.43% to CDK7 kinase.

Finally a comparison between the LC-MS profiles of the invertebrate extracts and the profiles of the microbial symbionts was performed showing overlapping of 4 to14% indicating the contribution of microorganisms to the whole invertebrate metabolome.

Wednesday, 29th August, 2018 - Bibo BallroomC + Room1 - 11:10 - 12:10

Invited Lecture - Session 5-2

Session 5-2:-IL-01:

Metabolomics Assay identified a novel virulence-associated siderophore encoded by the High-Pathogenicity Island in uropathogenic Escherichia coli

Hai-tao Lv

Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China, Shanghai, China

Yersiniabactin is still identified as a unique siderophore encoded by the high-pathogenicity island (HPI) in uropathogenic Escherichia coli (UPEC). This does not fully describe the HPI as directing the biosynthesis of siderophores. We aimed to discover and identify new siderophores in the HPI-dependent biosynthetic pathway using a combinational strategy of metabolomics and genetics. A global metabolome assay of wild-type UTI89, UTI89ΔybtS and UTI89ΔybtS involving salicylic acid found numerous unknown metabolite features that were encoded by the HPI with an obvious substrate dependency on salicylic acid. One metabolite feature with a mass-to-charge ratio of 307.0206 was shown to have a similar phenotype as yersiniabactin. Furthermore, isotope mass spectrum calculations and ms/ms annotations were combined to identify this metabolite as 2'-(2-hydroxyphenyl)-4'-thiazolyl-2,4-thiazoliny-4-carboxylic acid (HPTzTn-COOH), and our study is the first to identify HPTzTn-COOH in UPEC UTI89. HPTzTn-COOH was verified as a new siderophore in this study, and it was observed to have a robust capacity to chelate different metals, including Al³⁺, Ni²⁺ and Ca²⁺, in addition to binding Fe³⁺. Our data revealed that HPTzTn-COOH has a much stronger diagnostic ability, characterized by a high production throughout the selected UPEC strains harboring the HPI. Altogether, our discoveries revise the siderophore family, and HPTzTn-COOH can be classified as an additional key siderophore along with yersiniabactin.

Session 5-2:-IL-02:

Using Chemical Proteomics Approaches to Identify Drug Targets and Monitor Nascent Protein Synthesis

Qian Zhao

The Hong Kong Polytechnic University, Hong Kong, Hong Kong

Chemical proteomics combines chemical biology and mass spectrometry-based proteomics, which is a powerful tool for studying small molecule-protein and protein-protein interactions. In chemical proteomics, synthetic chemical probes react with mechanistically related classes of proteins. The biological function of these proteins can be studied through perturbation or regulation using the probes.

We have developed novel chemical proteomics methods and applying them in biology studies including drug target profiling^{1,2}, nascent protein synthesis^{3,4} and protein post-translational modifications (PTMs)⁵. In particular, we have identified the direct-binding protein targets of natural product triptolide, celastrol and withaferin A by combining multiple mass spectrometry-based techniques. We have also developed sulfonyl fluoride-based probes that covalently label a broad swath of the intracellular kinome under physiological condition. We were able to simultaneously identify low-abundant endogenous kinases that bind to clinical kinase inhibitor dasatinib and accurately measure its target engagement rate by mass spectrometry. Based on previous experience, we have also developed “OPP-ID” method for monitoring protein synthesis by using O-propargyl puromycin (OPP) to label newly-synthesized proteins. OPP-ID has been the key technique for us to identify proteins that are synthesized locally in injured sciatic nerve, which has been a key question in Neurobiology.

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4. Science. 2018, 359, 1416-1421.
5. Angewandte Chemie International Edition, 2013, 52 (18):4883–4886.

Session 5-2:-IL-03:

National Third -party Testing Platform for TCM Quality

Yao Shen¹, Peng Zhang ¹, Xiao-mei Fu ¹, Yu-miao Luo ¹, Gang Xiao ¹, De-an Guo ^{1,2}

¹ Genchim Testing (Shanghai) Co., Ltd., Shanghai, China

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With the increasing TCM market inside and outside of China, the need for TCM testing is drastically changing to match this growth. The market demands TCM testing, especially testing has to be done by a third-party organization in case the first- and second-party laboratory perform divergent results. In EN ISO/IEC 17025, a third-party testing laboratory has to be qualified as independent in that it is free from any undue commercial, financial and other pressures which might influence their technical judgement. Chinese Government,

Sate Administration of Traditional Chinese Medicine and National Development and Reform Commission, launched a project to cultivate third-party platform for TCM testing. National third-party testing platform for TCM (the South) – Genchim Testing is the only platform that obtained this grant so far. Genchim is a full-service TCM testing laboratory, its service covers from physicochemical property analysis, exogenous contamination analysis to biology analysis. It with an area of 5000 m² now located in Shanghai is applying for accreditation of CNAS and CMA and will obtain the certificate of conformity in the end of this year. Genchim Testing will work continuously towards improving TCM quality through professional testing service and leading TCM Standardization research.

Wednesday, 29th August, 2018 - The Chapel - 11:10 - 12:25

Invited & Short Lecture - Session 6

Session 6-IL-01:

Systemic Research and Industrial Promotion of *Cistanche deserticola*

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As one of the most famous tonic herbal medicines, *Cistanches Herba* (CH) has long been applied for the treatments of kidney deficiency, impotence, female infertility, morbid leucorrhea, profuse metrorrhagia, and senile constipation in traditional Chinese medical practices. There are four species, including *Cistanche deserticola* Y. C. Ma, *C. tubulosa* (Schenk) Wight, *C. salsa* (C. A. Mey.) G. Beck, and *C. sinensis* G. Beck, distributed in northwest China. Among them, two species, *C. deserticola* and *C. tubulosa*, are authorized as the original sources of CH in Chinese Pharmacopeia. All *Cistanche* plants are root holoparasitic herbs, and their hosts such as *Tamarix* and *Haloxylon ammodendron*, are sand-fixing plants. The wild sources of *Cistanche* plants are on the edge of extinction attributing to over-harvesting.

Because of continuous efforts in the past 28 years, we have in depth elucidated the active compounds in CH and revealed the pronounced benefits of phenylethanoid glycosides against neurodegenerative disorders, e.g. Alzheimer's and Parkinson's diseases; we have clarified the parasitic mechanisms of *Cistanche* plants and established high-quality cultivation protocols; and we have also widely advanced the wide cultivation of their hosts, such as *Tamarix* plants and *H. ammodendron*, for 341.3 km², and thereafter inoculated *C. deserticola* and *C. tubulosa* for 84 km². Currently, the source shortage of CH has been completely addressed to guarantee the clinical applications and TCM industry, and ecological civilization, economic development, wealth accumulation and social security are significantly promoted in the border and minority nationality areas. Overall, our continuous efforts create a new model for sustainable desert control as well as a novel

strategy for “Targeted Poverty Alleviation” in arid regions.

Session 6-SL-03:

Effect of *Bacillus subtilis* on morphology and bioactive compounds of saffron

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Bacillus subtilis is a plant growth-promoting rhizobacterium (PGPR) in the soil rhizosphere which has been shown to be one of the endophytes of saffron (stigmas of *Crocus sativus* L.). This plant is the most valuable medicinal food product in Iran and has a nutraceutical potential in industry. The pharmacological effects of saffron is associated with two major bioactive components, crocin and safranal.

In this study, the effects of *B. subtilis* ATCC 6633 on morphology and bioactive compounds of saffron has been investigated using two types of soils including clay and peat/perlite mixture. Three different bacterial suspensions (10^2 , 10^5 and 10^8 cfu/ml) were used in the 14-day-interval treatments of saffron planted in either sterile or unsterile soils. Flowering time was recorded and then the stigmas were collected and weighed. The amounts of α -crocin and safranal in the stigma extracts were quantified using high performance liquid chromatography (HPLC).

The longest stigma, petal and leaf was observed in the treated group with 10^5 and 10^8 cfu/ml in both sterile and unsterile soils. The highest weight of stigma per corm belonged to the treated groups with 10^2 cfu/ml in unsterile soil and 10^5 cfu/ml and 10^8 cfu/ml in sterile soil. Treatment with 10^2 and 10^8 cfu/ml caused 1.077 and 1.149-fold increase of safranal production in sterile peat/perlite and 2.270 and 1.851-fold increase in unsterile peat/perlite compared to the control group (no treatment). While upon treatment with 10^5 and 10^8 cfu/ml 1.092 and 1.120-fold increase of α -crocin was measured in sterile peat/perlite and 10^2 cfu/ml caused 8.87-fold increase in α -crocin in unsterile peat/perlite soil.

In conclusion, the data of this study showed that *B. subtilis* triggers the morphological and physiological processes in saffron. *B. subtilis* suspension can be used in agricultural settings to induce the production of important bioactive components, α -crocin and safranal.

Wednesday, 29th August, 2018 - Bibo BallroomAB - 11:50 - 12:25

Invited & Short Lecture - Session 4-2

Session 4-2-IL-01:

Discovery of the multi-targeted mechanisms of a natural diterpenoid eriocalyxin B in breast cancer

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Eriocalyxin B (EriB), a natural ent-kaurane diterpenoid isolated from *Isodon eriocalyx* var. *laxiflora*, has been reported to exhibit anti-tumor and anti-inflammatory activities. The mechanisms of action of its anti-angiogenic and anti-tumor activities were further elucidated by our research team using various in vitro and in vivo models. The anti-angiogenic activities of EriB were investigated in human endothelial cells, zebrafish and mouse models, while the anti-tumor effects were evaluated in both estrogen receptor (ER)-positive and ER-negative human breast cancer cells as well as breast xenograft mouse models. Results showed that EriB at 50 or 100 nM could significantly suppress vascular endothelial growth factors (VEGF)-induced cell proliferation, tube formation and cell migration in human endothelial cells, which involved down-regulation of VEGFR-2 signaling pathway [1]. The inhibitory activity on angiogenesis were also observed in zebrafish embryos and mouse matrigel plug models. While in human breast cancer cells, EriB at 1.5 or 2.25 mM induced apoptosis and triggered autophagy by inhibition of Akt/mTOR/p70S6K signaling pathway, as well as coordinated the crosstalk between apoptosis and autophagy [2]. Furthermore, EriB treatment (10 mg/kg) could induce autophagy and anti-angiogenic effects in breast xenograft-bearing mice. Our recent comprehensive transcriptome analysis in zebrafish embryos illustrated that EriB at 10 or 15 mM could enrich several pathways, such as glutathione metabolism, phototransduction, and metabolism of xenobiotics by cytochrome P450 [3]. The target genes involved in the regulation of signaling pathways by EriB were newly identified, which will provide new insight of the biological activities of this natural compound. In conclusion, the multi-targeted activities of EriB were further verified through the molecular studies and functional tests in cell-based and animal models. The great potential of this compound to be developed as an anti-cancer agent for breast cancer is warranted.

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Session 4-2-SL-02:

Ayurvedic treatment options in Diabetes Mellitus and allied bacterial infections, inflammation; a functional food based approach.

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Ayurveda of India, commonly practiced in subcontinent including Pakistan, has a published history preceding to 1500 BCE and its reliance is established most recently in a genomic SNP (single nucleotide polymorphism) based analysis, that confirms the genetic basis of Ayurveda. Moreover, it was also established that Prakriti approach (Genetic makeup of every human) of Ayurveda practice simply resounds with concept of modern personalized medicine. We explored a constituents of traditional herbal formulation that also serves as functional food. The *Ferula narthex* Boiss. (Apiaceae), *Ziziphus numularia* (Burm.f.) Wight & Arn. (Rhamnaceae) and six different Citrus species (*Citrus sinensis* (L.) Osbeck, Hybrid of *Citrus nobilis* and *Citrus delicense*, *Citrus paradisi* Macfad, *Citrus aurantifolia* Christm. and *Citrus limon* (L.) Burm. f. from Pakistan were explored for detailed phytochemical analysis and biological activities. The ligupersin A isolated from *Ferula narthex* presented highest antiglycation activity (IC₅₀ 0.414 mM) in BSA-glucose model whereas, being more active than in BSA-MGO assay, highest activity was shown by 8'-O-acetyl-asacoumarin A (IC₅₀ 1.03 mM). In case of *Z. nummularia* (in DPPH assay), the methanolic fraction presented highest activity (IC₅₀ 193.1 µg/ml), followed by ethyl acetate (IC₅₀ 220 µg/ml) and chloroform (IC₅₀ 263 µg/ml) fractions. The FRAP value for ethyl acetate fraction recorded as highest (370.2 µM). During the antibiofilm assay, n-hexane fraction presented highest inhibition (88%) followed by ethyl acetate (69%) chloroform (65%) fractions. The extracts also presented interesting results towards diabetes, and inflammation. Likewise the citrus peel extracts presented significant antidiabetic, antimicrobial, antiinflammatory properties that could be mainly contributed by flavonoids. It was therefore concluded that indigenous herbal formulation effectively helps in management of diabetes and allied comorbidities.

Wednesday, 29th August, 2018 - The Chapel - 12:05 - 12:20

Short Lecture - Session7

Session7-SL-01:

Nanosized liposomes of andrographolide for improved brain delivery: formulation and in vitro studies using pampa and hCMEC/D3 cells

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Andrographolide (AG), the major diterpenoid of *Andrographis paniculata* (Burm. f.) Wall. ex Nees (Acanthaceae), has received attention due to its pharmacologic activities including protection against damage induced by beta-amyloid, modulation of the formation of amyloid plaques and recovery of spatial memory functions in Alzheimer disease mouse model [1]. The high lipid solubility of AG permits penetration of the blood–brain barrier but it reduces its bioavailability. Continuing our studies on the development of drug delivery systems able to cross the BBB [2], in this work we developed nanosized liposomes to deliver AG and ameliorate its biopharmaceutical properties.

The surface properties of the liposomes were modified by adding Tween 80 (LPs-AG) alone or with didecyldimethylammonium bromide (DDAB) (CLPs-AG) for enhanced penetration into the brain. The mean vesicle size resulted less than 100 nm with low polydispersity index. The entrapment efficiency was about 50%. TEM analyses showed spherical vesicles with smooth bilayer surface. Stability of liposomes was assessed over one-month period as suspension at 4°C and as lyophilized product at 25°C. The release of AG at pH 7.4 was prolonged and sustained and Higuchi model was shown to be the best-fit model to describe the kinetic of release. In vitro permeation studies, both PAMPA and hCMEC/D3 cells, revealed that LPs-AG and CLPs-AG increased the permeability of AG, about an order of magnitude, compared to free AG. Further studies indicated that active processes, in particular caveolae-mediated endocytosis, were involved in the uptake of liposomes.

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Wednesday, 29th August, 2018 - Bibo BallroomC+Room1 - 12:10 - 12:25

Short Lecture - Session 8

Session 8-SL-01:

The effect of clove essential oil and some of its active ingredients on swine jejunal preparations

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Herbal extracts and preparations are a constantly growing group of feed additives. The introduction of essential oils (EOs) to animal diet represents one of the strategies aimed at replacing the use of antibiotics as growth promoters for pig production. EOs, including clove oil, have been used in pig breeding with the main focus on digestive system functions. The beneficial effect of clove oil on swine production performance results from its antioxidant activity, the ability to modulate intestinal microbiota and increasing feed digestibility. The occurrence of gastrointestinal signs like diarrhea requires causative and symptomatic treatment in animals. The symptomatic therapy should include the regulation of gut motility disorders. Therefore, the aim of the study was to evaluate the effect of clove oil and its active ingredients on swine jejunal smooth muscle.

The study was conducted on jejunal segments collected from adult pigs which underwent routine slaughter procedure. Intestinal preparations were examined under isometric conditions. The effect of clove essential oil and four of its active ingredients (eugenol, thymol, carvacrol, cinnamaldehyde) were analyzed.

The results showed that clove oil, eugenol, carvacrol, thymol and cinnamaldehyde induce a dose-dependent modification of the spontaneous contractility of swine jejunum. The most advanced myorelaxant effect was generated by intact clove oil used in a dose of 10 μ L/mL. The reaction exceeded the magnitude of the relaxation induced by the reference relaxant agent. Among the individual components, eugenol and thymol turned out to be the most potent antispasmodic agents, followed by carvacrol. Cinnamaldehyde produced an opposed contractile effect of a mild intensity.

In conclusion, the obtained data suggest that clove oil extract and eugenol are responsible for the main effect on jejunum contractility. The antispasmodic effect desired in diarrheic animals can be expected only if clove oil is used as feed additive in relatively high doses.

Wednesday, 29th August, 2018 - Pavilion - 14:00 - 15:45

Plenary Lecture - Plenary Lecture

Plenary Lecture-KNL-01:

Contextualized Metabolomics Transforms Pharmacognosy – A Paradigm Shift in Natural Product Research

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The rapid innovations in metabolite profiling, bioassays and chemometrics lead to a paradigm shift in natural product (NP) research. Indeed, having at hand partial/full structure information of possibly all secondary metabolites and an estimation of their levels in plants, provides a way to perform pharmacognostic investigations from a new and holistic perspective. The increasing amount of accurate metabolome data that can be acquired on massive sample sets, notably through data dependent HRMS/MS, allows mapping natural extracts at an unprecedented precision level [1,2]. In this context, data contextualization is however still a lagging process [3]. For this, we investigated methods that could provide enhanced annotation confidence level through multiple scores integrating taxonomy information and molecular network (MN) structural consistency as well as other orthogonal analytical data. Benchmarking of such approaches is currently assessed by profiling mixtures of herbs with well-studied composition. We also investigate the best way to integrate extracts bioactivity data in MN and shortcut bioactivity guided isolation for an efficient targeted identification of bioactive NPs [4]. To this end, accurate chromatography gradient methods at various scales have been developed for MS-targeted purification of biomarkers and their full de novo structure identification by NMR. Different recent applications of our metabolomics/phytochemical investigations will illustrate these aspects. Evaluation of what is readily implemented and is still required in NP research will be made, notably in terms of contextualisation of the data.

Keywords :

Dereplication, metabolite profiling, metabolomics, MS-targeted isolation

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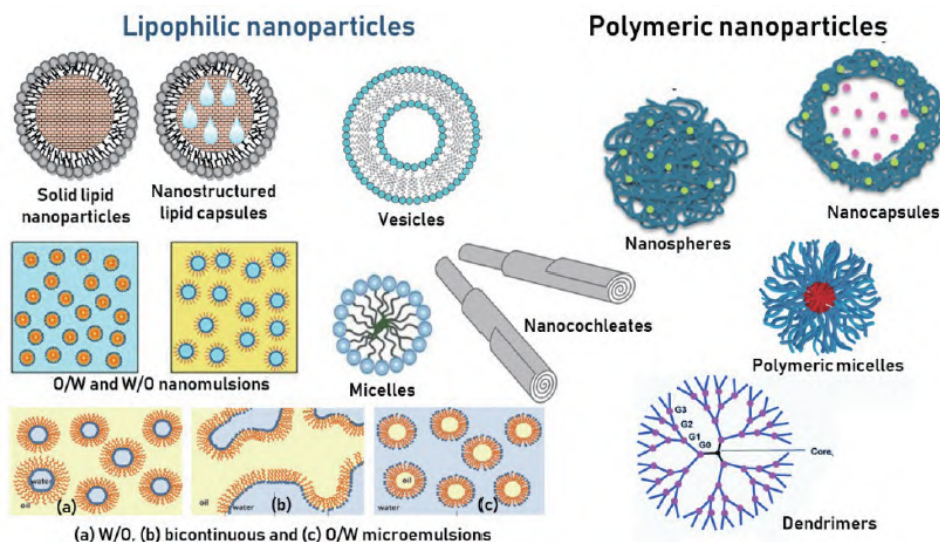
Plenary Lecture-KNL-03:

Nanocarriers to improve solubility, stability, and optimise bioefficacy of natural products

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Over the millennia, plants have represented for Humankind the main source of food, but also for prophylactic properties or to cure human and animal diseases. Presently, between 65 and 80% of populations in developing countries use medicinal plants as therapeutic remedies for their primary healthcare and in Europe and USA there is an increasing demand of botanical products. Botanicals on the market are mainly based on traditional, conventional and innovative extracts, but the number of marketed isolated constituents is also increasing. Conversely, the clinical use of many of these isolated constituents and several extracts is limited due to the need of repeated administrations or high doses because of low hydrophilicity and intrinsic dissolution rate(s), or physical/chemical instability. Other limits are low absorption, poor bioavailability, trivial penetration and accumulation in the organs of the body. Nowadays, the design and production of appropriate drug delivery systems, in particular nanosized ones, have already entered into clinical use and can offer an advanced approach to optimize the therapeutic efficacy of extracts and isolated constituents [1-4]. Novel nanoformulations (Figure 1), namely polymeric nanoparticles and lipid based-nanocarriers represent successful examples overcoming these limitations. Emerging molecules with pleiotropic functions and several extracts have been successfully formulated in nanocarriers.



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Poster session-PO-36:

Comparison of the Content of Polysaccharides and Triterpenoids in Sporoderm-broken Spores of Ganoderma Lucidum and Ganoderma lucidum from different producing areas

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Ganoderma Lucidum(GI), known as a precious traditional Chinese medicine which can nourishes and strengthens the body. The broken germ cell of GI called Sporoderm-broken Spores of Ganoderma Lucidum(SSGI) which is a new traditional Chinese medicine that can be collected artificially. The aim of this study was to investigate the differences of the content of polysaccharides and triterpenoids between SSGI and GI. Twelve batches of SSGI samples and twenty batches of GI samples were collected in different producing areas including shandong, jilin, heilongjiang, anhui, sichuan, zhejiang and fujian provinces. Samples were assayed according to Chinese Pharmacopoeia of 2015 year by Ultraviolet Spectrometry. The polysaccharides were quantified by anthrone-sulfuric acid method, the triterpenoids were quantified by vanillin-glacial acetic acid method. As a result, the content of polysaccharides in SSGI was between 0.92% and 2.97%, average value(AV) \pm standard deviation(SD) was 1.61% \pm 0.63, with the RSD was 39.44%; while the content of triterpenoids was between 1.18% and 2.98%, AV \pm SD was 1.96% \pm 0.60, with the RSD was 30.72%. Corresponding, the content of polysaccharides in GI was between 0.55% and 2.16%, AV \pm SD was 1.04% \pm 0.42, with the RSD was 40.66%; while the triterpenoids was between 0.44% and 1.30%, AV \pm SD was 0.73% \pm 0.20, with the RSD was 27.75%. According to the results, the content of polysaccharides in SSGI was lower than that of triterpenoids. Conversely, the content of polysaccharides in GI was higher than that of triterpenoids. Otherwise, the content of polysaccharides and triterpenoids in SSGI were all higher than that in GI. Although SSGI and GI are homologous, but the material basis is different which can provide reference for the inner quality evaluation and pharmacological difference of SSGI and GI.

Sporoderm-broken Spores of Ganoderma Lucidum; Ganoderma Lucidum; different producing areas; polysaccharide; triterpenoids; content

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Additional Poster session-PO-01:

Chemical Characterization and Antibacterial Activity of *Nepeta cadmea* Boiss.

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The genus *Nepeta* (Lamiaceae) is represented in Turkey by 33 species and altogether 38 taxa, 17 of these being endemic in Turkey [1,2]. It grows in Central and Southern Europe, West and Southern Asia. *Nepeta* species are widely used in folk medicine because of their antispasmodic, diuretic, antiseptic, antitussive, antiasthmatic activities [3].

Nepeta cadmea Boiss., endemic to Turkey was collected during the growing season from Denizli in 2017. The aerial parts of *N. cadmea* were hydrodistilled for 3 h using Clevenger-type apparatus to obtain essential oil. The essential oil was analyzed by GC, GC/MS, simultaneously to determined the chemical characterization of its. 4α,7α,7β-nepetalactone (74.0%), 4α,7α,7β-nepetalactone (4.5%) and caryophyllene oxide (2.5%) were found as major components for essential oil. The potential *in-vitro* antibacterial activity of the essential oil was evaluated using the broth microdilution assay. A panel of human pathogenic strains *Escherichia coli* NRRLB-3008, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL-B3711 and *Streptococcus sanguinis* ATCC 10556 were used. Minimal Inhibitory Concentrations (MIC) of the sample was determined, where ciprofloxacin was used as a positive control in the experiments. MIC values were found 2500, 1000, 600, 600, 600 µg/mL against *E. coli*, *P. aeruginosa*, *S. typhimurium*, *B. cereus*, *S. sanguinis* respectively. Compared the literature the essential oil had lower effective against these pathogens.

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Additional Poster session-02:

Bio-guided Isolation of Volatile Compounds with Repellent Properties against *Aedes albopictus* (Diptera: Culicidae) using CPC technology

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Aedes albopictus is an invasive mosquito species, with wide spread in many countries worldwide. The

protection from mosquito bites still remains the most effective way to prevent infections associated with moboviruses, such as Zika, Dengue and Chikungunya. In the effort to develop potent mosquito repellents less harmful for humans and the environment, research on natural products with strong bioactivity, such as essential oils, has been prioritized.

In the present work 6 essential oils (EOs) obtained from wood of *P. heldreichii* and *P. nigra*, needles of *P. pinea*, old and fresh fruits of *J. phoenicea* and fruits of *J. oxisedrus* have been prepared using Microwave-assisted hydrodistillation. Chemical composition of all EOs were determined by GC/MS analysis. Additionally their repellent properties were evaluated against *Aedes albopictus* showing that the essential oil of *P. pinea* and *J. phoenicea* presented high activity at the dose of 0.2 $\mu\text{L cm}^{-2}$. Comparing the results of GC/MS analysis and biological assays we decide to further analyze the EOs of *P. pinea* and *J. phoenicea* in order to isolate the main volatile compounds and to identify those responsible for the repellent activity. The fractionation of the selected EOs took place by CPC using for the analysis the biphasic system n-Heptane/ACN/BuOH in ratio 1.6/1.6/0.2 (v/v/v). The results of this step were the isolation of (-) limonene and guaial from EO of *P. pinea* and myrcene and germacrene-D from EO of *J. oxycedrus*. All isolated compounds were tasted for their repellent activity at the dose of 0.2 $\mu\text{L cm}^{-2}$. The results of repellent bioassays, employing fractionated compounds, revealed that (-) limonene, guaial and germacrene D presented high repellent activity, while myrcene was almost non-active.

It is worth noting that the use of CPC for bio-guided isolation of active volatile compounds from EOs is presented for the first time.

Additional Poster session-PO-03:


STUDIES ON THE ESSENTIAL OIL BEARING PLANTS OF CYPRUS

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Cyprus is the 3rd largest island in the Mediterranean sea with a land cover of 9.251 sq.km. Being in eastern Mediterranean it is under the influence of the floras of Asia, Africa and Europe. Flora of Cyprus is well documented with 1.610 species and altogether 1.738 taxa. 108 species (143 taxa) are endemic plants comprising 6.7% (8.2%) of the flora. The families Asteraceae (66), Lamiaceae (39) and Apiaceae (29) are important for the aromatic flora.

We have been systematically investigating essential oils from aromatic plants of Cyprus and here I shall present our results on the essential oils of the following species: *Anthemis tricolor*, *Asphodelus aestivus*, *Chenopodium murale*, *Chrysanthemum coronarium*, *Helichrysum conglobatum*, *Helichrysum italicum*, *Lagoecia cuminoides*, *Lathyrus* spp., *Origanum cordifolium*, *Origanum majorana*, *Origanum dubium*, *Phlomis brevibracteata*, *Phlomis cypria* var. *cypria*, *Pimpinella cypria*, *Sideritis cypria*, *Teucrium cyprium*, *Teucrium kyreniae*, *Teucrium micropodioides*, *Teucrium salaminium*, *Thymus capitatus*, *Thymus integer*, *Zosima absinthifolia*.



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