Thieme

67th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) in cooperation with the French Society of Pharmacognosy AFERP

Date/Venue:
September 1–5, 2019, Innsbruck, Austria

Congress president:
Univ.-Prof. Dr. Hermann Stuppner

Organizing societies:
GA (Gesellschaft für Arzneipflanzen- und Naturstoff-Forschung e.V./Society for Medicinal Plant and Natural Product Research) in cooperation with the French Society of Pharmacognosy (AFERP)

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Editorial

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The 67th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) took place in Innsbruck, Austria, from September 1-5, 2019 in cooperation with the French Society of Pharmacognosy AFERP.

The main scientific topics of the conference were:

- Natural products chemistry
- Biological and pharmacological activities of natural products
- Analytical methods for quality control of Herbal Medicinal Products
- Metabolomics and molecular networking
- Identification and authentication of plant material
- Ethnobotany and ethnopharmacology
- Herbal drug formulations
- Biosynthesis and biotechnology of natural products
- Biodiversity and chemical ecology
- Clinical studies with natural compounds and Herbal Medicinal Products
- Phytopharmaceuticals, dietary supplements, functional food, cosmeceuticals
- Medicinal plants and natural products in animal healthcare and veterinary medicine

The symposium started on Sunday, September 1st 2019 with four pre-congress events: the Young Researchers' Workshop, the Workshop on African Research Network and the one-day pre-congress Symposia on Animal Healthcare and Veterinary Phytotherapy as well as Economic Adulteration of Botanical Ingredients.

The scientific program of the Main Conference included 10 plenary lectures, 2 keynote lectures, 45 contributed short lectures and 492 posters. The Regulatory Affairs Workshop was held during the main conference and served as a forum for industry to update on the latest trends in regulation of phytomedicines and related areas. The meeting was also an excellent platform for exhibitors, who presented their latest products and services.

We would like to thank everyone who has made this GA meeting possible, in particular the members of the Organizing and Scientific Committees, as well as the representatives of the PCO Tyrol Congress. We are also grateful to the University of Innsbruck for providing the meeting place and infrastructure for the pre-symposia and the get-together event, as well as the sponsors for their financial support.

Last, but not least, we want to thank Thieme Publishers for publishing the conference abstracts in Planta Medica.

On behalf of the Organizing Committee

Univ.-Prof. Dr. Hermann Stuppner

ABBREVIATIONS

IL: Invited lecture
ISL: Invited short lecture
SL: Short lecture
KL: Keynote lecture
PL: Plenary lecture

Pre-Congress Symposia

African Research Workshop

IL AR-01 Revisiting African traditional medicine within the 21st century context – past, present and future perspectives

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DOI 10.1055/s-0039-3399630

Although Africa appears culturally heterogeneous with thousands of distinct tribes, dialects and languages, it is united by a common thread of traditional medical systems and spiritual attributes. African biodiversity, coupled with this deeply rooted African ethnobotanical heritage, has already contributed a number of novel chemicals entities resulting in potent pharmacotherapeutics, and Africa still remains a promising untapped reservoir for the discovery of more diverse chemical entities. African flora have already demonstrated their chemical diversity by providing a series of novel chemical entities [1].

The pharmacotherapeutic potential of the African biodiversity has not yet been realized since much of the research carried out is fragmented with little or no focus on drug discovery. African researchers are not only challenged by communicable and non-communicable diseases encroaching upon the continent, but they are also confronted with meagre financial resources, poor infrastructure and non-accessibility to modern technological platforms which are crucial elements for drug discovery. While the African continent is virtually considered as the epicentre of pathogens endemicity, the African scientific community is very poorly represented in setting the research agenda and priorities.

The recent initiative of GA to streamline African research is undoubtedly a move in the right direction, provided that concerted and concomitant efforts are made to maintain a sound scientific capacity in Africa. Such efforts are a major requirement for stopping the brain drain, further consolidate the African ethnobotanical heritage and building a critical mass of young African scientists to guarantee ownership and sustainability of long-term control programmes.

References [1] Khalid, SA. Natural products-based drug discovery against neglected diseases with special reference to African natural resources. In: Chibale, K et al. Drug Discovery in Africa. Heidelberg: Springer-Verlag Berlin; 2012 doi:10.1007/978-3-642-28175-4_9

SL AR-01 Antioxidant and anti-inflammatory activities of Cameroon nutritional spice extracts in human gastric epithelial cells

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In Cameroon, many medicinal plants, including spices, are used as herbal medicines and traditionally employed for the treatment of gastric diseases, in which oxidative stress is involved.

The present work chemically characterizes and investigates the antioxidant and anti-inflammatory effect of hydro-alcoholic extracts of eleven Cameroonian spices at gastric level, focusing on Nuclear Factor (NF)-κB pathway.

Prepared hydro-ethanolic extracts were characterized by HPLC-DAD and GC/MS analysis, then screened for their ability to inhibit tumor necrosis factor (TNF)α-induced IL-8 and IL-6 release, in human gastric epithelial cells (GES-1 and AGS), assessing the involvment of NF-κB driven transcription. The antioxidant activity of the extracts was evaluated as well.

After a preliminary screening, *Xylopia parviflora, Tetrapleura tetraptera, Dichrostachys glomerata, Aframomum melegueta,* and *Aframomum citratum* extracts were chosen for in-depth studies. They reduced in a concentration-dependent fashion the cytokines release (IC_{50}s between 0.19 µg/mL and 20 µg/mL) and the NF-κB driven transcription (IC_{50}s between 0.33 µg/mL and 20 µg/mL). They also showed a highest antioxidant capacity measured by ORAC (range: 2.52–11.88 µM Trolox Eq/g of extract), FRAP (range: 40.23–233.84 mg gallic acid Eq/g of extract) and Total phenols (range: 8.96-32.96% mg gallic acid Eq/g of extract) assays. Chemical analysis suggested that their secondary metabolites (androstenone, chlorogenic acid, pimaric acid, catechin, caffeic acid and its derivatives, 4′,5,7-trihydroxyflavanone, gingerol, shogaol, paradol and gallotannins) could potentially justify the biological properties observed.

Results obtained from this study showed that the extracts reduce oxidative stress and inflammatory markers by scavenging free radicals and impairing NF-κB signaling at gastric level. However, other molecular mechanisms cannot be excluded, and further studies are needed to better clarify their biological activities.

SL AR-02 Chemical composition and *in vitro* investigation of the antibacterial activity of identified compounds from fungus-growing termites *Macrotermes bellicosus*

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DOI 10.1055/s-0039-3399632

The fungus growing termites' species *Macrotermes bellicosus* is 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 [1]. Previous *in vitro* studies revealed that *Macrotermes bellicosus* is efficient against various pathogenic microorganisms [1]. However, the determination of active compounds that contribute to the bioactivity remains unexplored. Aim of the present study was to perform a chemical profile of an ethanolic *Macrotermes bellicosus* extract and determine active compounds that contribute to the antibacterial activity. Chemical profile and structural elucidation of compounds were performed using a pattern recognition strategy based on ^{13}C NMR. The antibacterial activity of fractions and major identified compounds from *Macrotermes bellicosus* was investigated using the broth

microdilution method. Minimum inhibitory concentrations (MIC) were derived to characterize the antibacterial activity. **Major compounds have been identified including benzohydroquinone, 2-methylhydroquinone, niacinamide, ethyl-hexopyranoside,** 3,4-dihydroxyphenethyl glycol and N-[2-(3,4-Dihydroxyphenyl)ethyl]acetamide. Benzohydroquinone and methylhydroquinone exhibited an antibacterial activity against *Staphylococcus aureus* with an MIC of 680 µM and 100 µM respectively. Fractions containing N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide and 3,4-dihydroxyphenethyl glycol showed a growth inhibition of *S. aureus* with IC_{50} of 24.04 µg/mL and 37.28 µg/mL respectively. *Macrotermes bellicosus* used as a traditional medicine could be a therapeutic option for infectious diseases. The study demonstrates that methylhydroquinone and benzohydroquinone are contributing to the antibacterial activity of *Macrotermes bellicosus.* Moreover, antibacterial activity could be observed in *M. bellicosus* extract fractions containing N-[2-(3,4-dihydroxyphenyl)ethyl]acetamide and 3,4-dihydroxyphenethyl glycol.

References [1] Hammoud D, Chougourou D, Vissiennon Z, Ahyi V, Nieber K, Vissiennon C. In vitro evaluation of the antimicrobial activity of fungus-growing termites Macrotermes bellicosus used in traditional medicine in Benin. Conference paper, PMIO 2017 4 (S 01): S1–S202. doi:10.1055/s-0037-1608532

SL AR-03 Identification of glycogen phosphorylase as molecular target for antidiabetic action of *Nauclea latifolia* Smith fruits

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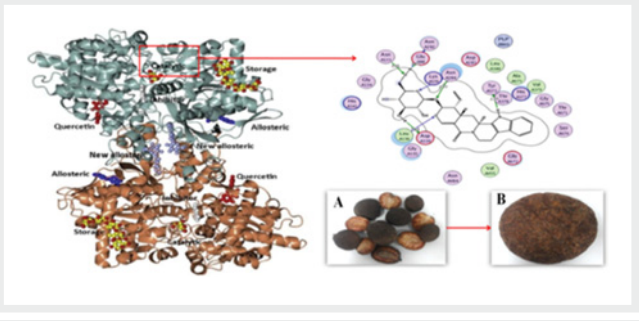
Type 2 Diabetes (T2D) is a metabolic disease characterized by a persistent increase in blood glucose above normal values due to a progressive insulin secretory defect. Diabetes is a leading cause of the pathophysiological conditions associated with the generation of reactive oxygen species and metabolic abnormalities resulting in microvascular and cardiovascular complications [1]. Glycogen phosphorylase (GP) has recently emerged as an important therapeutic target to discover potent antidiabetic drugs for T2D. We aimed to validate the traditional use of *Nauclea latifolia* (Rubiaceae) fruits as antidiabetic agent and identify its main chemical components, molecular target(s), and possible mechanism of action. Both the intact and processed fruits crude extract/fractions were screened for GP inhibition *in vitro.* Antioxidant activity was performed according to standard methods using DPPH and ABTS radicals compared with Ascorbic acid and Trolox. LC-PDA-ESIMS analysis and Molecular Operating Environment (MOE) were used for chemical profiling and *in silico* studies, respectively. Screening of *N.latifolia* fruits revealed a remarkable GP inhibition for the intact fruit (95.57%) compared to the processed fruit. The intact fruit ethyl acetate fraction exhibited the most prominent antioxidant activity which far exceeding its respective processed counterpart (89.50± 0.009, 83.57±0.02%RSA± SD,respectively). The chemical profiles of the fruits comprise β-carboline alkaloids and polyphenols. GP catalytic site was proved to be the most appropriate to accommodate alkaloids and phenolics identified in the fruits (▶**Fig. 1**).The intact fruit revealed superior antidiabetic and antioxidant capacity than the processed fruit. The catalytic site has been identified as the main molecular target.



▶**Fig. 1** 2D interaction of Strictosamide identified in *N.latifolia* intact **(A)** and processed **(B)** fruits with catalytic site of the GP

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SL AR-04 Phytochemistry of Zulu medicinal plants

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In South Africa, the Maputaland-Pondoland-Albany biodiversity hotspot is an important center of plant endemism, and the second richest floristic region in Africa after the Cape Floristic Region. In total, about 8,100 species of plants from 243 families occur within this hotspot, and nearly a quarter of these, at least 1 900 species, are endemic to the area. The Zulu people, who live within this biodiversity hotspot, has a long history of medicinal plant use. In our research, we investigate the chemistry and activity of indigenous medicinal plants, with the focus on plants that are used for illnesses of importance in Africa, i.e. infectious diseases and diabetes. In this contribution, a short overview will be given on important Zulu medicinal plants. In a specific example, the structure and antiplasmodial activity of metabolites of the African fever tree (Vachellia xanthophloea) will be presented. Several flavonoids were isolated, with the most active compound being tri-*O*-methylgallic acid.

SL AR-05 Effects of garlic (*Allium sativum* L.) feed additive on experimentally-induced hepatotoxicity and nephrotoxicity in commercial chickens

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Intoxication, as well as, infectious and metabolic diseases resulting in liver and kidney damage are major causes of losses in poultry species. While there are no specific drugs for the reversal of pathophysiology of the liver and kidney, certain herbs, such as garlic, have been shown to improve liveability of livestock and poultry [1, 2]. Its probable potential at enhancing the functionality of these organs in the face of injuries/insults by intoxication was investigated Chicken subgroup supplemented with 0.25% garlic-meal and administered 300mg/kg acetaminophen at 8 week-old (G1) and subgroup without acetaminophen (G2), those not supplemented and without acetaminophen (NG2) and those not initially supplemented but administered acetaminophen and later supplemented (NG1g) had higher Newcastle disease vaccinal antibody titers (8.0±0.25, 7.5±0.24, 7.6±0.23, 8.14±0.31, respectively) in comparison with the subgroup without supplementation and administered acetaminophen (NGI - 7.13±0.38). Serum protein levels had a similar pattern. AST levels (U/L) were significantly higher ($p<0.05$) in acetaminophen subgroups G1 (79.4 ±5.79) and NG1 (83.7±7.5) than their respective controls G2 (75.1±7.85) and NG2 (65.2±6.84) with no corresponding increase in CK levels. At 2, 7 and 14 days post-administration of acetaminophen (paa), creatinine (mg/dl) was significantly higher in G1 (1.44±0.01, 1.42±0.01, 1.44±0.02) and NG1 (1.47

±0.01, 1.51±0.01, 1.47±0.01) than in G2 (1.16±0.05, 1.23±0.01, 1.26±0.06) and NG2 (1.31±0.06, 1.29±0.02, 1.31±0.05). A similar pattern was observed at 21 days paa. Clinical signs and pathological lesions associated with toxic dose of acetaminophen in liver and kidneys were reversed. Thus exhibiting the potential of garlic in protection against hepatorenal damage or injury

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[2] Tollba AAH, Hassan MSH. Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions 2- black cumin (*Nigella Sativa*) or garlic (*Allium sativum*). Poult Sci 2003; 23: 327–340.

SL AR-06 Tyrosinase TLC-autography for South African indigenous tea, *Athrixia phylicoides* DC, and potential use as a cosmeceutical extract

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Athrixia phylicoides DC (bush tea), a South African indigenous tea, has been consumed in rural communities with its nutritional and medicinal attributes well documented [1]. The low consumption of bush tea in more urbanized areas means the tea can not immulate the success of other South African herbal teas such as rooibos and honeybush [2]. Therefore, the aim of the study was to assess plant metabolites of bush tea for their inhibition of tyrosinase using a TLC (thin layer chromatography)-based autography assay [3]. Ethanol extracts were spotted on TLC (silica gel 60 F_{245}) plate and separated using eluent systems EMW [(Ethyl acetate: Methanol: Water, 40:5.4:4), CEF (Chloroform: Ethyl acetate: Formic acid, 5:4:1), BEA (Benzene: Ethanol: Ammonium hydroxide, 90:10:1)] and their inhibition of tyrosinase evaluated. The competitive and non-competitive inhibitory activity of active metabolites were further tested using a microtiter plate. Metabolites were identified by their retention factor (Rf) value. Gallic acid, chlorogenic acid and quercetin separated with the EMW eluent system expressed anti-tyrosinase activity similar to kojic acid. The prevalence of a dark ring on the TLC plate was indicative of melanin formation while a clear spot indicating inhibition of tyrosinase. Active metabolites with Rf values different from kojic acid were identified with the CEF eluent system. Furthermore, metabolites separated with the BEA eluent system were found not to inhibit tyrosinase. Active metabolites expressed a non-competitive behaviour with a K_m equivalent to kojic acid. The findings from the study reported an alternative usage of bush tea as a natural cosmeceutical extract.

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SL AR-07 Bioactive natural products of endophytic fungi from medicinal plants growing in Timor Island

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Timor Island is located in Eastern Indonesia with high intensities of sun light for the whole year and it is a tropical very dry land that gives high ecological stress for medicinal plants growing in this tropical dry land area. Endophytic fungi has been known to contribute the host plant adaptation to biotic (e.g. pathogens, herbivores) and abiotic (e.g. drought tolerance) stress factors [1, 2] by producing unusual and bioactive secondary metabolites. Research has been undertaken to investigate the bioactive natural products produced by the endophytic fungi isolated from Timorese medicinal plants. Antibiotic compounds and UVB protector compounds have been isolated in high yield from the endophytic fungi *Corynespora cassicola, Diaporthe melonis* and *Aspergillus flavus*. Corynesidones compounds had a strong inhibition to the growth of MRSA bacteria with MIC value of 16 µg/mL while flavomannin dimethyl ether inhibited the growth of streptococcus pneumonia with MIC value of 2 µg/mL. In addition, the UVB protector compound, kojic acid was isolated in high quantities (10 gram) by simple VLC fractionations from small scale fermentation. This finding confirmed the role of ecological factors in directing the specific metabolites production by endophytic fungi for supporting the plant adaptions.

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[2] Redman RS, Sheehan KB, Stout TG, Rodriguez RJ, Henson JM. Thermotolerance generated by plant/fungal symbiosis. Science 2002; 298: 1581.

SL AR-08 Metabolomic profiling and *in vivo* toxicity of essential oils as promising hits and affordable bioactive agents against *Madurella mycetomatis*

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There is an increasing demand to develop antifungal agents to combat *Madurella mycetomatis*; the major causative agent of mycetoma. This infection is currently treated by triazoles, antifungal agents with limited efficacy and thereby high morbidity rates [1]. Hence, there is an urgent need to identify novel and affordable fungicidal agents with fewer side effects. A dozen of essential oils (EOs) of taxonomically diverse aromatic medicinal plants were extracted by hydrodistillation followed by GC/MS analysis. EOs were screened for antifungal activity *in vitro* and for toxicity *in vivo* on *Galleria mellonella* larvae model [2]. The biological and chemical data generated were subsequently subjected to chemometric analysis.

Seven fungal cultures from diverse geographical origin were exposed to various concentrations of essential oils ranging from 0.25-0.0039%v/v employing resazurin viability assay [3]. Itraconazole was used as positive control.

Ten out of twelve tested oils exhibited remarkable *in vitro* antifungal activity (MIC 0.125- 0.0078%v/v) with no toxicity at 0.5-1%v/v. GC/MS analysis identified diverse monoterpenes and sesquiterpenes associated with antifungal activity. Chemometric analysis revealed that the most active essential oil, *Croton zambezicus*, was clearly separated by chemical data as well as *Xylopia aethiopica* and *Boswellia papyrifera*. Some of the identified pure compounds (*B*-caryophyl, *p*-cymene, sabinene, 1,8-cineole, linalool, thymol and borneol) previously exhibited varying degrees of antimycetomal activity as single compounds. Chemometric analysis of the EOs constituents identified by GC/MS coupled with the antimycetomal activity revealed that the following monoterpenes, terpinen-4-ol, *n*-octyl acetate, *p*-cymene, 1,8-cineole, α-terpineol and linalool are most likely the active compounds against *M. mycetomatis*.

References [1] Ahmed AO, vande Sande WWJ, van VianenW, van Belkum A, Fahal AH, Verbrugh HA, Bakker-Woudenberg I. *In vitro* susceptibilities of *Madurella mycetomatis* to itraconazole and amphotericin B assessed by a modified NCCLS method and a viability-based 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) assay. Antimicrobial Agents and Chemotherapy 2004; 48 (7): 2742–2746.
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Animal Healthcare and Veterinary Phytotherapy

IL VET-01 Phytochemicals in animal nutrition – their potential as functional feed

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DOI 10.1055/s-0039-3399639

Phytochemicals are highly versatile in plants. They are also termed secondary plants compounds, a terminology that might be outdated taking into account the primary role metabolites for plant physiology, reproduction, and protection. From the perspective of ecological mutuality between plants and animals, these compounds are of crucial importance because they not only protect but also serve as visual attraction for animals, especially herbivores, signaling nutritional and/or health-promoting values. In animal nutrition, the phytochemicals are commonly considered as phytogenic feed additives without essential nutritional value as are the primary nutritive ingredients. However, the perception of the diet has changed during the last years both in animal and human nutrition. One now expects that the diet delivers both nutritive and health-promoting values. The knowledge of the last years suggests essential nutritive and health-promoting properties of many phytochemicals, making them good functional feed candidates. Inclusion of such functional feed ingredients in the animal diet may provide precursors for synthesis, protect the animal against oxidative stress, and, most importantly, many compounds enhance key metabolic pathways both at metagenomics and host phenotypic level, being essential for animal nutrition and health. Using such examples, this talk will highlight the potentials of phytochemicals as functional feeds, particularly focusing on the promotion of animal health.

IL VET-02 Let feed and food be our medicine

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The health of livestock, humans, and environments is tied to plant (phytochemical) diversity. Health is enhanced when livestock forage on phytochemically rich landscapes, is reduced when livestock forage on low-diversity pastures or eat high-grain rations in feedlots, and is greatly reduced for people who eat highly processed diets. The shift away from phytochemically and biochemically rich plant and animal foods to processed foods enabled 2.1 billion people to become overweight or obese and increased incidence of diet-related disease.
Circumstantial evidence supports the hypothesis that phytochemical richness of herbivore diets enhances biochemical richness of meat and dairy, which is linked with human health. Among many roles they play in health, phytochemicals in herbivore diets protect meat and dairy from protein oxidation and lipid peroxidation that cause low-grade systemic inflammation implicated in heart disease and cancer. Yet, epidemiological studies critical of red meat do not discriminate among meats from livestock fed high-grain rations as opposed to phytochemically rich diets.
While conventional agriculture adds 25% of greenhouse gas (GHG) emissions, regenerative agriculture can play a sizeable role in mitigating climate change. Of 80 ways to diminish these effects, regenerative agriculture—managed grazing, silvopastoralism, agroforestry, conservation agriculture, and farmland restoration—jointly rank number one as ways to sequester GHG.
We can enable human and planetary health by forsaking processed foods and by sourcing, growing, and eating wholesome foods. We must learn we are members of nature's communities. What we do to them, we do to ourselves; only by nurturing them can we nurture ourselves.

Veterinary Phytopharmacology

KL VET-01 Pharmacological and toxicological insights into veterinary phytotherapy

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DOI 10.1055/s-0039-3399641

Increasing interest of society in natural products, disappointing and dissatisfying results of conventional therapy, as well as the "One Health" concept encouraged by authorities are only some of the causes of enhanced significance of phytotherapy in animals. Despite the long and well documented tradition of herbalism veterinary medicine, some pharmacological and toxicological aspects must be carefully analyzed while considering the application of a plant-derived product into treatment protocol.
In contrast to human medicine, veterinary medicine consists of various species with distinct differences in anatomy and physiology. Consequently, safe and efficient phytotherapy is a challenge for many veterinarians. Significant interspecies differences are reflected in diverse pharmacokinetics of phytomedicines. The complexity of gastrointestinal tract, various intensity of gut microorganisms' activity and the activity of enzymatic systems, predominantly CYP450, are responsible for variable bioavailability of phytocompounds in animals of different species. In cats, special attention must be paid to drugs requiring conjugation with active glucuronic acid since these animals are unable to perform this reaction. Similarly, urine excretion is species-dependent due to various urine pH which may either facilitate or decrease elimination of plant-origin substances and their metabolites. Another animal specific aspect of veterinary phytotherapy includes doping issue. Some herbal constituents are not allowed in sport animals, some other may increase the concentration of concurrently applied drugs and cause a positive result of anti-doping testing. Herb–drug and herb-nutrients interactions, as well as unconscious use of counterfeit phytomedicines are other matters to discuss. Altogether, only rational phytotherapy guarantees the safety and effectiveness people expect.

ISL VET-01 Effect of sustained dietary application of thyme oil on antioxidant parameters and thymol content in plasma and tissues of broilers

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DOI 10.1055/s-0039-3399642

Antioxidants are usually understood as chemicals which could eliminate oxidative stress by removing intracellular reactive oxygen species but high doses may block their beneficial effect and moreover, could be toxic. Understanding exact mechanism of action of plant components in animal organism help to propose dose, method and duration of their application.Thymol with strong antioxidant

properties is the main component of thyme oil. After absorption from the intestine, thymol enters the bloodstream and is transported to the tissues. We found significant correlation between thymol levels in plasma and feed and duodenal wall, which points to the efficient thymol absorption from the digestive tract into circulation. Erythrocytes play a crucial role in the distribution of circulating polyphenols due to binding to their surface and being able to act like depots. That is why the real thymol concentration in blood could be higher than it was found in plasma. Thyme oil (0.1%, thymol 460 µg/g DM feed) significantly increased superoxide dismutase activity, had tendency to increase glutathione peroxidase activity and significantly decreased malondialdehyde as a product of lipid peroxidation in plasma which points to modulation of antioxidant enzymes activity and improvement of the oxidative stability. Obtained the lowest thymol concentrations in muscle (139 ng/g DM) in comparison with plasma (854 ng/mL), liver (568 ng/g DM) and kidney (5541 ng/g DM) could be due to low thymol penetration or high activity of efflux transporters, hence insufficient to affect antioxidant defence system. In conclusion, thymol effectiveness depends on its systemic availability for the targeted organ.

Acknowledgement The current experiment was financed by funds from the Scientific Grant Agency of the Ministry for Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (Vega 2/0052/13, 2/0078/16, 2/0069/17), ITMS project No. 26220220204 and financialy supported by Austrian Federal Ministry for Science, Research and Economics.

SL VET-01 A combination of saponins and essential oils of Asteraceae, Lauraceae and Myrtaceae plants counteracts coccidiosis-related production losses in fast-growing broilers

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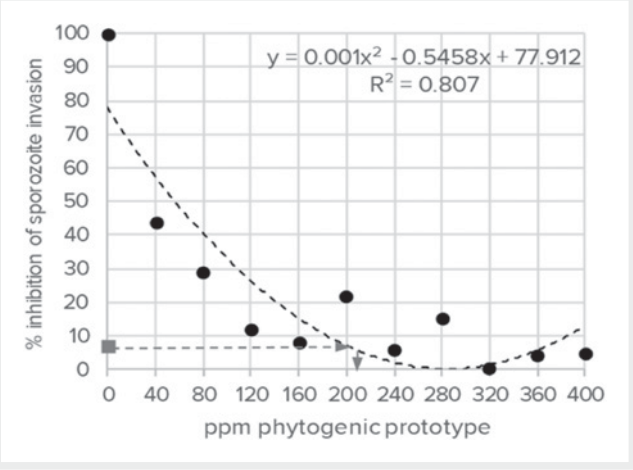
Eimeria spp. triggered coccidiosis is a protozoal disease of the intestinal tract relevant for different avian species. Infections result in diminished nutrient absorption and cause economic losses through performance depression [1]. Frequently, they are accompanied by secondary infections with *Clostridium perfringens*.

Despite rotation of chemical coccidiostats, *Eimeria* become resistant to these substances. The present study investigates how phytogenic feed additives can support health status of coccidiosis and *C. perfringens* challenged broilers. Extensive screening of EO-combinations, different saponin sources and combinations thereof [2], revealed two prototypes for testing in broiler challenge trials. Prototypes consisted of a saponin core combined with EO mixes, from Asteraceae and Lauraceae plants (PT1) or from Myrtaceae and Asteraceae plants (PT2).

1750 day-old male chickens were spray-vaccinated against coccidiosis and assigned to 5 groups of 350 birds in 7 repetitions for a 42-day feeding trial. Except non-infected non-treated group 1 (NINT), all birds were individually inoculated at days 19, 20 and 21 with *C. perfringens* to induce necrotic enteritis (NE). The further dietary groups were: 2) infected non-treated, 3) infected + Bacitracin-methylene-disalicylate, 4 and 5) infected + PT1 or PT2. Both prototypes compensated infection related growth depression. Feed Conversion Ratio was even improved compared with NINT. In an *in-vitro* dose response test only 208 ppm of PT2 were equally effective to 10 ppm Monensin regarding *Eimeria* sporozoite invasion (▶**Fig. 1**).

In conclusion, the supplementation of phytogenics based on saponins and specific EO mixes can be considered as a promising and save alternative in coccidiosis and NE control.



▶**Fig. 1** Dose response effects of PT2, basing on saponins and an EO mix from Myrtaceae and Asteraceae plants, on inhibition of Eimeria sporozoite invasion.

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Clinical Research

KL VET-02 Mistletoe in adjuvant cancer treatment of companion animals

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Cancer diseases are frequent in veterinary practice and overall there is a 23% – 45 % risk for dogs to die from cancer. Oral malignant melanoma (OMM) as well as canine mammary tumor (CMT) show high malignancy and adjuvant treatment is needed. The aim of both controlled trials was to explore if *viscum album* L. extract (VAE) therapy might be a potential treatment of OMM and CTM. In OMM the adjuvant VAE treatment showed a significant longer median survival time (MST) with 236 days compared to the control (radiation only) with 49 days (p = 0,0047). Adjuvant VAE treatment in CMT showed not significant differences in MST compared to the control group (surgery only). A tendency (p= 0,7) of decrease in tumor-related death risk (TRDR) to 25% (HR 0.251, 95% CI 0.056–1.122; p = 0.07) was observed for the treatment group, with stable Quality of life during entire treatment.

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ISL VET-02 Devil's claw (*Harpagophytum procumbens*) – Pharmacokinetics of harpagoside in horses

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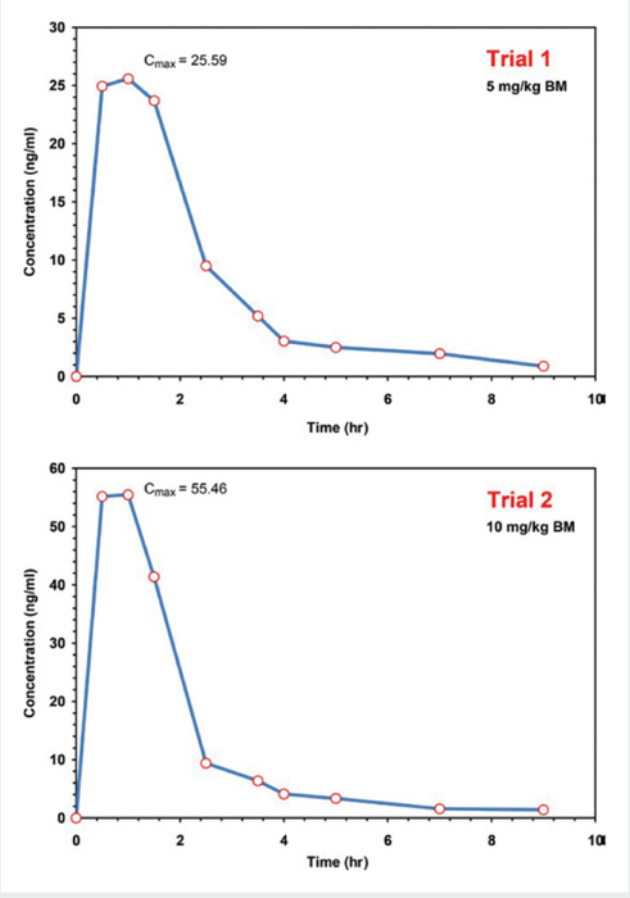
DOI 10.1055/s-0039-3399645

Devil's claw is used for the treatment of chronic inflammatory symptoms and degenerative disorders [1] in horses since many years, but without the substantive equine pharmacokinetic data [2,3,4].

The pharmacokinetic parameters of harpagoside, the main active constituent of *Harpagophytum procumbens* DC ex Meisn., were evaluated in equine plasma after administration of *Harpagophytum* extract FB 8858 in an open, single-dose, two-treatment, two-period, randomised cross-over design. Six horses received a single dose of *Harpagophytum* extract, corresponding to 5 mg/kg BM harpagoside, and after 7 days washout period, 10 mg/kg BM harpagoside via nasogastric tube. Plasma samples at certain time points (before and 0-24 h after administration) were collected, cleaned up by solid-phase extraction and harpagoside concentrations were determined by LC-MS/MS using apigenin-7-glucoside as internal standard. Plasma concentration-time data and relevant parameters were described by non-compartmental model through PKSolver software. Harpagoside could be detected up to 9 h after administration. C_{max} was found at 25.59 and 55.46 ng/ml, $t_{1/2}$ at 2.53 h and 2.32 h, respectively and t_{max} at one hour in both trials. $AUC_{0\text{-}inf}$ was 70.46 and 117.85 ng h ml^{-1}, respectively. A proportional relationship between dose, C_{max} and AUC was observed. Distribution (V_z/F) was 259.04 and 283.83 L/kg and clearance (CL/F) 70.96 and 84.86 L h^{-1} kg^{-1}, respectively.

Treatment of horses with *Harpagophytum* extract did not cause any clinically detectable side effects. The knowledge of basic equine pharmacokinetics, based on the results of this study, will help to link results from *in vitro* assays and clinical studies and optimise therapeutic efficacy.



▶ **Fig. 1** Mean plasma concentration-time profile of harpagoside after single intragastric dosing of Harpagophytum procumbens extract FB 8858 corresponding to 5 mg/kg BM harpagoside (trial 1) and 10 mg/kg BM harpagoside (trial 2) to six horses

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SL VET-02 *Aloe vera* gel and *Coriandrum sativum* seeds: traditional medical plants and their role as anti-diabetic agents in dogs

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DOI 10.1055/s-0039-3399646

This study aims to evaluate acute anti-diabetic or anti-obesity effects of *Aloe vera* gel and milled *Coriandrum sativum* L. seeds as oral treatment for several diabetes related blood parameters including glucose (G) and insulin (I) in dogs. 4 female and 5 male healthy beagle dogs were used in 2 experimental phases each 5 weeks 1 week of control diet (cd), 2 weeks of phytotherapy (cd + herbal preparation) and 2 weeks of wash-out period (cd). The herbal preparations were dosed 2g/kg body weight (BW)/day. Fasting and postprandial blood parameters were assessed after control period, first-, and second week of phytotherapy period. The BW was measured weekly and a general examination was carried out two times per week. The BW decreased 1.4% and 0.8% after two weeks of treatment with *A. vera* (p=0,001) and *C. sativum* (p=0,112), respectively. Hemogram and blood chemistry revealed no impact of herbal preparations. Blood G peaked at 160 up to 183 min after the meal containing *A. vera* and *C. sativum* respectively. Max I concentrations of 37 up to 55 µU/ml refers to the I response on the meal and showed no treatment effect. *A. vera* gel and *C. sativum* seeds exert in a 2-week oral treatment period no significant side effects on general conditions of dogs and on blood parameters. In healthy dogs, no effect was detectable on blood G and I responses. However, significant positive effects on weight regulation could be confirmed.

Animal Self Medication and Ethnoveterinary Medicine

KL VET-03 After ten thousand years of domestication, can livestock still self-medicate?

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DOI 10.1055/s-0039-3399647

To determine if livestock can self-medicate, we first showed that sheep fed high-grain diets ingest sodium bicarbonate or bentonite, substances that attenuate acidosis and restore acid-base balance. We then showed that sheep and goats regulate intake of polyethylene glycol (PEG), which alleviates the aversive effects of consuming high-tannin diets, in accord with the amount of tannin in their diet. Finally, we showed that sheep ingest dicalcium phosphate to counteract foods high in oxalic acid. To learn whether sheep make multiple illness-medicine associations, we conditioned sheep to use three medicines – bentonite, PEG, and dicalcium phosphate – and then offered them grain or food with tannins or food with oxalic acid and gave them access to the three medicines. Sheep chose the medicine that rectified the malady.

Sheep and goats infected with internal parasites eat more tannin-rich forage than non-infected animals. As parasite loads increase, they increase their intake of plants with tannins, which decreases parasite loads. Livestock are less inclined to self-medicate when they are provided with anti-parasitic drugs. Parasitized sheep reduce intake of high-tannin food when their parasite infection is terminated with ivermectin. Likewise, goats treated with anthelmintic drugs eat less tannin-containing heather than do goats infected with internal parasites.

Collectively, these findings show livestock self-medicate, even after 10,000 years of domestication. Biochemically mediated flavor-feedback associations, where cells and organ systems alter liking for foods as a function of needs, enable livestock to self-medicate. To do so, however, livestock must have access to phytochemically rich foods and learn to use them.

ISL VET-03 Medicinal diet of Tibetan macaques in Southern China

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Dietary selection is important to assure the balance of nutrients for energy, growth, maintenance, and reproduction. The innate response of an animal is to avoid eating plants with toxic secondary metabolites, but these properties can be medicinal when ingested in appropriate amounts. It is proposed that under physical conditions that require direct intervention (health maintenance or self-medication), animals ingest even such toxic plants for their medicinal benefits to regain health homeostasis. Previous studies on primate plant food selection have found that 15~25% of the items in their diet could be classified as 'medicinal food' (plants with bioactive, physiology modifying properties). Here we focus on identifying prospective medicinal foods in the diet, in order to investigate the potential properties and possible roles of such items for a wild group of Tibetan macaques (Macaca thibetana) at Mt. Huangshan, Anhui Province, China. Using a 50 species food list of this group, a pharmacological database search was conducted. We identified 12 species (24%) with significant pharmacological potential. Across the 12-month study, medicinal foods accounted for 18~23% of the total diet in any one season. The reported activities in these items included anti-parasitic, anti-bacterial, anti-rheumatic, neuroprotective, osteoprotective, reproductive stimulant and wound healing, among others. While the actual medicinal benefits to macaques gained from ingesting these plants are yet unknown, based on the available evidence for their parasite infection ecology, reproductive behavior and possible stress reduction, we hypothesize on the possible scope of self-medication in Tibetan macaques, and suggest future avenues for research.

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SL VET-03 A survey of plant remedies for livestock diseases in the Mnisi community, South Africa, and investigation of their biological activities

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Ethnoveterinary medicine (EVM), although not well documented, serves as an alternative or complementary medication for infectious diseases and parasites in South African livestock. In this study, plant species and traditional remedy formulation methods used by the Mnisi community, Bushbuckridge, in EVM were documented using Rapid Rural Appraisal techniques. Three plant species were selected for evaluation of antimicrobial, antibiofilm and cytotoxic activities based on their frequency index and lack of published information on their bioactivity. Traditional methods were used for plant extraction using water. Acetone was used as an organic solvent to compare traditional and organic solvent methods of extract preparation. The extracts were tested for their antimicrobial activity and cytotoxicity. *Elephantorrhiza obliqua* acetone extract had the best antibacterial activity with a minimum inhibitory concentration (MIC) value of 0.09 mg/ml against *Pseudomonas aeruginosa*, while *E. obliqua* water extract had the best antifungal activity with MIC of 0.02 mg/ml against *Aspergillus fumigatus*. Some extracts also inhibited biofilm formation by at least 50% and were active against mature biofilms. Only two of the nine plant extracts were relatively toxic against Vero cells. Interestingly, traditionally prepared remedies were generally more active against fungi and mycobacteria and less cytotoxic than organic solvent extracts. *In vivo* studies are necessary to support the traditional use and safety of the remedies against livestock diseases.

SL YRW-01 Prioritization of high-value natural products from a large chemo-diverse plant extracts collection: a focus on structural novelty

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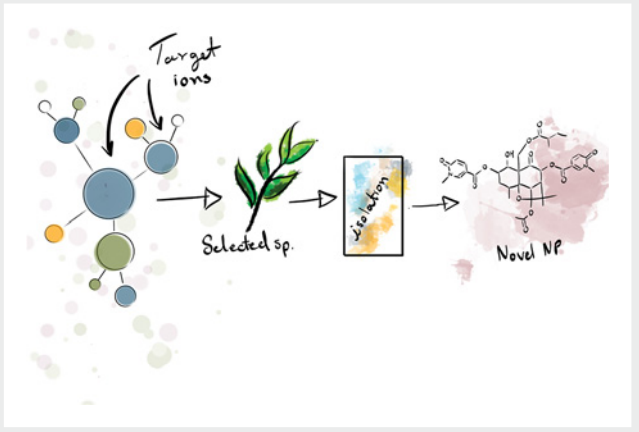
DOI 10.1055/s-0039-3399650

Novel natural products (NPs) identification is key for innovation in pharmacognosy but recurrent isolation of known compounds discourage further studies [1]. Comprehensive computational dereplication approaches could help to overcome this problem. This is the case for early metabolite identification workflows combining MS^2 spectral organization (MN) [2], MS^1 molecular formula determination, MS^2 *in silico* spectral match [3], annotations based on taxonomical consistency, chemotaxonomical and chemical space exploration. Such protocols allow highlighting NPs of interest in crude extracts, and target their solation in the sub mg range with efficient optimised high resolution preparative approaches for full structure and bioactivity assessment [4].

We performed a massive metabolite profiling on a biodiverse set of 1,600 extracts (156 families, 533 genera, 783 species) from the plant part library of Pierre Fabre Laboratories made available through a collaboration. After dereplication, individual clusters were examined and we were able to highlight a cluster with several possible new analogues of *β*-sesquiterpene pyridinic alkaloids, which was further studied.

Selected species was subjected to a straightforward one-step isolation procedure using a gradient transfer combined with a dry-load injection of the sample at a semi-preparative HPLC scale. This approach allowed the targeted isolation of a series of 14 original dihydro-*β*-agarofuran sesquiterpene alkaloid derivatives, which were fully characterized by 2D NMR and HRMS.

Our workflow is able to quickly spot possible new NPs from a large and chemodiverse collection of plants. They are then efficiently isolated in the required amounts for structural and bioactive characterization without extensive and time-consuming purification steps.



▶ **Fig. 1**

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SL YRW-02 Identification of *C. elegans* lipid lowering constituents form chaga by correlation of ^{1}H NMR spectra with phenotypic screening

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Natural products and phenotype directed screening in animal models have been prolific fields for the discovery of innovative small molecule drugs – also in the field of metabolic disease [1–2]. Since the screening of extracts and natural products (NP) in classical obesity rodent models is hampered by several disadvantages, e.g. financial efforts, legal and ethical considerations and the requirement of large quantities of test materials, we established an obesity model in the nematode *Caenorhabditis elegans* (*C. elegans*). The *C. elegans* obesity assay is based on lipid staining of the mutant strain SS104 with the fluorescent dye Nile Red. It can be performed with little sample amounts in 96-well plates with medium throughput. An ethanolic extract of chaga, i.e. the fruit bodies of the mushroom *Inonotus obliquus* (Ach. ex Pers.) Pilát, significantly inhibited the fat accumulation in *C. elegans* at 10 µg/mL, without having a negative effect on the nematodes' lifespan and was therefore selected for further mycochemical investigations. The extract was separated via flash chromatography into 37 micro-fractions with quantitative variances of constituents over several consecutive fractions. The effect on fat accumulation was determined for each fraction and the increases and decreases of bioactivity were correlated with the fractions' ^{1}H NMR spectra following a recently developed biochemometric approach named ELINA (Eliciting Nature's Activities) [3]. Together with UPC^2-ELSD-QDa data we were able to pinpoint several constituents and their chemical features responsible for the observed phenotypic effect, which enabled their targeted isolation.

Acknowledgement We thank Dr. Agnieszka Kowalska, Department of Pharmacognosy, University of Vienna, for her excellent technical support, and Pakuso LLC, Lieto, Finland for providing the mushroom material.

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SL YRW-03 Feature-based molecular networking and network annotation propagation applied to natural antiviral compound research from tropical Euphorbiaceae.

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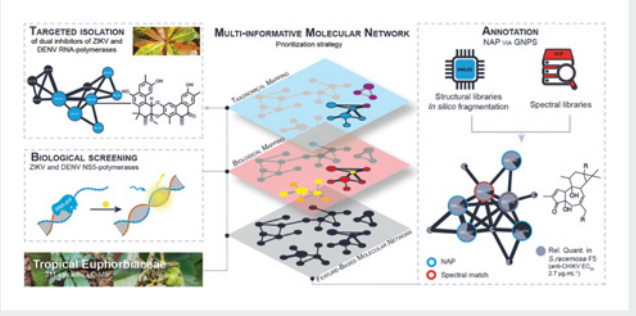
DOI 10.1055/s-0039-3399652

In an effort to identify novel inhibitors of Chikungunya (CHIKV), Dengue (DENV) and Zika (ZIKV), a systematic study with 311 extracts from tropical Euphorbiaceae species was performed in a virus-cell-based assay for CHIKV and DENV and ZIKA NS5 inhibition assays.

The French Guianese species *Sandwithia guyanensis* and *Sagotia racemosa*, from which bark extracts exhibited significant anti-CHIKV activities, were first investigated. Following a classical bio-guided isolation workflow, more than 20 new diterpenes were characterized but none of them showed significant antiviral activity. [1]

To address this issue, a Feature-Based Molecular Network (FBMN) was built from the LC-MS^2 data acquired from the chromatographic fractions of both extracts. FBMN is a computational method that bridges data processing tools for LC-MS^2 and molecular networking (MN) analysis on GNPS [2]. Then the Network Annotation Propagation (NAP) workflow that improve *in silico* fragmentation candidate structure ranking using spectral networks to propagate information from spectral library matching, allowed highly reliable identification of phorbol analogues. Those compounds present in trace amounts in both extracts, provides a plausible explanation for the loss of the biological activity observed during the bioassay-guided isolation procedure [3].

In a second study, a bioactive prioritization approach based on the merging of taxonomical and bioassays data over the MN built from the initial set of Euphorbiaceae extracts led to the targeted isolation and characterization of several dual inhibitors from *Codiaeum peltatum* [4]. Both studies exemplify how MN and recent advances in molecular annotation can be implemented in phytochemical studies to understand drawbacks and improve the bioactive compound discovery process.



▶ **Fig. 1**

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SL YRW-04 Oxidised juncuenin B analogues with increased antiproliferative activity on human adherent cell lines: semisynthesis and biological evaluation

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Juncuenin B is a phenanthrene belonging to a relatively small group of plant secondary metabolites. Phenanthrenes have become of great interest in the last 20 years because of their wide-range of structural and pharmacological diversity like antioxidant, anti-inflammatory, antimicrobial and antiproliferative activities [1].

Protoflavones are a unique, relatively oxidized class of flavonoids. They bear a non-saturated B-ring, and a hydroxyl group is connected through C-1′. These flavonoid analogues with a *para*-quinol ring are described in the literature with considerable antiproliferative activities [2].

The aim of our work was to merge the characteristics of the mentioned metabolites, according to the structural similarities of phenanthrenes and flavonoids. As juncuenin B contains phenolic hydroxyl groups, during oxidation reactions formation of *p*-quinol ring containing semisynthetic derivatives were expected. To accomplish these reactions, organic oxidizing agents, namely PIFA ([bis(trifluoroacetoxy)iodo]benzene) and PIDA [(diacetoxyiodo)benzene] were applied under different reaction conditions. From the reaction mixtures, thirteen compounds were prepared by using combined chromatographic techniques (prep. TLC, MPLC, and HPLC). The components are substituted with methoxy-, ethoxy-, and butoxy groups; majority of them contain a *p*-quinol ring. The structure elucidation of the compounds was carried out by 1D and 2D NMR spectroscopic methods. The antiproliferative activity of the compounds was investigated on different human cancer (MCF-7, T47d, HeLa, SiHa, C33a, and A2780) and mouse embryo fibroblast cell lines. Five compounds possessed remarkable inhibition [IC_{50} values 1.93–30.03 μM] on the tested cell lines, higher than the original compound, juncuenin B.

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SL YRW-05 *Melissa officinalis* essential oil loaded glycerosomes: preparation and *in vitro* activity evaluation against herpes labialis (HSV-1)

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DOI 10.1055/s-0039-3399654

Essential oils (EOs) are complex mixtures of strongly active compounds, but very volatile and sensitive to light, oxygen, moisture and temperature. Loading inside nanostructures can be a strategy to stabilize them and use them in therapy [1]. In the present study, *Melissa officinalis* L. (Lamiaceae) essential oil was loaded inside glycerosomes (GS) at a concentration of 10 μL/mL and was

evaluated for its anti-herpetic activity [2]. GS were prepared by the thin layer evaporation method and characterized by Light Scattering techniques, determining average diameter (about 60 nm), polydispersity index (= 0.2) and ζ-potential (= -30 mV). From the morphological observation by transmission electron microscope, GS appeared as small vesicles with several lamellae and a spherical shape. Moreover, the EO encapsulation efficiency inside the GS, in terms of citral and β-caryophyllene, obtained by HPLC-DAD, was =63 % and =76 % respectively and the EO release, by the dialysis bag method, was very low (=15% of citral) within 24h. The same instruments and analytical techniques were adopted to monitor the GS long-term stability until 4 months and no relevant changes were observed in the chemical-physical parameters. Successively, GS were tested with an *in vitro* antiviral assay against the HSV-1/strain vCLIDA61. From these studies, the antiviral activity of loaded-EO was found to be comparable to free-EO, for high concentrations of EO. Hence, at present, GS seems to be a promising tool in order to administer the EO and replace the conventional anti-herpetic drugs, when drug-resistance forms take place.

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SL YRW-06 How plant shape their root associated microbiome to acquire resilience against pathogen infection? What is the mechanism behind?

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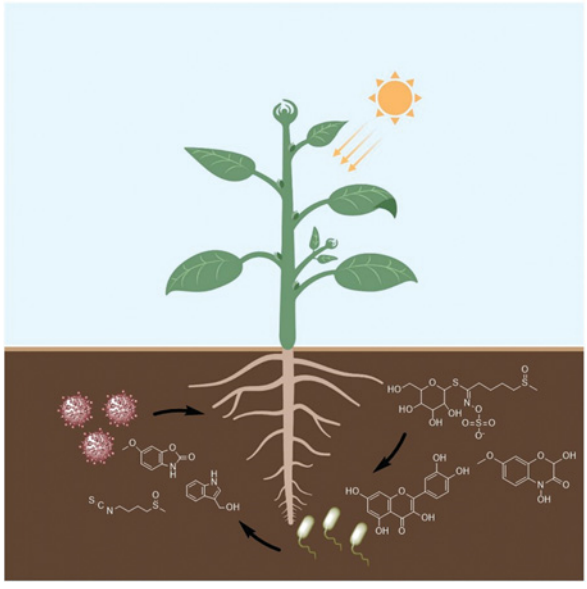
DOI 10.1055/s-0039-3399655

Plants may utilize bioactive chemical compounds which exude from their root to shape their associated beneficial microorganism in the rhizosphere to acquire resistance against pathogen infection (▶**Fig. 1**). However, the underlying mechanism regulating this interaction has been poorly elucidated. The aim of the present study was to investigate the functional role of root exudate (from different host genotypes) in mediating belowground indirect plant defence. To elucidate the molecular mechanism underlying plant interaction with health-promoting microorganism, *Arabidopsis thaliana* (Col-0 and Ler-0 accessions) were grown for three weeks in field soil (non-sterile). Subsequently, the roots were exposed to *Fusarium oxysporum matthioli* (FOM). Sampling was done every five days after the infection for 25 days. The collected samples were subjected to both targeted (aliphatic and indole glucosinolates, plant hormones, and mycotoxins) and untargeted analysis by employing LC-MS/MS and GC-TOF/MS platforms as well as metabarcoding (Illumina MiSeq). Phenotypic characterization of the plants displayed that the Col-0 accessions had developed a resistance to FOM exposure whereas the Ler-0 ecotype was susceptible. The disease progression was also evaluated on a molecular level and resulted in the identification of beauvericin in both root and shoot tissues of the susceptible plants which likely was associated with promoting pathogenicity in FOM. The result from targeted and untargeted metabolomics as well as analysis of the root microbial community composition (bacteria and fungi) revealed the role of novel natural products in shaping the distinct member of the microbial community leading to diseases suppression.



▶**Fig. 1** Tritrophic interactions between root of the plant, beneficial microbes (green color) and the detrimental pathogen (red color), in the rhizosphere.

SL YRW-07 Phyloactivity-based screening of ethnomedically inspired plant extract libraries against *Trypanosoma cruzi* and Chagas disease

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Trypanosoma cruzi is the etiologic agent of Chagas' disease, a neglected tropical disease prevalent in America. Chemotherapy with benznidazole or nifurtimox is effective when administered in the acute stage but the efficacy rapidly decreases in the chronic phase and these drugs become toxic [1]. Intervention with botanical drugs seems to be a possible alternative against parasitic diseases, especially among rural communities where triatomine bugs still infect people [2]. However, a systematic analysis to challenge this hypothesis is lacking. We performed a one-year ethnobotanical/pharmacological fieldwork among indigenous groups in the Bolivian Chaco where the Chagas disease is hyperendemic. Our hypothesis was that ethnopharmacologically inspired plant extract libraries show a higher degree of specific antitrypanosomal activities than a comprehensive Dioscoroides medicinal plant extract library without association to trypanosomiasis. To that aim, we have established a BSL3* lab in Bern to assess selective toxicity towards the different stages of the parasite during host infection and in comparison, with *T. brucei* and mammalian host cells. So far, more than 750 plant taxa were assessed. Few plants have been subjected to bioactivity-guided isolation using LC-MS and structure elucidation. Based on our most active clusters we found naphthalene, sesquiterpene lactones and anthraquinones. A library of 20 anthraquinones was tested. Some of the anthraquinone derivatives showed nM potencies in the infection assay but did not exhibit antitrypanosomal activity on the insect stage, indicating a stage-dependent mechanism of action specific to the host-parasite interaction. We will present a phyloactivity-based screening including activity-based protein profiling and LC-MS/MS on parasite and host biochemistry.

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SL YRW-08 Optimization of growth media for leafcutter ant-associated antimicrobial producing bacteria using cuticular hydrocarbons

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DOI 10.1055/s-0039-3399657

Cuticular hydrocarbons (CHC) on the surface of ants have multiple functions as adaption to environmental conditions, interspecific recognition and intraspecific nest mate recognition [1]. Therefore, the CHC profile can be adjusted by the metapleural gland and Dufour's gland. In leafcutter-ants, which are living in symbiosis with antimicrobial-producing bacteria like *Pseudonocardia* inhabiting the ants' surface, the CHC profile may influence the growth of the bacteria. To investigate the impact of CHCs in this symbiosis we analyzed the CHC profiles of three different species of leafcutter ants containing two genera, *Atta* and *Acromyrmex*, by GC-EI-MS. These results were compared with CHC profiles of the closely related ant *Messor aciculatus* and the tropic ant *Polyrhachis dives*. Our research could show high quantitative and qualitative differences in the CHC profile of the investigated ants. The CHC profiles of all leafcutter ants contained more amides like tetradecanamide, hexadecanamide and hexadecenamide. The similarity between the two different tropic genera of leafcutter ants living in symbiosis with *Pseudonocardia* and the difference of those ants to the tropic *Polyrhachis* and the closely related *Messor* suggests an impact of the identified compounds on the symbiosis. Our findings allowed an optimization of growth media for leafcutter ant associated, antimicrobial producing bacteria by testing different CHC and chitin concentrations, different temperature conditions and pH values. The comparison of 36 growing conditions pointed out that the best growth for *Pseudonocardia* appeared at pH 7, 28°C with 2 % chitin and addition of tetradecanamide, hexadecanamide, hexadecenamide and their corresponding acids.

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SL YRW-09 Immunosuppressive activity of *Artemisia argyi*

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There is a need for novel immunosuppressive drugs, given that currently used small molecule drugs and biologics exhibit side effects including increased susceptibility to infections, paradoxical inflammation or autoimmune diseases [1]. Aiming at the discovery of natural products with potentially new mechanisms of action we screened a library of 435 extracts prepared from plants used in Traditional Chinese Medicine. Immunosuppressive activity of extracts was assessed in a proliferation-based assay utilizing physiologically relevant anti-CD3 and anti-CD28 stimulated primary human T lymphocytes [2]. An ethyl acetate extract of *Artemisia argyi* H. Lév. & Vaniot (Asteraceae) was found to be highly active with an IC_{50} of 16.2 µg/mL. Apoptosis and necrosis induction analysis of T lymphocytes showed that the inhibitory effect on T cell proliferation was not due to toxic effects of the extract.

Treatment with 3 – 30 µg/mL *A. argyi* extract significantly lowered the expression of activation markers and suppressed the cytokine secretion of activated T lymphocytes in a dose-dependent manner. The effect of *A. argyi* extract on transcription factors AP-1 (activator protein 1), NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and NFAT (nuclear factor of activated T-cells) was investigated. Results point to a specific suppression of transcription factors leading to a diminished expression of IL-2 and, as a consequence, to inhibition of T cell proliferation. Compounds responsible for the effects are currently being tracked by HPLC-based activity profiling [3], mode-of-action studies are ongoing.

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SL YRW-10 Novel dual-function type III polyketide synthase from *Hypericum polyphyllum*

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Benzophenone synthase (BPS) and biphenyl synthase (BIS) are benzoic acid-specific type III polyketide synthases (PKSs). They use the rare starter substrate benzoyl-CoA and three molecules of malonyl-CoA as extender substrate to form the same linear tetraketide intermediate (▶**Fig. 1**), BPS catalyzes intramolecular C6→C1 Claisen condensation to form 2,4,6-trihydroxybenzophenone [1], whereas BIS catalyzes intramolecular C2→C7 aldol condensation to form 3,5-dihydroxybiphenyl [2]. Polyprenylated benzophenones with interesting pharmacological features, such as cytotoxic [3] and antibacterial [4] activities, are specialized metabolites in some *Hypericum* sp. Biphenyls and related dibenzofurans are phytoalexins of the Rosaceae subtribe Malinae [5].

A type III polyketide synthase was cloned from *H. polyphyllum,* which forms 2,4,6-trihydroxybenzophenone and 3,5-dihydroxybiphenyl in *in vitro* assay. Therefore, it is a good candidate for investigating the two different cyclization mechanisms by undertaking mutations in the active center of the enzyme.

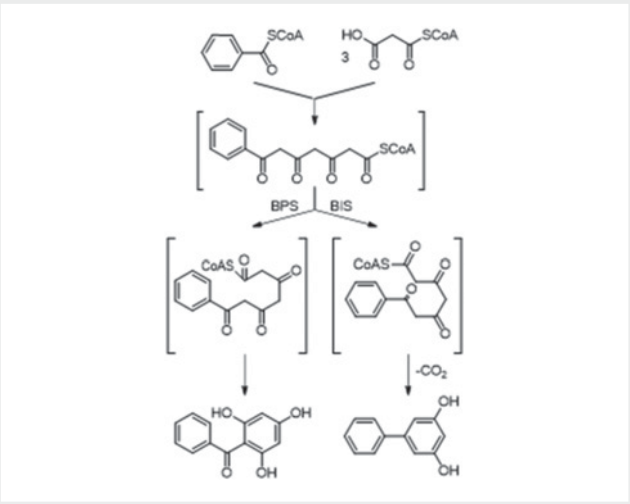
The incubation temperature influenced the product ratio. The enzyme produced only 3,5-dihydroxybiphenyl until 30 °C. Above, 2,4,6-trihydroxybenzophenone formation started and both products were observed in equal amounts at 45 °C. Under optimum conditions (45 °C, 300 mM KH_2PO_4, pH 7,5), V_{max} value was 300 nkat/mg and *K*m values were 4.5 µM for benzoyl-CoA and 26.4 µM for malonyl-CoA.

Two single amino acid mutations in the active center changed the product ratio

(2,4,6-trihydroxy-benzophenone:

3,5-dihydroxybiphenyl) to 1: 0.5 and 1: 0.2, respectively. The double mutant produced only 2,4,6-trihydroxybenzophenone.

Thus, the PKS is a promising candidate for crystallization which will provide in-depth information about the active site structure and the switch in the cyclization modes.



▶ **Fig. 1** Reactions of benzo-phenone synthase (BPS) and biphenyl synthase (BIS) [2].

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SL YRW-11 Simulating human gastrointestinal and colonic biotranformation pathways through an *in vitro* assay reveals insight on hydroxytyrosol and oleuropein metabolism

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Hydroxytyrosol (HT) and oleuropein (OL) are significant components of olive oil and olive fruits (*Olea europaea* L.), inextricably linked to the Mediterranean diet. Both of them are considered as some of the chemical constituents relevant to the biological activity of olive oil, leading to a global upsurge in the investigation of their diverse health promoting and disease preventing abilities, including several chronic diseases, mainly cardiovascular and neurodegenerative disorders, and cancer. Moreover, a large body of evidence suggests that the potential biological effects of edible phenolic compounds in the human body are partly consequence of their biotransformation by the colon microbiota.

Aiming towards a detailed investigation into the absorption, metabolism and microflora-dependent transformation of HT, and its conjugated form, OL, and within the framework of the international MediHealth project, the GIDM-Colon Model was applied. The aforementioned model is an optimized, validated *in vitro* continuous flow dialysis system, simulating the absorption from lumen to mucosa; followed by the colon phase using pooled human faecal suspensions, which mimics the physiological conditions during human gastrointestinal digestion [1]. Throughout GIDM-Colon digestion, different samples were collected of both dialysate and retentate solutions, after gastric and small intestinal digestion, and at five different time points of colonic digestion. Data analysis and metabolite profiling were conducted using a UPLC-Orbitrap HRMS along with chemometric approaches. Results of this study provide significant insight into the bioavailability and biotransformation of HT and OL in the human gut using a validated *in vitro* Gastrointestinal Dialysis Model with Colon phase.

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SL YRW-12 Identification of transcription factors from *Radula marginata* TAYLOR

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The liverwort *Radula marginata* TAYLOR belongs to the Radulaceae family within the division of bryophyte. Since the identification of cannabinoid-like compounds, various studies including phytochemical, morphological and transcriptomics analysis suggest liverwort as an alternative source of cannabinoid-like natural compounds. However, lack of information regarding transcription factors (TFs) hinders understanding of the regulation of gene expression relevant for the biosynthesis of these secondary metabolites.

Therefore, we decided to capture the transcriptome and to exploit next-generation sequencing approach for the prediction of TFs in *R. marginata*. In-depth transcriptomic analysis revealed a total number of 3449 unigenes that have potential binding sites for 1085 transcription factors (TF) from 39 families. Moreover, comparative analysis from related moss species showed that six TF families like BSD, CSD, DbpA, FHA, LIM, tify and TIG had uniquely predicted in *R. marginata.* On the other hand, we could not find any target site from B3 and trihelix TF families which might have been due to evolutionary contraction. In addition, 156 TFs from MYB (66) bZIP (39) AP2/ERF (9/22) NAC (10) bHLH (8) DOF (2) that are primarily involved in regulating the secondary metabolism also predicted.

To sum it up, it is the first ever micro transcriptomic study predicting the TFs in *R. marginata*. However, further functional characterization of TFs especially for the secondary metabolism and terpenoid biosynthesis is vital to unraveling the expression of gene regulation.

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Economic Adulteration of Botanical Ingredients Abstracts

ISL EA-01 Preventing adulteration and fraud in botanical ingredients in the international marketplace: the ABC-AHP-NCNPR Botanical Adulterants Prevention Program

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DOI 10.1055/s-0039-3399662

The global market for herbal medicines, dietary and food supplements, and other botanically-based natural products has increased, as have confirmed reports of economically motivated adulteration of botanical raw materials, extracts, essential oils with undisclosed, non-authentic, lower-cost ingredients. These adulterated ingredients are used – sometimes by unwitting manufacturers – in finished botanical-based consumer products, reducing the activity and expected efficacy of these products, and, in some cases creating potential safety problems.

Detailed reviews on adulteration of specific botanical ingredients are currently being compiled in a series of publications by an independent consortium of nonprofit organizations consisting of the American Botanical Council (ABC), the American Herbal Pharmacopoeia (AHP), and the National Center for Natural Product Research (NCNPR) at the University of Mississippi. The ABC-AHP-NCNPR Botanical Adulterants Prevention Program (BAPP) is an international educational program that has been supported and endorsed by over 200 botanical industry companies, third-party analytical laboratories, nonprofit professional organizations, industry trade associations, research centers, and others. As of July 2019, BAPP has published a total of 51 extensively peer-reviewed documents. These include Bulletins confirming adulteration of specific ingredients and Laboratory Guidance Documents in which laboratory analytical methods are evaluated for their ability to detect the types of confirmed adulteration currently present in the marketplace. This presentation will give an overview of recent cases of adulteration and BAPP's efforts to help prevent adulteration and fraud by educating the herb industry, researchers, and regulators via documents available on BAPP's free-access website at http://cms.herbalgram.org/BAP/index.html.

ISL EA-02 Combining analytical tools to identify adulteration: some practical examples

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DOI 10.1055/s-0039-3399663

The European Pharmacopoeia (Ph. Eur.) is the legal and scientific reference for the quality control of medicines, including herbal drugs (HDs) and their preparations (HDPs). Typically, quality is defined using markers or active constituents by the means of HPTLC, HPLC, GC. Nevertheless, substitution or adulteration of HDs and HDPs either intentionally, e.g. motivated by the desire to maximize financial gains, or unintentionally, e.g. by clerical errors or lack of knowledge, is not a rare occurrence and can have tragic consequences.

Diverse analytical methods including DNA fingerprinting, Nuclear Magnetic Resonance (NMR), Near InfraRed (NIR) and (bio)sensors can be very useful as integrative/ alternative analytical methods. Identification of plants at the species level can be successfully based on genome-based methods, using DNA barcodes, the nucleotide sequence of a short DNA fragment, as in the case of different species of goji berry. NMR can provide direct NMR fingerprint determination (complete assignment of the signals by 1D and 2D experiments), and fully characterize different commercial extracts of ginkgo or even complex HDPs mixtures. NIR spectroscopy is a fast qualitative and quantitative analytical method, getting knowledge about substitution of plant species, i.e. star anise and the neurotoxic Japanese star anise, *Stephania tetrandra* and the nephrotoxic *Aristolochia fangchi*. Finally, chemical and biological sensors represent one of the most interesting analytical tool because of the versatility of the recognition element able to recognize the presence of undefined DNA intercalating constituents or specific classes of metabolites could be related to the substitution or adulteration [1, 2].

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ISL EA-03 Authenticity versus botanical specificity: the use of interchangeable species

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DOI 10.1055/s-0039-3399664

For centuries, botany predominantly existed as a subspecialty of medicine and medicinal plants were chosen specifically for their medicinal properties, oftentimes with a lack of exactness to botanical specificity. Greater sophistication in analytical techniques, including genetics, has allowed for greater levels of exactness in differentiating species than was historically possible. However, the medicinal plant trade has not kept up with this increasing level of sophistication, often using multiple species as the medicinal article of trade. Some of these interchangeably used species are codified in pharmacopoeias internationally, others are not. Some species are readily identifiable as genuine articles of trade whereas botanical specificity may be elusive or questionable. Pharmacopoeias provide the suite of identity, purity, and quality tests, that when complied with, gives a high level of confidence for ensuring an authentic botanical drug of trade, but may lack botanical preciseness. The occurrence of hybrids raises additional challenges that may or may not result in clinically relevant changes in the botanical drug.

Presented are examples of closely related species, some of which can be readily identified as authentic articles of medicinal trade according to pharmacopoeial identity tests but may not be identified with 100% botanical specificity. Included are points of discussion regarding the challenging nature of proper nomenclature and use of interchangeable species in the trade of medicinal plants with implications for traditional health care practitioners, medicinal plant traders, regulators, and medicinal plant manufacturers.

ISL EA-06 The truth behind herbal products: how HPTLC can help herbal industry detect adulteration?

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DOI 10.1055/s-0039-3399665

Adulteration of herbal drugs, ingredients and products is a growing concern of industry, regulators and consumers worldwide. The last few years have seen an increase in negative press reports targeting the herbal industry. A famous example was the 2015 case of the New York State Attorney General who launched his own investigation of the quality of herbal products sold as dietary supplements. Although the validity of the study was questioned, such case raised several discussions regarding conformity of herbal ingredients and products with specifications and the issue of adulteration.

Our group has participated in several market surveys looking at black cohosh, ginkgo, St. John's wort, milk thistle and echinacea products by HPTLC [1, 2, 3]. In most of these cases, we found a significant number of samples either adulterated or not in full compliance with their labels.

This presentation will illustrate the role of HPTLC in ensuring quality herbal drugs, ingredients and products in a simple and pragmatic way. HPTLC is not only an identity tool, but also is used to determine purity and potency of the investigated material. The concept of "comprehensive HPTLC fingerprinting" combines visual image data and quantitative information that becomes available, when such data are converted into peak profiles. This information comes at no extra cost.

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ISL EA-07 How some suppliers attempt to fool commonly used analytical methods

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As the global market for herbal medicines and supplements has increased, so have reports of undisclosed ingredients being added to botanical raw materials, extracts, and finished consumer products. Often, adulteration is carried out for financial gain (economically motivated adulteration), where raw materials are intentionally substituted or diluted with undisclosed lower-quality ingredients. This represents a challenge to the global botanical medicine marketplace and, in some cases, impacts consumer safety. The American Botanical Council (ABC), the American Herbal Pharmacopoeia (AHP), and the National Center for Natural Product Research (NCNPR) at the University of Mississippi have initiated the ABC-AHP-NCNPR Botanical Adulterants Program, a program to educate members of the herbal and dietary supplement industry about ingredient and product adulteration.

The chemically complex nature of botanically-derived ingredients calls for unique quality control processes. Appropriate testing for identity and authenticity of botanical materials is a universal requirement in countries around the world. However, unscrupulous suppliers often take advantage of a lack of specificity in test methods used to confirm the identity of a botanical ingredient by providing materials that comply with these identity tests even if they do not correspond to the material declared on the label. Ingredients for which adulteration has been reported include, e.g., extracts of cranberry (*Vaccinium macrocarpon*) fruit, ginkgo (*Ginkgo biloba*) leaf, saw palmetto (*Serenoa repens*) fruit, St. John's wort (*Hypericum perforatum*) herb, and turmeric (*Curcuma longa*) root/rhizome. The presentation gives an overview on botanical ingredient adulteration with examples how fraudulent suppliers or manufacturers attempt to fool standard analytical testing.

ISL EA-08 The 2019 curcumin crisis in Italy: what we know so far, and early lessons

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On May 10, 2019, the Italian Ministry of Health publicly announced that 4 cases of acute cholestatic non-viral hepatitis had been reported, and associated with the consumption of food supplements. Investigators suspected a causal association with curcumin obtained from turmeric (*Curcuma longa* L.). By July, 21 cases had been reported by health authorities, involving 22 different food supplements, mostly by Italian brands. A significant proportion of products also included piperine as ingredient; it has also been reported that several products were adulterated with synthetic curcumin, presented to consumers as natural. Few cases of acute non-viral hepatitis associated with curcumin had been reported in the US, Australia and, apparently, Japan. The aim of the presentation is to summarize available information on the case-series, and in the literature; to report on the risk assessment and risk management decisions in Italy, and being debated in other Member States; to reflect on early lessons, particularly in reference to adulteration. Surveillance bias may have contributed to the clustering of cases, but risks to individuals with liver or biliary disease or undergoing medicinal therapy could have been anticipated. Both authorities and industry have shown to be only partially prepared to investigate and address case-reports. While synthetic curcumin is not believed to be the cause of the case-series at this stage, its safety is untested, and its labeling not appropriate. In this case, authorities and industry have become aware of the challenge posed by adulteration, but struggle to respond in an effective and decisive manner.

ISL EA-09 Authentic and non-adulterated botanical ingredients in an unethical market situation. Full traceability, high quality production and exhaustive analytical control

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Adulteration and fraud are a menace to the botanical ingredients sector and generate growing demand for transparency for all the processes associated with production [1,2]. Manufacturers cannot ultimately avoid market scrutiny. Full traceability of the entire process is key, starting with the plant material from cultivated or wild sources, and including quality and sustainability criteria.

The multilayer set of control methods of plant material starts with the taxonomic and phytochemical identity, continues with purity controls, including contaminants, and ends with the determination of active or marker compounds. Besides classical methods, modern techniques such as metabolomic evaluations and DNA barcoding should be employed. These methods allow a more robust ingredient identification and lower instances of adulteration. The subsequent physical and chemical production processes, and the set of specifications and control methods, will finally determine the quality and authenticity of the botanical ingredient.

Saw Palmetto Lipidic Sterolic Extract (SPE), made from *Serenoa repens* berries, is the most expensive oil of the pharmaceutical and health food market, hence, its adulteration is frequent. Substitution of the saw palmetto berry with fruit from closely-related palm species, dilution of products with exhaustively extracted berry powder, the use of unripe berries, the addition of vegetable oils to extracts, and/or full substitution of SPE with other lower-cost vegetable or animal oils, have all been detected and documented by Euromed[3]. Wide variation of fatty acid content in authentic and adulterated saw palmetto extracts can explain differences in clinical trial outcomes and threaten the market reputation of the extract.

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ISL EA-11 Herbal product analysis: are analytical standards your achilles heel?

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In controlling the supply chain of botanical raw materials, extracts and other dietary ingredients, there are different challenges that must be addressed compared to other industries, especially in the traceability and sustainability of plant sources. However, there are also universal principles that may apply: testing of starting materials, intermediates and finished products for identity, contamination and potency.

Among many testing techniques, the determination and quantification of chemical constituents of herbs or herbal products is of tremendous value beyond meeting regulatory requirements. Chromatographic techniques have been available for years to address these needs, whether the purpose is material identification, potency assurance, or the detection of contamination and adulteration.

Because the results of chromatographic techniques are calculated directly against the reference standards chosen, these reference standards have direct impact on the ability to accurately assess the safety, quality, and potency of the raw materials and finished products that have been tested. It is critical that companies understand and incorporate reference standards as part of their increased commitment to valid, fit for purpose product testing as required by 21CFR 111.320 (a-b).

As industry companies work to develop unassailable testing programs, and reference standards are increasingly understood to be a vital component of herbal product testing, the sourcing of quality reference standards is as important as increased commitment to sourcing quality ingredients.

ISL EA-12 How to compete with adulterations in Chinese medicine

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In samples from Chinese medicine clinics and pharmacies in Taipei, from 372 traditional Chinese medicines 2.42% were adulterated [1]. In material from herbal markets in China, adulterants were detected in 3.7-13.3 % of the cases [2]. Public health issues due to confusion with toxic herbs have been increasing [3], why there is an urgent need for proper authentication.

Chromatographic fingerprints are a powerful tool for identification [4]. Mixing up Aristolochiae fangchi radix and Stephaniae tetradrae radix can be avoided by simple TLC tests [5]. Toxicological risks occur also from mixing up *Arenebia euchroma (Ruan Zicao)*, low in toxic pyrrolizidine alkaloids (PAs), with *Lithospermum erythrorhizon* (*Ying Zicao*) or *Onosma paniculata* (*Dian Zicao*), both high in PAs. By DNA and HPTLC fingerprint analysis, they can be discriminated [6]. Also, confusion of Acanthopanacis gracilistyli Cortex (Wu Jiapi) and Eleutherroocci senticosi radix (Ci Wu Jia) with Periplocae sepiae Cortex (Xiang Jiapi) is hazardous, because periplocin, the major constituent of Xiang Jiapi is a potent cardenolide [7,8]. Other adulterations are more of therapeutic relevance, like mixing up different Angelica roots [9] and Lonicerae japonicae Flos (Jinyinhua) [10]. They can be detected by chromatographic or DNA analysis.

Expensive Chinese herbs are adulterated for commercial reasons. Near-infrared (NIR) spectroscopy has been suggested for differentiation of various grades of *Panax ginseng* (Ren Shen) [11], and for identifying adulterants of *Panax notoginseng* (Sanqi) [12], as well as for authentication of Cordiceps sinensis [13]. By using species-specific nucleotide signatures, adulteration of Cistanches Herba (*Rou Cong Rong*) can be detected [14].

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Abstracts of Plenary Lectures

PL-01 The NCI program for natural product discovery

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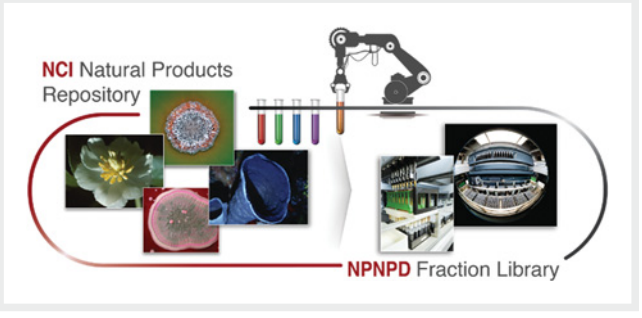
The US National Cancer Institute's Natural Product Repository is one of the world's largest, most diverse collections of natural products containing over 230,000 unique extracts derived from plant, marine and microbial organisms that have been collected from biodiverse regions throughout the world. Importantly, this national resource is available to the research community for the screening of extracts and the isolation of bioactive natural products. However, despite the success of natural products in drug cancer discovery, compatibility issues that make crude natural product extracts challenging have reduced enthusiasm for the high-throughput screening (HTS) of crude natural product extract libraries in targeted assay systems. To address these limitations and make the NCI's Natural Products Repository more amenable to HTS, we have initiated the prefractionation of extracts using an automated, high-throughput robotics platform capable of generating a library of 1,000,000 partially purified extracts. The talk will discuss this and other mechanisms to increase the utility of the NCI Natural Products Repository in cancer-related drug discovery.



▶ **Fig. 1**

PL-02 Digitizing historical collections of natural products to further explore the monoterpene indole alkaloids chemical space

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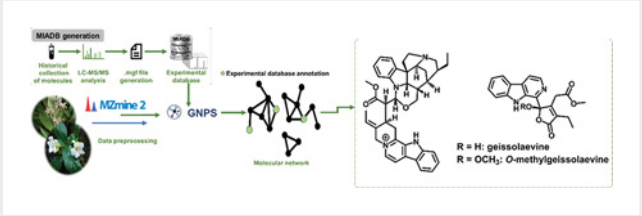
DOI 10.1055/s-0039-3399672

Monoterpene indole alkaloids (MIAs) constitute a broad class of nitrogen-containing plant-derived natural products composed of more than 3000 members.[1] This natural product class is found in hundreds of plant species from the Apocynaceae, Loganiaceae, Rubiaceae, Icacinaceae, Nyssaceae, and Gelsemiaceae plant families. Throughout the six past decades, the structural intricacies and biological activities of these molecules have captured the interest of many researchers all over the world.

As part of our continuing interest in MIA chemistry, we recently implemented an in-house MS/MS database, constituted of a cumulative collection of alkaloids from the global natural products research community. These endeavors led to the construction of the largest MS/MS dataset of MIAs to date, that we named: Monoterpene Indole Alkaloids DataBase (MIADB).[2] Thanks to this database, we developed a streamlined molecular networking dereplication pipeline directed toward the reinvestigation of previously studied MIA-containing plants. This pipeline allowed to prioritize the isolation workflow toward new alkaloids bearing unprecedented carbon skeleton and interesting biological activities. This presentation is intended to describe the most significant results gleaned from the reinvestigation of several forgotten plants by this new generation of sophisticated tools.



▶ **Fig. 1**

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PL-03 Integrative analysis of bioactive compounds from frogs, plants and microorganisms from the Brazilian biodiversity

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DOI 10.1055/s-0039-3399673

Living organisms have been used as source for different traditional medicines from several ethnic groups since antiquity. In recent years, one of the greatest challenges within the field of natural products is to understand its functioning for future rational applications. Mass spectrometry methodologies are important strategies for investigating the complex chemistry of natural products. During the last years, the increasing power of informatics for data analysis has increased our capacity to inventory many biological systems [1]. In our laboratory, for instance, we have investigated neuroactive and antimicrobial compounds from skin secretions of anuran amphibians [2]. We have found that some components of the rich secretion can be associated with chemical communication between frogs at intra and interspecific levels [2, 3]. In this talk we will present and discuss the potential relevance of plants and bacteria as a source of chemical signals in frogs, which represent one component of the vast Brazilian Biodiversity.

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PL-04 Will perplexity prevail over complexity in biomedical natural products research?

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The inherently complexity of bioactive natural products (NPs) requires new analytical technology, trans-disciplinary approaches, and an integrative pharmacognosy approach for advancement. Recent research reveals that complexity comes in three main flavors: *obvious, less obvious*, and *residual*. While analytical chemistry and (bio)synthetic biology progress renders chemodiversity an *obvious complexity*, other chemistry aspects are *less obvious*: mechanistic explanations for postulated "botanical synergy" are mostly elusive; monomers w/well-understood 3D (stereo)chemistry of (±)-catechin overwhelms analytical tools and analysist building SARs of 30,000+ perceivable tetrameric grape seeds tannins. As Nature's permutations outstrip scientific capabilities, perplexity prevails over complexity when developing rationales for synthesis/isolation of promising analogues and pharmacophore definition. Correlating ClpC1-target interaction with anti-*M.tb.* bioactivity profiles of rufomycin anti-TB leads exemplify *less obvious complexity*. Despite being an *obvious complexity*, impurity ranks high among factors that confound NP research. Searches for bioactives (phytoestrogens, antimicrobials) frequently lead to *residuals* explaining the bioactivity of crude NPs: finding that 0.24% within a "pure" NP explain the entire anti-*M.tb.* "lead" potential was a perplexing demonstration of *residual complexity*. Apparent simplicity of hyper-popular NPs hide the *less obvious* and *residual complexity* of their promiscuous properties. Well-founded rationales that designate NPs such as curcumin as *invalid/improbable panaceas* (IMPs) are less complex than the impact of basic tenets of pharmacognosy, scientific publishing, or marketing practices. Advertised globally, the therapeutic potentials ascribed to IMPs are in perplexing contrast to the rigor of available efficacy evaluations. The present contribution structures the three complexity flavors and identifies approaches for overcoming perplexity in NP research.

PL-05 The evolution of animal self-medication and lessons for the development of medicine and new medicines

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Parasites, viruses and other pathogens cause a variety of diseases that affect the behavior and reproductive fitness of an individual. While the study of animal self-medication as a science is relatively new, to date, research has classified health maintenance and self-medicative behaviors into four levels: 1) optimal avoidance or reduction of disease transmission: 2) the dietary selection of items with a preventative or health maintenance affect: 3) ingestion of a substance for the curative treatment of a disease or the symptoms thereof: and 4) external application of a substance to the body for the treatment or control of disease bearing insects. Of any species studied thus far, chimpanzees have provided the most details for level 2 and 3 behaviors, exemplified by such behaviors as bitter pith chewing and whole leaf swallowing used in response to parasite infection [1, 2]. This presentation will review the progress to date in primates, and compare these strategies with examples from other species to illustrate the wide and deep evolutionary origins of self-medication in the animal kingdom and show how this bio-rational approach can aid in the search for new natural plant compounds and has found new uses for well known compounds in human and livestock health care.

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PL-06 Chemistry, mode of action and clinical efficacy of the anticancer diterpenoid tigilanol tigliate (EBC-46)

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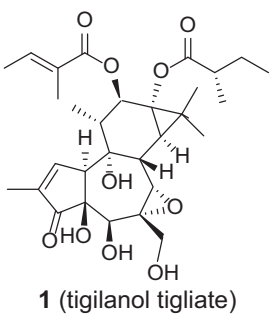
Tigilanol tiglate (EBC-46, **1**), a novel diterpene ester isolated from the seeds of the endemic Australian rainforest tree *Fontainea picrosperma* (Euphorbiaceae), is being developed for intratumoral treatment of cancers in humans and companion animals [1,2]. Here we summarise our current understanding of the chemistry and mechanism of action of the compound, and provide results from recently completed veterinary (Phase III) and human (Phase I/II) clinical trials.

Tigilanol tiglate has a multi-factorial mechanism of action. A single intratumoral injection (1) induces a rapid, highly localised and transient inflammatory response surrounding the tumour mass, (2) significantly increases permeability of the tumour vascular endothelium, and (3) causes rapid tumour cell death by oncosis. In combination, these result in tumour haemorrhagic necrosis, eschar formation and complete tumour destruction within 4 to 14 days. Localised inflammation and increasing permeability of tumour vasculature are associated directly with the activation by tigilanol



1 (tigilanol tigliate)

▶ **Fig. 1**

tiglate of specific isoforms of protein kinase C (-βI, -βII, -α, -γ), while tumour cell death via oncosis requires PKC/C1 domain mediated signalling. The compound also induces changes in cytokine signalling and gene expression that promote wound healing following tumour destruction.

In a Phase III fully-randomised, controlled and blinded veterinary clinic trial, a single treatment with tigilanol tiglate resulted in complete and enduring tumour destruction in more than 75% of canine patients with mast cell tumours. In a clinical Phase I/II human dose-escalation study, maximum tolerated dose was not reached and signs of efficacy were observed in 9 tumour types, including complete response in 4 patients.

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PL-07 GPCR-targeted drug discovery

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G-protein coupled receptors (GPCRs), also known as 7 transmembrane receptors, are the largest family of cell surface receptors. They are involved in a wide variety of physiological and pathological processes and are the largest family of druggable targets. Our group is interested in the biological functions of GPCRs and their roles in major diseases including autoimmune disease, neurodegenerative diseases, metabolic diseases and etc. In addition to the mechanism study, we also screen and develop drugs targeting GPCRs. Natural compounds may exert their effect by targeting GPCRs.

For example, Herbal-based food supplement is widely used to treat obesity. Among them, the *Hoodia gordonii* (Asclepiadaceae) supplements are extremely popular. The African cactiform has been used for thousands of years by Xhomani Bushmen as an anorexant during hunting trips and has been proposed as a new agent for the management of body weight. However, the true active components and molecular targets of *Hoodia* remain unclear. We have demonstrated that Gordonoside F, a steroid glycoside isolated from *Hoodia gordonii*, but not the widely known P57, activates specifically GPR119, a receptor critically involved in metabolic homeostasis, and leads to increased insulin secretion and reduced food intake. These results not only demonstrate that the activation of GPR119 receptor is an important mechanism underlying *Hoodia gordonii*'s therapeutic effect, but also suggest that Gordonoside F or its congeners could be developed into new drugs in treating metabolic disorders.

PL-08 The Amaryllidaceae alkaloid narciclasine as modulator of endothelial activation processes

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Narciclasine is an isocarbostyril alkaloid that was first isolated from *Narcissus* species (Amaryllidaceae) more than 50 years ago. In the last decades, narciclasine attracted huge attention due to its high potency as anti-tumor compound. However, beyond cancer research, knowledge on the pharmacological activity of narciclasine is largely lacking. Only few studies reported about an anti-inflammatory potential of the alkaloid. Since both solid tumor growth and inflammation strongly depend on activation processes of vascular endothelial cells (ECs), we hypothesized that narciclasine might interfere with signaling pathways that regulate two crucial pathophysiological functions of ECs: the formation of new blood vessels (angiogenesis) and the interaction of leukocytes with ECs (extravasation and tissue infiltration). Indeed, by using a battery of both *in vitro* and *in vivo* models, we showed that narciclasine strongly inhibits angiogenic processes as well as leukocyte-endothelial cell interaction. Most interestingly, we were able to elucidate the molecular mechanisms involved in these pharmacological activities: In human ECs, narciclasine blocked *de novo* protein biosynthesis by approx. 50% without inducing considerable cytotoxicity. This led to a loss of the short-lived cell membrane proteins tumor necrosis factor receptor 1 (TNFR1) and vascular endothelial growth factor receptor 2 (VEGFR2), which explains the observed inhibitory actions of narciclasine on several major signaling events in ECs. Taken together, our investigations highlight the Amaryllidaceae alkaloid narciclasine as an interesting anti-inflammatory and anti-angiogenic compound that is worth to be further evaluated in preclinical studies.

PL-09 The application of classic and modern pharmacognosy in monographing African traditional medicines – a botanical travelogue

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Southern Africa harbours a unique flora comprising of over 24 000 species of flowering plants. Woven within this tapestry of botanical diversity is the traditional use of indigenous plants as ethnomedicines. Developing official monographs, establishing a national repository of botanical standards, and producing validated analytical methods is a fundamentally important prerequisite to encourage research and commercialisation of Africa's medicinal flora. Furthermore, the availability of reference standards, analytical methods and comprehensive monographs would be highly beneficial to the regulator, consumer and nascent industries. The unfortunate underrepresentation of pharmacognosy in the curricula of many pharmacy schools has left a void of expertise, which has hampered the development of a comprehensive herbal Pharmacopoeia. A further challenge involves the inherent complexity of medicinal plants, exacerbated by extensive chemotypic variation. Chemical fingerprinting is a crucial component in characterising plant material and requires a dedicated approach to develop analytical methods for the profiling of complex herbal extracts. Funding from the National Research Foundation (NRF/DST SARChI Initiative) and the South African Medical Research Council has catalysed initiatives at the Tshwane University of Technology to develop herbal monographs that aid in the identification and quality control of important South African herbal medicines. Selected examples will be presented to illustrate the daunting workflow, which includes extensive sampling, the development of analytical methods to profile volatile and non-volatile compounds using GC-MS and LC-MS, HPTLC, vibrational spectroscopy, as well as the use of preparative chromatography to isolate biomarkers. The powerful tandem application of analytical chemistry and chemometric modelling will be highlighted. Developing comprehensive species monographs requires a multidisciplinary collaborative effort which will inevitably contribute to the safety, efficacy and quality of African Traditional Medicines and commercial herbal formulations.

PL-10 Endocannabinoid signaling across species – evolution and perspectives for drug discovery

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DOI 10.1055/s-0039-3399680

Endocannabinoids are arachidonic acid derived lipids that show a broad range of biological effects in mammals. In the brain they are key modulators of neurotransmission and in the immune system they modulate acute and chronic inflammatory processes. Endocannabinoids have evolved early in evolution, prior to the classic mammalian cannabinoid receptors CB1 and CB2 [1] and seem to play important functions also in lower organisms [2, 3]. Currently, only two very distant plant taxa are known to exert psychoactive effects via CB1 receptors *in vivo* [4], but numerous non-psychoactive endocannabinoid system modulating natural products exist. In this lecture, I will discuss the biochemical convergence and how lipids from plants can inspire probe design and help to identify new biochemical pathways in mammals and how this can foster translational research. Results from an ongoing comprehensive "phylo-activity" screening program of plant extract libraries will highpoint the potential link between nutrition and the endocannabinoid system [5], warranting a more pharmacological inquiry into the relevance of plant secondary metabolites for human health.

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Abstracts of Key Lectures

KL-01 Natural ingredients of skin lightening cosmetics

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DOI 10.1055/s-0039-3399681

Hyperpigmentation is a common aesthetic problem caused by increased melanogenesis and uneven distribution of melanin pigment in the skin. It is estimated that over 90% of the European population struggle with this disorder at some point of their lives [1] and approximately 15% of the world population invest in skin lightening cosmetics [2]. The primary target of skin lightening agents is tyrosinase (EC 1.14.14.1), a metaloenzyme catalyzing rate-limiting first two steps of melanogenesis - the convertion of L-tyrosine to L-DOPA and subsequently to dopaquinone [3, 4]. Kojic acid, arbutin and glabridin are examples of tyrosinase inhibitors isolated from natural sources that are commonly used in skin whitening cosmetics. However, due to their poor efficacy *in vivo*, low formulation stability and possible adverse effects novel cosmetic ingredients preventing and reducing hyperpigmentation are needed [5].

An interesting source of effective and safe skin-whitening agents are plants extract. The efficacy of plant-derived ingredients as skin lightening agents might be analysed and compared using various recently established *in vitro* methods that are more accurate and reliable than the commonly used mushroom tyrosinase activity assay. Advantages and disadvantages of these methods will be discussed. Examples of novel skin-whitening extracts and compounds found during the original research and described in recent publications will be presented and compared with currently used cosmetic ingredients. The advantages of the application of plant extracts rather than purified compounds in skin whitening cosmetics will also be discussed.

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KL-02 Novel noscapine derivatives as potent anticancer and antiprotozoal agents

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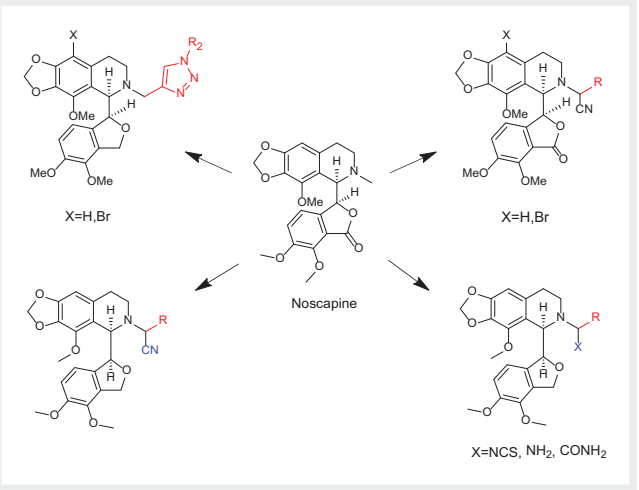
DOI 10.1055/s-0039-3399682

Noscapine as an opium alkaloid has several advantages over present anticancer drugs such as low toxicity, safety, high bioactivity, oral administration and unlike other opium alkaloids, noscapine is non-addictive, non-narcotic and non-analgesic [1].

Novel noscapine derivatives were synthesized via Huisgen and Strecker multicomponent reactions to achieve the best cytotoxic and antiprotozoal compounds.

The MTT assay was done against MCF7 cell line and results showed that triazole derivatives had substantial lower cell viability comparing with noscapine.



▶ **Fig. 1**

According to MTT results and to have a better idea concerning the mechanism of action, the surface plasmon resonance (SPR) analysis was done and the results indicated that there are promising bindings between the selected ligands and tubulin.

Furthermore, the antiparasitic activity of the synthesized compounds was studied against four unicellular protozoa, *i.e., Trypanosoma brucei rhodesiense, T. cruzi, Leishmania donovani,* and *Plasmodium falciparum.* Interestingly, seven isothiocyanate analogues displayed excellent antiparasitic activity against *L.donovani* with IC_{50} values between 0.4 -1.0 μM and selectivity indices (SI) ranged from 7.8 to 18.4, comparable to the standard drug miltefosine (IC_{50} =0.7 μM).

Regarding molecular modeling studies, there was a good compatibility between the calculated and experimental data on both specific cancer and protozoa receptors.

Therefore, noscapine can be considered as an efficient lead compound in medicinal chemistry for drug design studies.

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Abstracts of Short Lectures

Short Lectures Monday, September 02, 2019

Short Lectures A: Biological and Pharmacological Activities of Natural Products

SL A-01 *Cannabis sativa* L. extract reduces inflammatory markers in human fibroblasts and keratinocytes

Authors Sangiovanni E[1], Fumagalli M[1], Pacchetti B[2], Piazza S[1], Magnavacca A[1], Khalilpour S[1], Melzi G[1], Martinelli G[1], Dell'Agli M[1]

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DOI 10.1055/s-0039-3399683

Dermatitis and psoriasis are inflammatory skin diseases in which keratinocytes and fibroblasts play a key role in the release of pro-inflammatory mediators (e.g. IL-8, MMP9, VEGF, and NF-κB). The flowered tops of *Cannabis sativa* L. (hemp) contain the highest concentration of cannabinoids including cannabidiol (CBD), the second major cannabinoid without psychotropic activity.

The aim of the present study was to investigate the potential effect of a *Cannabis sativa* L. ethanolic extract (CSE), standardized in CBD, as anti-inflammatory agent in human keratinocytes and fibroblasts.

CSE and CBD (99.5% HPLC purity) were prepared by LINNEA SA (Riazzino, Switzerland) and assayed in fibroblasts (HDF) and keratinocytes (HaCaT), stimulated by TNFα or UVB.

CSE reduced TNFα-induced IL-8 secretion (HDF, IC_{50} 15.13 μg/ml), VEGF (HaCaT, IC_{50} 26.8 μg/ml) and MMP-9 (IC_{50} 18.0 and 7.21, for HaCaT and HDF, respectively), while CBD showed low or no inhibitory effect, but reduced the NF-κB driven transcription.

CSE (25 μg/mL) and CBD (4 μM) were tested on the expression of 84 genes involved in inflammation and wound healing. CSE decreased the mRNA levels of the TNFα-up-regulated genes, whereas CBD was not able to fully explain the activity of the extract.

These results suggest that CSE inhibits the release of pro-inflammatory mediators in human fibroblasts and keratinocytes, acting on the NF-κB pathway. The down-regulation of genes involved in wound healing and skin inflammation, were not strictly associated to the presence of CBD, suggesting that other unknown compounds occurring in the extract my exert anti-inflammatory effects.

SL A-02 Protective effect of Plumericin in inflammatory bowel disease: regulation of inflammatory and oxidative stress response *in vitro* and *in vivo*

Authors Rapa SF[1], Waltenberger B[2], Di Paola R[3], Autore G[1], Cuzzocrea S[3], Stuppner H[2], Marzocco S[1]

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DOI 10.1055/s-0039-3399684

Inflammatory bowel diseases (IBDs) are characterized by chronic relapsing intestinal inflammation. Although IBDs etiology remains unknown, the mechanisms of inflammation and oxidative stress as well as dysbiotic conditions and aberrations in the epithelial barrier are recognized as involved in the pathogenesis of these diseases [1]. Conventional therapy in IBDs uses anti-inflammatory and immunosuppressive corticosteroids, as well as biological drugs, however, the low remission rate and the severe side effects of these therapies are not satisfactory for IBDs pharmacological treatment. Thus, there is a great need for new drugs with anti-inflammatory and anti-oxidant activities.

In this study we evaluated the effect of Plumericin, one of the main bioactive components extracted from the bark of *Himatanthus sucuuba* (Woodson) on intestinal inflammation, both *in vitro*, on rat intestinal epithelial cells (IEC-6), and *in vivo*, in an experimental model of DNBS-induced colitis.

Our results indicated that Plumericin significantly reduces pro-inflammatory factors with a pivotal role in IBDs, such as tumor necrosis factor-α (TNF-α) levels, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) expression and nitrotyrosine formation. Plumericin was also able to inhibit nuclear factor-kB (NF-kB) activation and to reduce reactive oxygen species (ROS) release. Moreover, Plumericin activates the nuclear factor erythroid-derived 2 (Nrf2) anti-oxidant pathway and inhibited inflammasome activation in IEC-6. The anti-inflammatory and antioxidant effects observed *in vitro*, associated with a reduced intestinal macroscopic damage, were confirmed *in vivo* in DNBS-induced colitis in rat.

Plumericin shows anti-inflammatory and antioxidant activities at intestinal level and therefore, could be a promising agent the treatment of IBDs.

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SL A-03 Saponins from saffron corms inhibit the secretion of pro-inflammatory cytokines at both protein and gene levels

Authors Keller M[1], Fankhauser S[2], Giezendanner N[2], König M[2], Keresztes F[1], Danton O[1], Hamburger M[1], Butterweck V[2], Potterat O[1]

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DOI 10.1055/s-0039-3399685

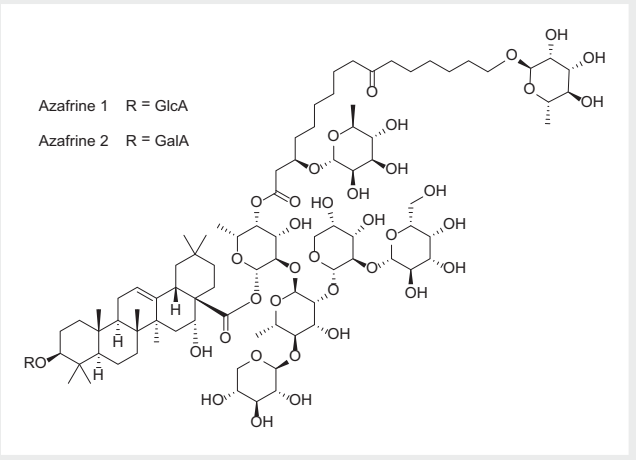
Corms are obtained as a by-product during the cultivation of saffron (*Crocus sativus*). In a project aimed at the valorization of this waste product, we observed that a 70% EtOH extract of the corms and particularly a Diaion HP-20 methanolic fraction thereof inhibited the TNF-α/IFN-γ-induced secretion and gene expression of the chemokines IL-8, MCP-1 and RANTES in human HaCaT cells. The effects were partly stronger than those of the positive control hydrocortisone.

After semi-preparative HPLC separation of the methanolic fraction, the activity could be assigned to a major broad peak in the ELSD trace. For

preparative isolation, the 70% EtOH extract was partitioned between *n*-butanol and water. Separation of the *n*-butanol-soluble fraction by centrifugal partition chromatography (CPC), followed by preparative HPLC on RP-18 and HILIC columns afforded a series of bidesmosidic glycosides of echinocystic acid bearing a fatty acid residue attached to the glycosidic moiety at C-28. The main components were identified as azafrines 1 and 2 [1].

Saffron saponins significantly inhibited TNF-α/IFN-γ-induced secretion of RANTES in human HaCaT cells at 1 μM ($p < 0.001$). Some of them further lowered TNF-α/IFN-γ-induced gene expression.

Saffron corm extracts and their saponin constituents may have a potential for the development of new cosmetic and/or medicinal products against inflammatory skin conditions.



▶ **Fig. 1**

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SL A-04 Therapeutic and analgesic effects of ephedrine alkaloids-free Ephedra Herb extract on complete Freud's adjuvant-induced arthritis model mouse

Authors Nakamori S[1,2], Miyajima N[1,2], Hyuga S[2], Minami Y[1,2], Kazama H[1,2], Hiyama M[1,2], Endo M[2], Yang J[3], Oshima N[4], Uchiyama N[5], Amakura Y[6], Hakamatsuka T[5], Goda Y[5], Odaguchi H[2], Hanawa T[2], Kobayashi Y[1,2]

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DOI 10.1055/s-0039-3399686

Ephedra herb is one of the most important crude drugs and is the component of Kampo formulae such as maoto, makyoyokukanto, and eppikajutsuto that have been treat myalgia, rheumatism, and arthralgia in Japan. We recently reported the ephedrine alkaloids-free Ephedra Herb extract (EFE) [1] which has developed to remove the side effects caused by ephedrine alkaloids, attenuates formalin-induced inflammatory pain in the same manner as Ephedra Herb extract [2]. In this study, we evaluated the effectiveness of EFE on arthritis using a complete Freud's adjuvant (CFA) induced arthritis model mouse. Oral administration of 28–700 mg/kg/day of EFE for 4 weeks (5 days/1 week) significantly reduced the articular swelling of the model mice in dose-dependent manner. We also measured the change of sensitivity against the mechanical stimulation using von Frey filaments to evaluate the analgesic effects of EFE. A 50% paw withdrawal threshold (PWT) of the model mouse was lowest at 7 days after CFA inoculation. Then EFE (175–700 mg/kg) was administrated the mice at 7 days after CFA inoculation, and the sensitivity against the mechanical stimulation was measured at 6 h after oral administration. EFE dose dependently improved the 50% PWT in the von Frey test without hampering the physical performance in the rota-rod test. These results suggested that EFE is useful for treatment of arthritis. Now, we are evaluating the efficacy of EFE against a cartilage damage in CFA-induced arthritis model mouse.

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Short Lectures B: Analytical Methods for Quality Control of Herbal Medicinal Products

SL B-01 The use of vibrational spectroscopy in medicinal plant analysis: current and future directions

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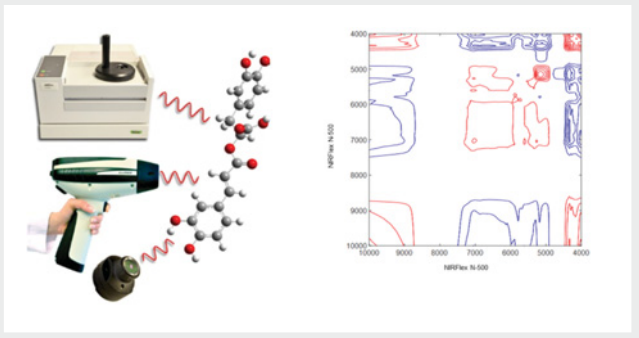
DOI 10.1055/s-0039-3399687

The field of molecular vibrational spectroscopy applied to medicinal plant analysis is developing very dynamically. Even though, traditional separation and mass spectrometric (MS) techniques offer analytical investigations with high selectivity and sensitivity, vibrational spectroscopy benefits from the short analyses times, non-invasiveness and the simultaneous analysis of chemical and physical parameters.

Furthermore, chemometric univariate and multivariate data treatment enables efficient spectral interpretation and the establishment of sufficient calibration/validation models. Advanced quantum chemical approaches can further support the challenge of band assignment [1]. Near-infrared (NIR, 4.000-10.000 cm^{-1}), attenuated total reflection (ATR, 400-4.000 cm^{-1}) and Raman spectroscopy have been demonstrated as being very efficient for even complex qualitative and quantitative attempts in combination with selective reference analytical methods. Qualitative attempts comprise analysing, e.g., species and in some cases also origin, quantitative analysing chemical and physical parameters. Two-dimensional correlation spectroscopy (2D-COS) has been developed towards a powerful analysis tool for monitoring the dynamics of a spectrometer system [2]. The miniaturization of spectrometers is a highly demanding trend, enabling to carry out investigations at any independent place including the field [3]. Imaging and mapping spectroscopic attempts (MIR, NIR, Raman) enable high-resolution analysis of potent ingredients down to approximately 4 μm and 1 μm, respectively [4].

This contribution highlights recent advances of molecular spectroscopy in medicinal plant research. The latest technical developments will be discussed followed by several selected applications. Their limits and advantages over traditional methods will be critically evaluated to point out the future trends.

This work was supported by the Austrian Science Fund (FWF), P32004-N28, and by the Federal Ministry of Ministry of Education, Science and Research (Vienna, Austria) (Novel analytical tools for the quality assessment of Chinese herbs with metabolic, immune related neuromodulatory effects, BMBWF-402.000/0017-WF/V/6/2016).



▶ **Fig. 1**

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SL B-02 Quantum mechanical modeling of NIR spectra of thymol

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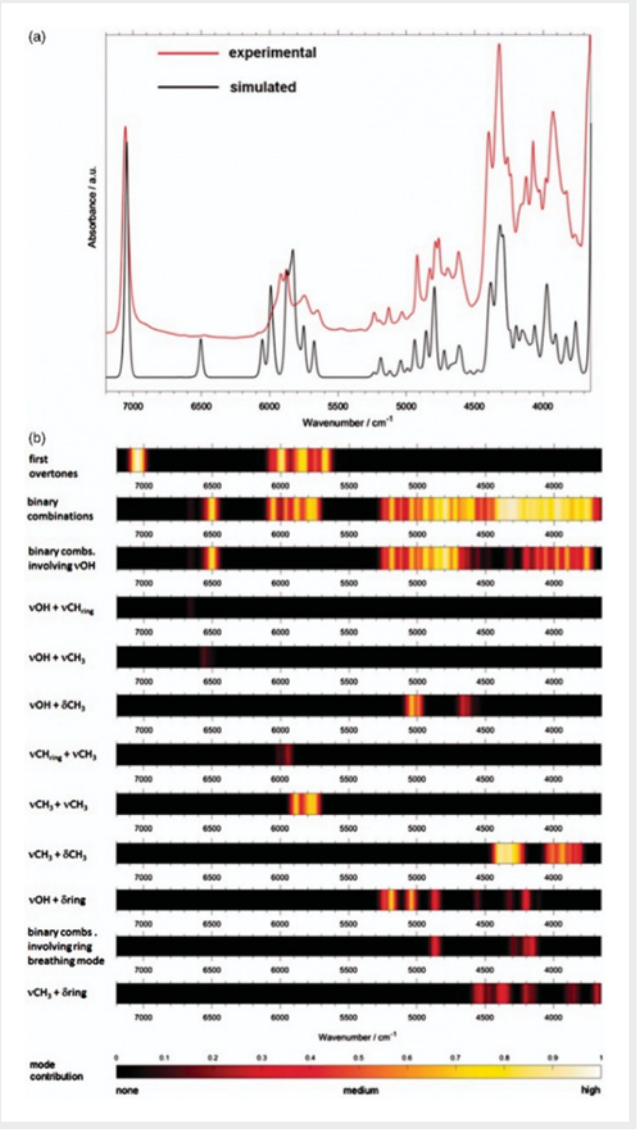
DOI 10.1055/s-0039-3399688

Near-infrared (NIR) spectroscopy is a major analytical tool with marked importance in various applications including the qualitative and quantitative analysis of herbal medicines. Yet, the physical background for the measured spectra routinely remains un-interpreted. Quantum mechanical calculations are capable of providing deep and independent insights and form a strong support for applied spectroscopy. An example of thymol [1] is presented here, an important constituent of a traditional herbal medicine *Thymi herba*.

NIR spectra of thymol in dependence of the sample state (solid, melted and soluted) and concentration (for soluted sample) were analyzed. Patterned spectral changes were observed, indicating that the differences between the bands in their sensitivity to the intermolecular interactions are reflected in the quantitative models. The analysis of PLS regression coefficients vector in the quantification of thymol content in *Thymi herba* based on NIR spectroscopy was conducted. Surprisingly, the vibrations which are the most significant spectra forming factors and which are the most sensitive to the chemical surrounding (i.e. those of OH group) do not correlated well with the sample content.

This work was supported by the Austrian Science Fund (FWF), P32004-N28.



▶ **Fig. 1**

References [1] Beć KB, Grabska J, Kirchler CG, Huck CW. NIR spectra simulation of thymol for better understanding of the spectra forming factors, phase and concentration effects and PLS regression features. J Mol Liq 2018; 268: 895–902

SL B-03 Purification & isolation of compounds from natural products using an SFE-SFC workflow

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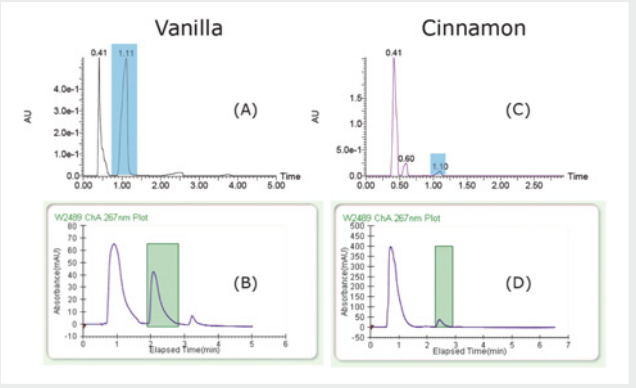
DOI 10.1055/s-0039-3399689

In a supercritical fluid workflow, CO2 with or without the addition of an organic modifier, is used to extract (SFE) and purify (SFC) target compounds. The CO2 used as a solvent is safe and the extracts produced by this process are free from biological contaminants, have longer shelf life,

high potency, and address major international concerns regarding residual solvent concentration.2 In this study, a complete SFE-SFC workflow will be demonstrated using the MV-10 ASFE system (MV-10) for extraction and the Prep 80a SFC System (SFC 80) for purification of two compounds; vanillin from vanilla planifolia and cinnamic acid from Cinnamomum Verum. This workflow includes SFE method development, analytical method development, scale-up, purification, and fraction analysis. The process can be adapted to purify target compounds from a variety of natural products and matrices.



▶Fig. 1

SL B-04 Vibrational spectroscopy and chemometric data analysis: the principle components of rapid quality control of herbal medicines

Authors Vermaak I[1,2], Mavimbela T[1], Tankeu S[1], Djokam M[1], Sandasi M[1,2], Chen W[1], Kamatou G[1], Viljoen A[1,2]

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DOI 10.1055/s-0039-3399690

Researchers are constantly exploring alternative methods to ensure the quality of raw plant materials and herbal products. Conventional quality control techniques may fall short on speed of analysis. Vibrational spectroscopy provides rapid results, uses no solvents and is non-destructive. The application of vibrational spectroscopy and hyperspectral imaging (HSI) in combination with chemometric data analysis as a quality control method was demonstrated using several examples: 1) species differentiation; 2) biomarker quantification and 3) percentage composition. Spectral data was acquired in the mid-infrared (4000-500 cm^{-1}) and near infrared (10 000-4000 cm^{-1}) wave regions and hyperspectral images were acquired in the shortwave infrared region (920-2514 nm). The spectral data obtained was processed using chemometric data analysis techniques.1) Differentiation between closely related species was demonstrated for mid-infrared (MIR) spectral data in the case of powdered *Agathosma betulina* vs. *Agathosma crenulata* leaves as well as for whole fruits of *Illicium anisatum* vs. *Illicium verum* using hyperspectral imaging; 2) Calibration models (R2≥0.75) based on spectral data were developed for seven major compounds identified in *Melaleuca alternifolia* essential oil; The biomarker Sutherlandioside B (SU1) was quantified in external samples of powdered *Sutherlandia frutescens* leaves using the calibration model (R2>0.95) developed; 3) The hyperspectral imaging model developed to determine percentage tea blend composition (*Aspalathus linearis*/*Agathosma betulina*) in intact teabags had an R2X_cum of 0.767 and Q2_cum of 0.932 showing good prediction ability. The results showed that these techniques have great potential to be implemented as non-destructive quality control methods depending on the application and the desired accuracy.

Short Lectures C: Biological and Pharmacological Activities of Natural Products

SL C-01 Root extracts from *Ononis spinosa* exert anti-inflammatory activity *in vitro* on IL-8 and TNF-α release by inhibition of TLR-4 receptor

Authors Spiegler V[1], Michalak B[2], Addotey J[3], Saenger T[1], Jose J[1], Kiss AK[2], Hensel A[1]

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DOI 10.1055/s-0039-3399691

Extracts from the roots of *Ononis spinosa* L. are traditionally used for urinary tract infections and rheumatic conditions. A reduction of oedema in the carrageenan-induced rat paw oedema test suggested an anti-inflammatory activity *in vivo* [1]. Recent investigations indicated the isoflavone sativanone to be a potent inhibitor of human hyaluronidase-1, an enzyme strongly related to induction of inflammatory cellular response [2]. During further investigations, a dichloromethane extract fully characterized by LC-MS studies showed concentration-dependent inhibition of IL-8 and TNF-α release from LPS prestimulated human neutrophils. This inhibitory activity was shown to be mainly due to the presence of the norneolignan clitorienolactone B and the triterpene α-onocerin. In addition, extract as well as clitorienolactone B and α-onocerine significantly decreased the expression of adhesion molecules CD11b/CD18 and conversely increased the expression of CD62L in LPS-stimulated human neutrophils, a finding that is in line with reduced inflammatory response by inhibition of adhesion and migration of immune cells.

As all of the observed effects are potentially mediated via the Toll-like receptor 4 (TLR4) signaling pathway, TLR4 transfected HEK293 cells and non-transfected HEK293 cells as control were incubated with the dichloromethane extract. LPS-induced IL-8 secretion was significantly inhibited in a concentration-dependent manner, confirming TLR-4 antagonism. An aqueous extract from the roots of *O. spinosa* also showed antagonistic effects.

This study rationalizes the traditional use of extracts from *O. spinosa* for therapy of urinary tract infections and rheumatic conditions, due to its potential anti-inflammatory effects that are in part mediated *via* TLR-4 receptor antagonism.

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SL C-02 Sesquiterpene lactones from *Siegesbeckia orientalis* inhibit pro-inflammatory functions of human neutrophils

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DOI 10.1055/s-0039-3399692

Siegesbeckia orientalis (Asteraceae), more commonly referred to as hy thiêm in traditional Vietnamese medicine (TVM), is used in Vietnam to treat arthritis, rheumatism, and gout [1]. In an initial screening of extracts from plants used in traditional Vietnamese medicine, the dichloromethane (DCM) extract from the aerial parts of *Siegesbeckia orientalis* showed distinct inhibitory effects on the release of interleukin (IL)-8 in human neutrophils.

The phytochemical investigation of the bioactive *Siegesbeckia orientalis* DCM extract led to the isolation of nine compounds. One diterpene, 17(13→14)-abeo-*ent*-3*S**,13*S**,16-trihydroxystrob-8(15)-ene, was identified as a new natural product.

The isolated compounds were tested *in vitro* to evaluate their effects on the pro-inflammatory functions of stimulated human neutrophils: IL-8, IL-1β, tumor necrosis factor (TNF-α), and monocyte chemoattractant protein 1 (MCP-1) release, reactive oxygen species (ROS) generation, and expression of adhesion molecules (CD11a, CD11b, and CD62L).

Four sesquiterpene lactones inhibited IL-8 production with IC_{50} values between 0.7 and 6.3 µM, and TNF-α production with IC_{50} values between 18.2 and 31.9 µM, respectively. Furthermore, they significantly inhibited IL-1β, ROS, and MCP-1 production and decreased the expression of the adhesion molecules CD11a, CD11b, and CD62L.

In conclusion, the bioactivity of the DCM extract could be traced back to the presence of sesquiterpene lactones, which seem to interact with several potential therapeutic pathways including the NF-κB pathway, a key regulator of inflammation in rheumatoid arthritis [2]. Therefore, these findings support the traditional use of *Siegesbeckia orientalis* in the treatment of joint pain and rheumatism.

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[2] Baldwin AS The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol 1996; 14: 649-683

SL C-03 Nutritional spices from Cameroon inhibit inflammatory markers from human gastric epithelial cells

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Several Cameroonian herbs/spices are traditionally used to remedy certain diseases. In this context different studies have demonstrated health benefits of Cameroonian spices, including anti-microbial and antioxidant properties, whereas their anti-inflammatory activity at gastric level has never previously been reported.

The present work chemically characterizes and investigates the anti-inflammatory effect of hydro-alcoholic extracts of eleven Cameroonian spices at the gastric level, focusing on Nuclear Factor (NF)-κB pathway.

Hydro-ethanolic extracts were prepared and characterized by HPLC-DAD and GC/MS analysis, then screened for their ability to inhibit tumor necrosis factor (TNF)α-induced IL-8 and IL-6 release, in human gastric epithelial cells (GES-1 and AGS). The activity of the extracts on NF-κB driven transcription was also evaluated.

After preliminary screening, six spices (*Xylopia aethiopica, Xylopia parviflora, Tetrapleura tetraptera, Dichrostachys glomerata Aframomum melegueta* and *Aframomum citratum*) were chosen among eleven plants for in-depth studies. The selected extracts reduced in a concentration-dependent fashion both the cytokines release (IC_{50} between 0.19 µg/mL and 20 µg/mL) and NF-κB driven transcription (IC_{50} between 0.33 µg/mL and 20 µg/mL). Chemical analysis suggested that their secondary metabolites (androstenone, chlorogenic acid, pimaric acid, catechin, caffeic acid and its derivatives, 4′,5,7-trihydroxyflavanone, gingerol, shogaol, paradol and gallotannins) could potentially justify the biological properties observed.

Results obtained from this study showed that six extracts reduced inflammatory markers by impairing NF-κB signalling at the gastric level, justifying, at least in part, the Cameroonian traditional use of these spices. However, other molecular mechanisms cannot be excluded, and further studies are needed to better clarify their biological activities.

SL C-04 Plant-endophyte communication: maytansine as an example

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DOI 10.1055/s-0039-3399694

Background: Studies on microbe-host interactions in plant and animal systems aimed at understanding the role of these associations and their utility in pharmaceutical and agricultural sectors are gaining impetus.[1] Several recent studies have lent evidence to the fact that certain so-called "plant metabolites" are actually biosynthesized by associated endophytic microorganisms [1].

Aims: We wanted to elucidate the biosynthesis of the important anticancer and maytansine in Celastraceae plants in order to elucidate its actual producer(s), which has been an open question since its discovery in the 1970s.

Results: We showed that maytansine is actually a biosynthetic product of root-associated endophytic bacterial community in *Putterlickia verrucosa* and *Putterlickia retrospinosa* plants [2]. This extremely interesting outcome provided the scientific basis to investigate the actual producer (s) responsible for maytansine biosynthesis in *Maytenus* plants. Endophytic communities harboring different tissues of *Maytenus serrata* originating from Cameroon were investigated using a combination of bioanalytical tools such as HPLC-$HRMS^n$ and MALDI-MSI, and targeted genome mining techniques to elucidate the source and sites of maytansine biosynthesis. We proved that the biosynthesis of maytansine in *M. serrata* is shared between the endophytic bacterial community colonizing the stem and the host plant containing non-culturable cryptic endophytes [3].

Conclusion: Our work demonstrates that maytansine is biosynthesized in *M. serrata* only when the host plant joins forces with its selected and very eco-specific endophytic bacterial community.

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Short Lectures D: Metabolomics and Molecular Networking

SL D-01 Development of an innovative molecular networking-based approach for the discovery and targeted isolation of new bioactive metabolites from higher plants

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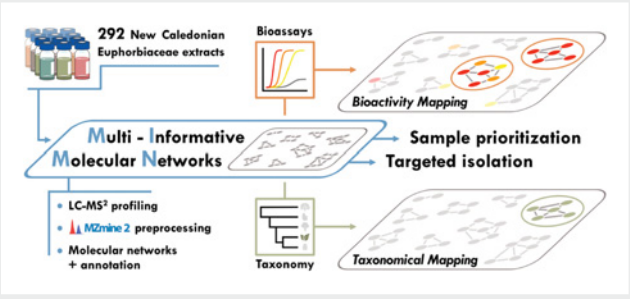
DOI 10.1055/s-0039-3399695

Natural products represent an inexhaustible source of new therapeutic agents. Their complex and constrained three-dimensional structures endow these molecules with exceptional biological properties, thereby giving them a major role in drug discovery programs. However, the pharmaceutical industry's lack of interest in studying natural resources has been apparent since the early 2000s. Two main reasons for this disaffection can be put forward. The methods providing information on the bioactivity potential of natural products before their isolation are still lacking, but they are of key interest to target the isolation of valuable natural products only. On the other hand, the procedures for isolating and characterizing bioactive secondary metabolites from complex mixtures are often long, costly and tedious.
The steps necessary to prioritize extracts and to isolate compounds of interest in a targeted, rapid and effective manner are therefore essential. To address these issues, we have recently developed a molecular networking-based strategy (▶**Fig. 1**) consisting in deciphering the relationship between spectral networks and biological activities and further exploiting it to prioritize the isolation of bioactive secondary metabolites [1–3]. The core concept of this approach is based on the cross-linking of various information layers within a massive molecular network to spot bioactive scaffolds. Assuming that spectral dissimilarity within a taxonomically homogeneous set of samples could imply chemical uniqueness, the generation of these multi-informative maps unifying structural data, taxonomical information, and bioassay results allows bioactivities to be associated with taxon-specific scaffolds.



▶**Fig. 1** Molecular Networking-based approach for sample prioritisation and targeted isolation of new bioactive metabolites from Euphorbiaceae extracts.

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[3] Olivon F, et al. ACS Chem Bio 2017; 12: 2644–2651

SL D-02 Molecular networks and CPC fractionation for rapid screening of bioactive natural molecules

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DOI 10.1055/s-0039-3399696

Natural products of plant origin have emerged as major source of bioactive compounds for the cosmetic and pharmaceutical industries. However, determination of active principle *i.e.* identifying, isolating and testing each molecule is complex, long and tedious taking into account molecular diversity of the extracts.
The objective of this study is to associate i) the dereplication information obtained by means of molecular networks and ii) a bioguided fractionation by Centrifugal Partition Chromatography (CPC). This combination allows developing a simple and fast approach to obtain and test simplified fractions, even pure molecules, thus facilitating screening and identification of the bioactive principles.
Firstly, the aerial parts of *Artemisia annua* and *Artemisia vulgaris* were extracted and their antioxidant activity was evaluated by DPPH, ABTS, CUPRAC and FRAP assays. Extracts with positive response were subjected to UHPLC-HRMS with autoMS/MS experiments in order to build molecular networks using GNPS platform. Molecular networks linked compounds according to spectra similarities (same ions and/or neutral losses) then grouping the numerous analytes by molecular families. Secondly, crude extracts were fractionated using CPC with an adapted Arizona solvent system according to molecular families (*e.g.* sesquiterpernes; phenolic derivatives). The numerous fractions were evaluated for antioxidant activity. Active fractions of the two plants were analyzed by FIA-HRMS in order to identify the isolated molecules, which can be located on the molecular network.
Therefore, this approach has rapidly revealed the few major phenolic compounds that are responsible for the antioxidant activity of the extracts: *Artemisia annua* (one compound) and *Artemisia vulgaris* (four compounds).

SL D-03 Constitution of a Lichen Metabolite Data Base (LDB) through HRLC-MS/MS analysis of 250 lichen compounds

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Since W. Zopf' pioneering works on lichen metabolite identification, physicochemical data of lichen compounds were accumulated during the twentieth century, culminating in the publication of S. Huneck & I. Yoshimura's Identification of lichen substances in 1996 [1]. This compendium summarized analytical data for lichen molecules (TLC, NMR, LC, MS/MS and microcrystallization). Nowadays, over thousand lichen metabolites have been described [2] among which one hundred are highly frequently produced in

large amounts by a variety of lichens species. While TLC is widely used in lichenology, mainly for chemotaxonomic purpose [3], the field remains underexplored in regards to the standards set in the era of metabolomics. Highly informative approaches through high resolution LC-MS and NMR are commonplace but their efficiency as a quick dereplication method for complex mixtures is highly reliant on databases. Consequently, with the aim to provide to the lichen chemist community a modern dereplication methodology using HRLC-MS/MS analysis, a database constituting of MS/MS spectra for lichen metabolites is under construction. A significant number of lichen molecules was collected, thanks to 300 pure compounds graciously donated by the Berlin Botanical Museum, completed by some molecules from the chemical library of University of Rennes. All molecules were then analysed by HRLC-MS/MS selecting the most appropriate ionization mode. In total 250 lichen compounds representing 8 main structural classes have been recorded. Data were converted to mzXML format using the ProteoWizard Toolkit [4] and manually curated to be treated using MZmine [5]. The resulting spectra are ready to be uploaded online and to serve as a public database for the community.

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SL D-04 Phytochemistry, quality control aspects and metabolomic approaches for the systematic investigation of *Pistacia lentiscus* L. var resin

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DOI 10.1055/s-0039-3399698

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, 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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Regulatory Affairs Workshop

ISL RA-01 DNA-testing: from research to regulation

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DOI 10.1055/s-0039-3399699

The use of DNA-testing for the herbal industry has been a hotly debated issue for several years [1]. During this time the methodologies have advanced, different approaches have been advocated and strengths and weaknesses highlighted. In 2016 the British Pharmacopoeia became the first to publish a DNA-based method for the identification of herbal drugs. This was intentionally placed in a non-mandatory section of the publication and focused on the provision of a 'reference sequence' for each herbal drug. The Chinese Pharmacopoeia has published ITS2 sequences for herbal drugs, and species-specific assays are being investigated by the US Pharmacopoeia. Examples of these techniques and their properties are described.

Harmonisation between the different global stakeholders would be a distinct advantage to the industry and facilitate compliance across the board; it is imperative that the methods proposed are transparent and accessible.

Through examples, the various issues that arise in the complex and varied supply chains for medicinal plants are described, arriving at the conclusion that DNA-based methods are a powerful piece of the puzzle for Quality Assurance but that this must be seen as part of a larger picture, along with phytochemical and other methods.

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ISL RA - 02 Hyphenated methods – potentials and limitations

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DOI 10.1055/s-0039-3399700

Herbal medicine exhibits specific challenges because of its complex multi compound composition and its inherent variance. Due to the progress of modern analytical technologies, more and more in-depth knowledge about the chemical composition of the plant material, the extract and its final dosage form can be obtained. This opens up fascinating opportunities from a scientific perspective but also from an industry point of view. At the same time questions arises how those modern technologies can potentially affect the regulation of future quality control. This topic has to be discussed in a balanced way, the aim should be to use those powerful tools for a better quality control, but with the condition that the technical and regulatory setting still should be realistic and practicable.

In this talk, the term "hyphenated methods" is at one hand used in a classic sense of connecting chromatographic techniques with detectors (LC-UV, LC-MS, GC-MS) and at the other hand in the sense of analyzing the complex data obtained from analytical techniques (MS, NMR, NIR, ...) with advanced multivariate methods.

ISL RA - 03 DNA-based authentication: incorrect use and exaggerated expectations damage a valuable approach

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DOI 10.1055/s-0039-3399701

Analysis of DNA with molecular methods is omnipresent in our everyday life. One of the many different applications is the identification of biotic materials useful for proofing presence (authenticity) or absence of certain organisms. The importance of molecular biology has led to the development of a plethora of methods and a wealth of information in public databases. However, application of sophisticated technology in a new field can take wrong turns, if expectations are wrong or too high, methods are incorrectly applied or results not critically interpreted.

Authentication of medicinal plants is a challenging task. DNA-based authentication is a wonderful supplementation and is already widely applied in risk management of critical medicinal plants. However, practical application in our sector during the last decade has uncovered vulnerabilities that are often not linked to the methods but rather to the way of their application.

DNA-based authentication needs expert knowledge: with advanced equipment and bioinformatics, many procedures of species identification can be automated to a high degree, which often misleads to an uncritical view on results. DNA-based authentication needs expertise in both, molecular methods and pharmacognosy.

Expectations: qualitative identification needs to be strictly distinguished from quantitative analysis. Although reliable quantitative molecular methods are available, and qualitative methods are run on quantitative equipment, standard barcode markers cannot be used for quantification at all.

Considering the peculiarities of medicinal plants raw materials, intermediates and products, DNA-based authentication is a useful approach but needs careful adaptation and harmonization. However, harmonization may not be so easy if freedom of action using molecular options should not be narrowed.

Short Lectures Tuesday, September 03, 2019

Short Lectures E: Applied NMR Session

SL E-01 Computer-assisted fully automatic structure revision of organic natural products based on their C13-NMR data using the CSEARCH-protocol

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DOI 10.1055/s-0039-3399702

Structure elucidation of organic natural products is a complex task mainly performed using spectroscopic methods. Among the techniques applied, NMR-spectroscopy plays a central role giving a detailed insight into constitution, configuration and conformation of the unknown compound. Despite the tremendous progress made in tailoring sophisticated pulse techniques to solve structural problems, many natural products have been published showing a wrong structure proposal leading to incorrect reference material for subsequent conclusions with the effect of making this erroneous knowledge base statistically more significant. A large number of comprehensive review articles [1,2] describes the actual situation in detail. From this analysis the necessity of massive software application during every step of the process of structure elucidation can be derived. The automatic peer-reviewing of some 700,000 C13-NMR spectra mainly taken from the public domain literature has revealed a significant number of wrong structure proposals and/or wrong signal assignments [3]. Subsequent application of a structure generator program based on the carbon-NMR data has created reasonable alternatives – in many cases in full agreement with an already known structure revision. Examples will be given showing the immediate need to change the workflow of structure elucidation along the line from spectrum interpretation to peer-reviewing of manuscripts [4].

References [1] McAlpine JB, Chen SN, Kutateladze A et al. The value of universally available raw NMR data for transparency, reproducibility, and integrity in natural product research. Nat Prod Rep 2019; 36: 35–107

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SL E-02 Eliciting Nature's Activities (ELINA): a biochemometric approach to unravel complex bioactive mixtures

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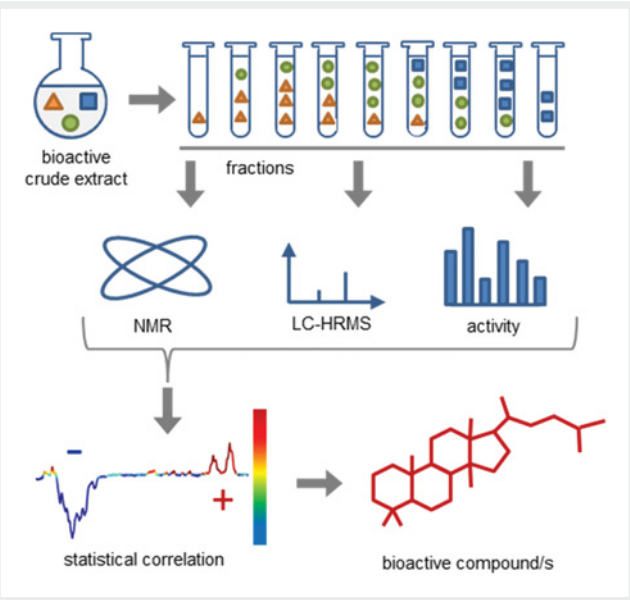
DOI 10.1055/s-0039-3399703

Although bio-guided isolation is a well-established method for the discovery of bioactive compounds from natural sources, it has many drawbacks, e.g. it is tedious, time-consuming, and not always successful. To improve this, biochemometric approaches have emerged in recent years. However, hit discovery from a complex extract containing close structural analogues remains challenging.

Hence, the aim of this work was to unravel a complex bioactive mixture of the same compound class. This was achieved by a ^{1}H NMR-MS workflow which we named ELINA (Eliciting Nature's Activities) [1]. ELINA detects chemical features that are positively ('hot') or negatively ('cold') correlated with bioactivity prior to any isolation. The approach is exemplified in the discovery of steroid sulfatase (STS) [2] inhibiting lanostane triterpenes (LTTs) from the polypore fungus *Fomitopsis pinicola* Karst.

To reduce the complexity of the extract, a single fractionation step was performed to give 32 fractions. This was done in a way to achieve varying concentrations of a constituent over several fractions. Aliquots of all fractions were forwarded to ^{1}H NMR, LC-HRESIMS, and bioactivity testing. Using statistical heterocovariance analysis (HetCA) [3], ^{1}H NMR spectra were correlated with bioactivity data and complemented with MS data.

The effectiveness of this approach was demonstrated by disclosing chemical features crucial for STS inhibition, thus taking advantage from the innate compound library produced by the polypore's biosynthetic machinery. As a proof of concept piptolinic acid D and pinicolic acid B equipped with these imperative features were isolated and showed IC_{50}s of 10.5 µM and 12.4 µM, respectively [1].



▶ **Fig. 1**

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SL E-03 Quantification of diterpene acids in copaiba oleoresin by UHPLC-ELSD and heteronuclear two-dimensional qNMR

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DOI 10.1055/s-0039-3399704

The oleoresin of selected *Copaifera* species, commonly known as copaiba oil, is a traditional Brazilian remedy for the treatment of various ailments, such as urinary tract infections, respiratory diseases, wound healing, rheumatism, herpes and tumors [1,2]. Due to its wide application in folk medicine, the oleoresin has been subject of various studies examining its chemical composition and the correlation with its health benefits.

As both, the diterpene acids and the biological activities vary between the different *Copaifera* species [2], the aim of the present study was the development of analytical methods for the quantification of diterpene acids.

The first method is an UHPLC assay using reversed-phase material and evaporative light scattering detection. With this method precise (RSD values below 4%), accurate (mean recovery rates of 91-105%) and sensitive quantification (LOQs of 10 and 20 µg/mL) was achieved. For the second assay, quantitative heteronuclear single quantum correlation spectroscopy (qHSQC) was applied. Here, calibration curves of eight different NMR cross-peaks were established and normalized with dimethyl terephthalate, which served as internal standard. This approach allowed the direct quantification of four major and one minor diterpene, whereas he contents of the remaining minor compounds were obtained with simple calculation procedures.

Comparison with the UHPLC assay showed good agreement for seven of out of eight diterpenoids. In terms of precision, the qHSQC method was advantageous for the quantification of the three major compounds, whereas UHPLC was superior in the determination of the minor components.

References [1] Veiga Junior VF, Pinto AC. O genero *Copaifera* L. Quim Nova 2002; 25: 273–286.
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Short Lectures F: Biological and Pharmacological Activities of Natural Products (Mixed)

SL F-01 *Rhodiola rosea* improves lifespan, locomotion, and neurodegeneration in a *Drosophila melanogaster* model of Huntington's disease

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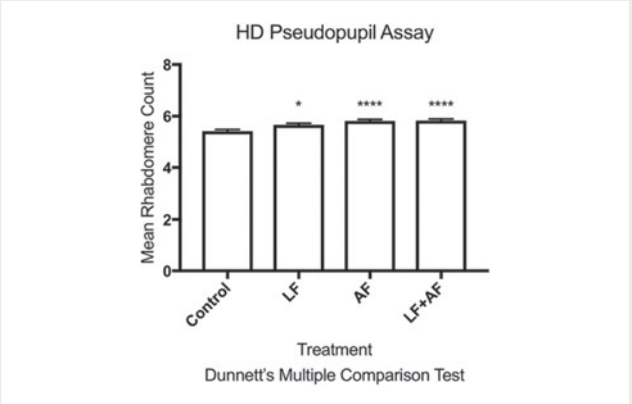
DOI 10.1055/s-0039-3399705

Huntington's disease (HD) is a dominant, late-onset disease characterized by choreiform movements, cognitive decline, and personality disturbance. It is caused by a polyglutamine repeat expansion in the Huntington's disease gene encoding for the Huntingtin protein (Htt) which functions as a scaffold for selective macroautophagy. Mutant Htt (mHtt) disrupts vesicle trafficking and prevents autophagosome fusion with lysosomes, thus deregulating autophagy in neuronal cells, leading to cell death. Autophagy has been described as a therapeutic target for HD, owing to the key role Htt plays in the cellular process. *Rhodiola rosea*, a plant extract used in traditional medicine in Europe and Asia, has been shown to attenuate aging in the fly and other model species. It has also been shown to inhibit the mTOR pathway and induce autophagy in bladder cancer cell lines. We hypothesized that *R. rosea*, by inducing autophagy, may improve the phenotype of a Huntington's disease model of the fly. Flies expressing HttQ93 which exhibit decreased lifespan, impaired locomotion, and



▶ **Fig. 1** The effect of R. rosea on the neurodegeneration of HD fly rhabdomeres. HD flies fed R. rosea exhibited significant rhabdomere count increases compared to control. Data analysis was performed using an analysis of variance (ANOVA) *p < 0.05, ****p < 0.0001, n =.

increased neurodegeneration were supplemented with *R. rosea* extract, and assays testing lifespan, locomotion, and pseudopupil degeneration provided quantitative measures of improvement. Based on our observations that the extract improves lifespan, locomotion, and neurodegeneration, *R. rosea* may be further evaluated as a potential therapy for Huntington's disease.

SL F-02 Regulations of ASIC 4, 5-HTR and SERT in the esophageal mucosa under STW5 treatment support a corporate role in pain sensing

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Background: Pain is a prominent symptom of gastroesophageal reflux disease (GERD) and commonly related to acidic reflux. 5-ydroxytryptamin (5-HT), a neurotransmitter and ligand for 5-HT receptors (5-HTR) is an activator of acid sensing ion channels (ASICs).We reported earlier [1] that gene expressions of ASIC4 and 5-HTR-subtypes were simultaneously regulated in the esophageal mucosa and hypothesized that both ASICs and 5-HTRs- play a role for the fast pain relief in responders to STW 5, a herbal multicomponent mixture, and the PPI Omeprazole (O).

Aims: Comparison of gene- and protein expressions of ASICs, 5-HTRs, 5-HT and serotonin transporters (SERT) in rat model GERD [2] by Western Blot (WB); detection of ASIC4 and 5-HT in human esophageal biopsies [endoscopically normal (n = 18), inflamed (n = 16)] by immunohistochemistry (IH).

Results: issues of animals suffering from GERD showed small, but significant increases in the gene expression of ASIC-subtype 4 (3.8f), of HTR2A (3fold), HTR2B (6.6f), HTR7 (9.3f) ($p < 0.001$) and a downregulation of SERT (−2.4f, $p < 0.001$) compared to 'normal' tissue. In tissues of animals treated with either STW5 (2ml/kg) or with O (30mg/kg), ASIC 4 (−4,8f, −4f; $p < 0.0001$), HTR2A (−5.4f, −3.9f) HTR2B (−7.9f, 3.7f) and HTR7 were downregulated ($p < 0.0001$) respectively. Reductions of HTR7 by O (−6.8f) was less compared to STW5 (−15.4f) and did not match the upregulation during inflammation. In human esophageal biopsies ASIC 4 was detected in normal and inflamed tissue, 5-HT only in inflamed tissue.

Conclusion: IH and WB of ASIC4, HTR-subtypes and SERT supported gene expression data and thereby a corporate role in the esophageal nociception.

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SL F-03 Benzoxazinoid phytochemicals, abundant in rye bread, are taken up in humans. Analytical method for plasma metabolites is highly needed

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DOI 10.1055/s-0039-3399707

Benzoxanoids (BXs) were identified for the first time in young cereal plants in 1957 and in medicinal plants in 1969. In 2008 our lab discovered that BXs are present in mature cereal grains and in food/feed based on cereal whole grains [1]. We showed that BXs are taken up in pigs, rats and humans [2–4], and that they appear in prostate tissue of humans consuming rye bread [5]. The aim of this study was to develop a method for identification and quantification of human phase 1 and phase 2 metabolites in plasma. The method included an initial protein precipitation step of the plasma with the purpose of reducing the matrix interference. The chromatographic analysis of BXs and their metabolites in the remaining extract was performed in LC-MS/MS. Pure standards were unavailable for most of the phase 2 metabolites. Phase 2 metabolites are present in much higher amounts in urine than in plasma. Thus urine metabolites of BXs for which structures could be assigned using data dependent acquisition were isolated and used for verification of MS/MS signals in plasma extracts. The isolation of approx 10 µg of each compound was done with consecutive HLB and mixed mode SPE's (Oasis, Waters) exploiting the variety of polarity and acidity of the metabolites. Glucuronide and sulphate metabolites of paracetamol were used as standards for semi-quantification due to their structural similarity and commercial availability.

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Short Lectures G: Biological and Pharmacological Activities of Natural Products

SL G-01 Chemical profiles and pharmacological properties of two Anthemis species: *Anthemis tinctoria* var. *pallida* and *A. cretica* subsp. *tenuiloba*

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DOI 10.1055/s-0039-3399708

Members of Anthemis genus are extensively used in the Turkish folk medicine to treat various ailments. In this present study, the ethyl acetate, methanolic and aqueous extracts of aerial the parts of Anthemis tinctoria var. pallida (ATP) and A. cretica subsp. tenuiloba (ACT) growing in Turkey were investigated for their antioxidant and key enzyme inhibitory potentials. Total phenolic and flavonoid contents were determined using colorimetric methods.

The antioxidant capacities of the studied extracts were evaluated using different assays including free radical scavenging, reducing power, phosphomolybdenum, and metal chelating. Additionally, we evaluated the putative protective effects of Anthemis extracts on 'Cortical Spreading Depression'

(CSD) paradigm, on rat cortex specimens treated with an excitotoxicity stimulus. To this regard, we assayed extract capability in blunting CSD-induced cortex 5-HT decrease. All extracts showed remarkable antioxidant activities, with the MeOH extracts being superior to the others (DPPH: 407.07 ± 8.88 and ABTS: 320.11 ± 5.67 mg TE/g). Enzyme inhibition was tested on AChE, BChE, α-amylase, α-glucosidase, and tyrosinase. Only the EtOAc and MeOH extracts were potent against AChE and BChE. The extracts showed remarkable enzyme inhibitory effects against tyrosinase and α-glucosidase, and modest activity against α-amylase. Finally, in agreement with the evaluation of antioxidant activity, Anthemis MeOH extracts revealed the most effective in restoring physiological 5-HT level, in cortex specimens subjected to an excitotoxic stimulus.

The results highlighted on the biological potential of the studied Anthemis species and warrant for further studies to explore their potential use in phytomedicines and cosmetics.

SL G-02 Potential anti-herpes and cytotoxic action of novel semisynthetic digitoxigenin-derivatives

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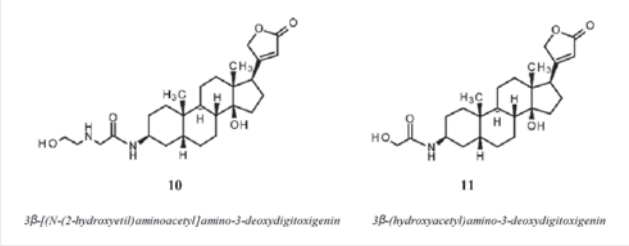
In recent years, new therapeutic possibilities, such as anticancer and antiviral activities [1, 2] were proposed for cardiac glycosides used to treat heart diseases. The present work aimed to synthesize the readily accessible 3β-azido-3-deoxydigitoxigenin from digitoxigenin. Two new series of compounds were obtained from 3β-azido-3-deoxydigitoxigenin: (i) O-glycosyl trizols through click chemistry with propargyl glycosides; and (ii) compounds substituted in the alpha carbonyl position with different residues linked via an amino-group. All obtained derivatives had their chemical structures confirmed, and their anti-herpes (against HSV-types 1 and 2 replication) and cytotoxic (against PC3, A549, HCT-8 and LNCaP cell lines) activities evaluated. Compounds *10* and *11* (▶**Fig. 1**) exhibited the most promising results against HSV-1 (KOS and 29-R strains) and HSV-2 (333 strain) replication with SI values > 1000. Both compounds were also the most cytotoxic for the human cancer cell lines tested with IC_{50} values similar to those of paclitaxel. They also presented reduced toxicity toward non-cancerous cell lines (MRC-5 and HGF cells). Promising compounds were tested in regard to their ability to inhibit Na^+/K^+-ATPase. The inhibition rate correlated suitably with the bioactivity demonstrated by both compounds against the different human cancer cells tested as well as against HSV replication. Moreover, the results showed that specific chemical features of compounds *10* and *11* influenced the bioactivities tested. In summary, it was possible to obtain novel digitoxigenin-derivatives with remarkable cytotoxic and anti-herpes activities as well as low toxicity and high selectivity. In this way, they could be considered potential molecules for the development of new drugs.



▶**Fig. 1** Chemical structure of 3β-[(N-(2-hydroxyetil)aminoacetyl] amino-3-deoxydigitoxigenin (compound 10) and 3β-(hydroxyacetyl)amino-3-deoxydigitoxigenin (compound 11).

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SL G-03 Standardized herbal extracts of Japanese Kampo medicine and their effects on human and murine pancreatic cancer cells

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DOI 10.1055/s-0039-3399710

Traditional Japanese Kampo prescriptions have anti-inflammatory and anti-cachexia effects. Epigenetic effects have also been described for herbal remedies. Here, we analyze the effect of standardized single and composite prescriptions of Japanese Kampo medicine in human and murine pancreatic cancer cells. PANC1, NKCII and KPCbl6 cells were cultivated in DMEM (+ 10% FCS, + 1% NEAA) until confluence. Standardized methanolic, ethanolic (25%) and traditional aqueous extracts of *Glycyrrhiza uralensis* root and *Scutellaria baicalensis* root were used. The concentration and time-dependent effect on cell viability was tested with MTT-at 24, 48, 72 and 96h. Protein expression of proliferation markers (CDK6, Cyclin D1, CyclinD3), transcription factors (NFATc1), tumor suppressor genes (p15, p21, p27, p53), and histone modifications were analyzed by Western Blot in whole protein lysates at 24 and 48h. Furthermore, gene/transcript expression of proliferation markers CDK6, Cyclin D1 and CyclinD3, and the tumor suppressor gene p21 were analyzed by qPCR. FACS analysis was used to study cell cycle arrest after treatment with *G. uralensis* root and *S. baicalensis* root. The different extracts of *G. uralensis* root and *S. baicalensis* root inhibited tumor growth in vitro. This effect was concentration dependent in all three tested cell lines. Cyclines and cell-cycle inhibitors were regulated accordingly. These results could be verified on both protein and transcript level. FACS analysis showed a G1 arrest. *G. uralensis* also induced histone H3K27 acetylation, indicating that specific genomic areas, e.g. enhancer regions, were activated upon treatment. The presented experimental data demonstrate that Kampo extracts have anti-proliferative effects in vitro.

Thieme

Short Lectures H: Biological and Pharmacological Activities of Natural Products

SL H-01 Innovative strategies for chemo-diversification of natural products and discovery of potential new leads

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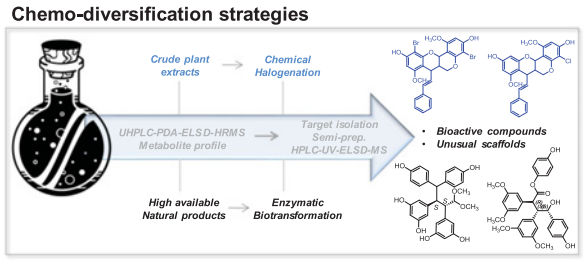
DOI 10.1055/s-0039-3399711

Chemical engineering of crude plant extracts and biotransformation of natural products (NPs) using enzymes could be an alternative way for obtaining bioactive compounds. The concept of these approaches is to start from extracts and abundant NPs to obtain active compounds using chemical and biological reactions. Given that 20% of marketed drugs contain halogen atoms, a methodology has been developed to allow controlled halogenation of compounds directly in plant extracts [1]. In addition, biotransformation chemical reactions of single NPs using enzymes secreted by a phytopathogenic fungus have been successfully used to obtain a wide variety of compounds [2]. A metabolite profiling based in the UHPLC-PDA-ELSD-HRMS analysis were used to monitor these different reactions and highlight the presence of the new generated compounds. In the majority of cases, it was possible to improve the chemical diversity of the original NPs leading to the acquisition of active compounds from inactive scaffolds. In order to isolate, characterize and study the biological activities of the generated compounds, the use of high-resolution preparative chromatographic methods was mandatory. At this level, significant improvements for the efficient targeted isolation of given NPs through dry load injection and chromatographic gradient transfer methods have been made [3]. The applications, possibilities and limitations of these latest technologies will be illustrated with recent investigations performed in our laboratory.



▶ **Fig. 1**

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SL H-02 Mushrooms – an unrevealed source for promising photopharmaceuticals

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A vast array of different pigments causes the splendid colors of mushrooms (Basidiomycetes). While plenty of these colorants are chemically elucidated, their pharmaceutical potential is only partly studied. Based on the structural similarity to well-known photosensitizers (e.g. bisanthrones and anthraquinones) we hypothesized that fruiting bodies of the subgenus dermocyboid Cortinarii are a promising source for new photosensitizers, which can be utilized as photopharmaceuticals.

To test this hypothesis, extracts of dried fruiting bodies of several European dermocyboid Cortinarii were prepared and submitted to a photo-activity screening workflow [1]. In detail, the chemical profile of light-absorbing metabolites was analyzed, the ability to produce singlet oxygen was tested, and the photocytotoxicity was evaluated.

Based on the photo-activity workflow, the most prominent dermocyboid species was selected and several photosensitizers isolated by applying a bioactivity-guided workflow. Chemical analysis disclosed that the most prominent photosensitizer is a biphyscion. While it is non-toxic in the dark, it showed e. g. an EC_{50} of 0.7 µM under blue light irradiation (468 nm, 9.3 J/cm^2) against cells of a lung cancer cell-line (A549).

In sum, starting with a hypothesis based on the structural similarity between well-established photosensitizers and the coloring principles of fruiting bodies we were able to assign a new pharmaceutical activity to selected fungal pigments.

The FWF (Austrian Science Fund project P 31,915, BS), the TWF (Tyrolean Science Fund), and the University of Innsbruck (Nachwuchsförderung,BS) are acknowledged for the financial support.

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SL H-03 Xanthones from the mangosteen fruit (*Garcinia mangostana*) disrupt androgen receptor functionality in prostate cancer

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DOI 10.1055/s-0039-3399713

The purple mangosteen fruit (*Garcinia mangostana*), native to Southeast Asia, has long been used in traditional medicine, however recent data has shown that xanthones, a class of chemical compounds isolated from the mangosteen, display anti-cancer activity [1]. α-Mangostin is the most abundant xanthone with more than 80 that have been isolated and identified from the fruit, roots, bark, and leaves of the mangosteen. Androgen receptor (AR) degradation is a novel strategy that would help

to overcome the rising drug resistance to FDA approved anti-androgens that are used to treat prostate cancer [2]. Our data suggests that α-mangostin interacts with the androgen receptor, and may disrupt AR functionality and degrade AR. Two different prostate cancer cell lines (22Rv1 and LNCaP) were used to evaluate efficacy and perform mechanism of action studies. Xanthone treatments promote a dose and time dependent decrease in AR, along with an additional increase in chaperone proteins involved in degradation. Additionally, there is a reduction in the phosphorylation profile of AR, suggesting these actions inhibit the nuclear translocation of AR. Consequently, these actions inhibit the transcription of downstream genes that are necessary for cell growth and proliferation. Our results suggest that α-mangostin promotes AR degradation by inhibiting nuclear translocation and may be effective against drug resistant prostate cancer cases.

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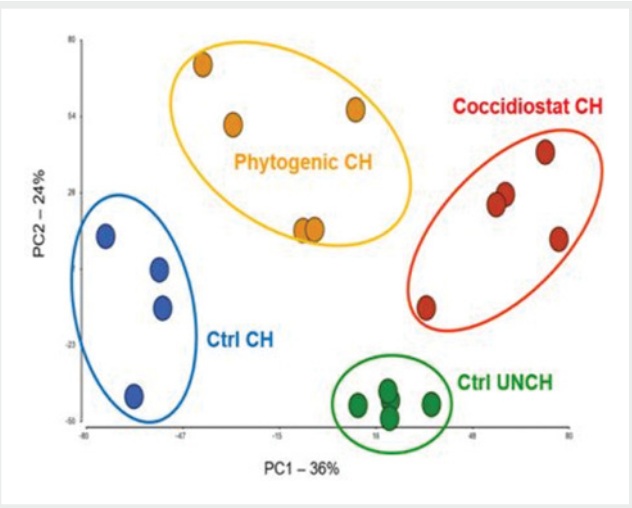

▶**Fig. 1.** Principal component analysis of cecal gene expression (RNA-Seq).

Short Lectures I: Veterinary Medicine

SL I-01 Regulatory effects on inflammatory signaling by a phytogenic feed additive assessed in vitro and in vivo including RNA-sequencing

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DOI 10.1055/s-0039-3399714

The worldwide trend towards a more prudent use of antibiotics has sparked the demand for alternative products to mimic the beneficial effects of feed additives. Restriction of the innate inflammatory response of animals might be one of the key mechanisms responsible for the observed growth promotion. Phytogenic feed additives harness the chemical richness offered by plants, which many are known to possess anti-inflammatory properties.

In the present study a phytogenic feed additive has been assessed for potential inflammation-restricting properties both in vitro and in vivo, the latter including novel RNA sequencing technology. First evidence for potential anti-inflammatory properties was obtained from TNF-α-stimulated IPEC-J2. Subsequently, a feeding trial with broiler chickens was conducted with an inflammatory challenge (coccidiosis vaccine overdosing) in order to compare effects on cecal gene expression when feeding the phytogenic product or a anticoccidial drug (positive control).

The inflammatory challenge significantly affected the expression of more than 500 genes compared to non-challenged birds. ost of these genes belonged to innate immune system or cytokine signaling pathways. This effect was almost completely reversed by feeding an anticoccidial additive, while in the phytogenic group approximately half of the genes were regulated back to the normal level of the unchallenged control group.

The findings corroborate the relevance of limiting inflammatory processes by phytogenic feed additives. Next-generation sequencing opens opportunities for studies on mechanisms of action of feed additives and may assist in further development.

Funding by the Austrian Research Promotion Agency (FFG) is gratefully acknowledged.

SL I-02 Evaluation of the traditional use of some Yemeni plants for the treatment of some livestock diseases

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DOI 10.1055/s-0039-3399715

Livestock is an integral component of agriculture production in Yemen. The aim of our work resides in the scientific substantiation of the ethnoveterinary use of some plants [1] based on the evaluation of their bioactivities and toxicological properties. Searching the scientific literature has revealed various pharmacological activities that may support the claimed healing activities of eleven (▶**Tab. 1**) out of fourteen remedies for some of their ethnoveterinary utilization. Moreover, three remedies were found to demonstrate toxic effects in experimental studies (▶**Tab. 1**). It can be concluded that our work has provided valuable scientific information on some Yemeni plants that could be utilized for the benefit of farmers to rational the use of plants.

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Table 1: The ethnoveterinary uses supported by scientific studies, and the toxicological properties of some plants used in ethnoveterinary medicine in Yemen.

Species/ Family	Part used	Indication	Toxic effects
Socotran Aloe species/ Asphodelaceae	Fresh latex	Constipation, colic and worms (internal use). Boils, suppurating abscess, large fresh lacerations and infected bites of ticks, lice or flies (external use).	Genotoxicity, mutagenicity and carcinogenicity of some constituents of Aloe such as aloe-emodin [3].
Boswellia sacra Flueck / Burseraceae	Underbark	Infected wounds (external use).	
	Oleo-gum resin	Mastitis (external use).	
Cissus rotundifolia Vahl/ Vitaceae	Unspecified part	Stomach pain (internal use).	
Cyphostemma digitatum (Forssk)Desc. / Vitaceae	Leaves	Appetite stimulation (internal use).	
Psiadia punctulate (DC.) Vatke / Asteraceae	Unspecified part	Bone fracture (External use).	
Pulicaria undulata (L.) C.A. Mey / Asteraceae	Unspecified part	Insect repellent.	
Rumex nervosus Vahl / Polygonaceae	Unspecified part	Pimples in the eye, mixed with salt.	
Aristolochia bracteolate Lam / Aristolochiaceae	Unspecified part	Bloating by giving *A. bracteolate* with *S. bicolor* grains (internal use).	Mutagenicity, carcinogenicity and nephropathy due to aristolochic acid I and II [4].
Sorghum bicolor (Poaceae)	Grains		
Trigonella foenum-graecum L. (Fabaceae)	Seeds	Constipation by giving the fenugreek seeds together with barley grains (Internal use).	Controversial results on the toxicity of fenugreek seeds such as antifertility, abortifacient, abnormal foetal development, and other cellular and molecular alterations [5].
Hordeum vulgare (Poaceae)	Grains		

▶Tab. 1

SL I-03 The effect of mixture of carvacrol, cinnamaldehyde and capsicum oleoresin on metabolic parameters in broiler chickens

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DOI 10.1055/s-0039-3399716

Among dietary phytonutrients, carvacrol, cinnamaldehyde, and Capsicum oleoresin are well known for growth promotion and gene expression influence particularly associated with lipid metabolism. Mostly of changes in gene expression were seen in the Capsicum-fed broilers with 98 upregulated and 156 downregulated genes [1]. Phytochemicals are potential feed additives possessing multiple functions, including immunomodulatory effect [2].

The effect of a mixture of carvacrol, cinnamaldehyde, and Capsicum oleoresin (Xtract®; XT, Pancosma S.A., Geneva, Switzerland) was tested on 96 broiler chicken hybrid Ross 308, during 42 days, divided in two groups. Chicken were fed with corn-soybean meal diet and offer as a starter (22% CP, 12.34 MJ ME; until 15 day), finisher 1 (20% CP and 12.76 MJ ME, until 24 day) and finisher 2 (17.7% CP and 12.63 MJ ME, until 42 day). Experimental group (E) had addition of plant extract mixture (100 mg/kg). Significantly($P < 0.05$) higher body weight had chicks from the E group 25th day of the trial, but until the end that differences were not significant [as published previously, 3]. Chicken from the E group had significantly ($P < 0.05$) lower glucose and LDL-cholesterol concentration, lower total protein, albumin and cholesterol concentration 25th day, but higher concentration of iron ($P < 0.05$), total protein, albumin, cholesterol, HDL and LDL at the end of the trial. Lower number of white blood cells ($P < 0.05$) and share of heterophyls but higher share of lymphocytes and iron concentration during the whole trial, show positive effect of plant additive on metabolism, better utilization of nutrients and possible immunomodulatory effect.

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SL I-04 Parasites and plants – bioactive compounds with anti-parasitic effect from *Cichorium intybus*

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Increasing resistance to the limited number of existing drugs against gastrointestinal parasites (GIP) has led to an urgent need to explore new control options. Cattle grazing on chicory (*Cichorium intybus*) have lower levels of *Ostertagia ostertagi* infection, indicating that chicory could be a promising anti-parasitic agent in cattle [1]. The putative active compounds in chicory are sesquiterpene lactones (SL) [1]. However, it is not known if the anti-parasitic effect is from the action of individual SL's or a combined synergistic effect of different compounds within the plant.

In this project, we aimed to isolate and characterize the active compounds from chicory and assess their anti-parasitic activity in a number of GIP.

SL enriched extracts from five different chicory cultivars were prepared and analyzed by HPLC-MS. The extracts were tested for anti-parasitic effects using a model *in vitro* system based on assessing viability of third stage larvae of the swine nematode *Ascaris suum*. PCA analysis of the molecular profiles from different cultivars led to identification of likely groups of active compounds. In parallel, bio-guided fractionation of chicory MeOH:DCM extract on *A. suum* using *in vitro* assays led to several active fractions. The active fractions were submitted to flash chromatography and purified compounds were identified by NMR. Indeed one specific SL showed a moderate effect in the *A. suum* assay. Given the magnitude of the effect, a synergistic effect with other SL's cannot be excluded. Further research is devoted to finding these synergistic combinations and explore the anti-parasitic effect of the identified single SL in other assays.

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SL I-05 Beneficial properties and mechanistic study of the medicinal plant, *Bidens pilosa*, for host health, growth and gut microbiota

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Edible plants and their compounds are now re-emerging as alternative medicine for human and animal health. An Asteraceae medicinal plant, *B. pilosa*, has been studied for over 40 diseases. In this presentation, we summarize the beneficial properties and mechanistic study of *B. pilosa*, based on our recent studies. First, we found that *B. pilosa* was therapeutically effective against coccidiosis in chickens as evidenced by a survival rate, gut pathology, fecal oocyst excretion and anti-coccidial index. Next, we showed that *B. pilosa* significantly increased body weight gain and decreased feed conversion ratio in chickens. Compared to anti-coccidial drugs, *B. pilosa* developed, if any, little drug resistance. Next, we performed pyrosequencing of the PCR ampilcons based on the 16S rRNA genes of gut bacteria in chickens. Metagenomic analysis indicated that the chicken gut bacteria belonged to 6 phyla, 6 classes, 6 orders, 9 families, and 8 genera. More importantly, we found that *B. pilosa* affected the composition of bacteria. This change in bacteria composition was correlated with body weight gain, feed conversion ratio and gut pathology in chickens. Collectively, our work suggests that *B. pilosa* has beneficial effects on growth performance and protozoan infection in chickens probably via multiple mechanisms, including prebiotic action and interference with protozoan life cycle.

Short Lectures Wednesday, September 04, 2019

Short Lectures J: Biological and Pharmacological Activities of Natural Products

SL J-01 New weapons against bacterial adhesion, invasion, *quorum sensing*, cell damaging facors of pathogens: natural products as new anti-virulence compounds

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Due to increasing antibiotic resistance, new antibaterial compounds have to be developed. As most clinically used antibiotics influence either cell wall assembly, protein formation or membrane funtionality, new molecular targets and specific inhibitors should identified. Receptor-mediated recognition between bacteria and host cell leads to adhesion between both partners, which can specifically be blocked by plant-derived natural products. FimH-mediated interaction of uropathogenic *E. coli* to uroplacin from human bladder cells can inhibited by polymethoxylated flavones (e.g. sinensitin from *Orthosiphon stamineus* [1], phtalides from *Apium graveolens* [2]) as shown within in vitro *studies* and evidenced by in vivo infection experiments. This is also accompanied by inhibition of bacterial *quorum sensing*. BabA-and LPS-controlled binding of *H. pylori* to Lewis^b antigens of human stomach cells can be blocked by acetylated rhamnogalacturonans [3]. Internalisation of adhering bacteria into the host cells by membrane-fusion can be inhibited by compounds changing membrane-fluidity of the host cells, as shown for hexadecyl coumaric esters [4]. Fimbriae-located host cell destructing proteases, e.g. arginin- and lysin-specific gingipains from *Porphyromonas gingivalis*, are the main virulence factors responsible for elimination of epithelial cells during active periodontitis. Galloylated dimeric procyanidins from *Rumex acetosa* interact specifically with Arg-gingipain by binding into the active center of the proteases, leading to inhibition of proteolytic activity. In vivo pilot study in humans, using a 7-day mouthwash with *R. acetosa* hydroalcoholic extract 0.8%, reduced significantly clinical symptoms [5]. Summarizing, pinpointing new and specific targets for innovative anti-virulence compounds can effectively be driven by screening of plant-derived natural products.

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SL J-02 Phytochemical and pharmaco-toxicological characterization on water hemp water extracts

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One of the most promising economic perspectives of hemp production chain is female inflorescence valorization. By contrast, scientific literature lacks on chemical composition or biological activity data from aqueous fraction obtained from industrial hemp flowers, which have long been considered as waste products. In this context, the main focus of the following study is the evaluation of antioxidant and anti-inflammatory effects related to aqueous flower extracts from four commercial hemp cultivars (Futura 75, Kc virtus, Carmagnola Cs and Villanova). We evaluated the extract phytochemical profile. Then, we studied the water extracts both in vitro and ex vivo in order to test protective effects in an experimental model of ulcerative colitis, constituted by isolated LPS-stimulated colon. All cultivar extracts displayed similar total phenol and flavonoid content. On the other hand, Futura 75 cultivar extract displayed a better antioxidant and anti-inflammatory profile. Considering this, Futura 75 extract has been subsequently tested to evaluate its effect on pathogen bacterial and fungal species involved in ulcerative colitis, finding a significant inhibition on the growth of C. albicans and selected Gram positive and negative bacterial strains.
Taken together, our results support the potential efficacy of Futura 75 water extracts in managing the clinical symptoms related to ulcerative colitis.

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SL J-03 Plant-derived products as antibiotic enhancers and antibiotic-resistance modifying agents

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Combination of conventional antibiotics with natural products represents a promising strategy in overcoming antibiotic resistance [1].

The aim of this work was to assess the interactions between plant-derived products and several antibiotics in order to identify potential synergistic antibacterial combinations. Both extracts and pure phytochemicals were included in this study. The effects of the combinations were evaluated against Gram-positive including methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria. Our studies on essential oils showed that coriander essential oil and its major constituent, linalool, acted synergistically with several antibiotics (oxacillin, gentamicin, tetracycline, ciprofloxacin) against both Gram-positive and Gram-negative bacteria with coriander essential oil reversing tetracycline resistance of *Staphylococcus epidermidis* [2]. Despite its moderate antibacterial activity, white mulberry leaf ethanolic extract was found to reverse oxacillin resistance of MRSA and to act synergistically with gentamicin against MRSA. Additionally, the extract showed synergy with gentamicin and tetracycline against *Staphylococcus epidermidis* [3]. Two of its constituents, namely morusin and kuwanon G, also reversed oxacillin resistance of MRSA and acted synergistically with gentamicin, tetracycline and ciprofloxacin against MRSA. Morusin also reversed tetracycline resistance of *Staphylococcus epidermidis*. Both morusin and kuwanon G increased the permeability of the bacterial membrane as shown by fluorescence and differential interference contrast microscopy. Conclusion: Plant-derived products are promising candidates for the development of novel treatment strategies in bacterial infections.

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SL J-04 Nature-derived peptides: a growing niche for GPCR ligand discovery

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G protein-coupled receptors (GPCRs) represent important drug targets, as they regulate pivotal physiological processes and they have proved to be readily druggable. Natural products have been and continue to be amongst the most valuable sources for drug discovery and development. Peptides from plants, insects or venomous animals are of interest as lead compounds for the development of GPCR ligands, since they cover a chemical space, which differs from that of synthetic small molecules. Peptides, however, face challenges in drug development, some of which can be overcome by studying plant-derived compounds. We argue and will present examples that utilizing circular plant peptides, called cyclotides present an opportunity for GPCR ligand discovery and development [1-3].

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Short Lectures K: Clinical Studies

SL K-01 Effect of an oil-free hydroethanolic pumpkin seed extract on symptoms of benign prostatic hyperplasia

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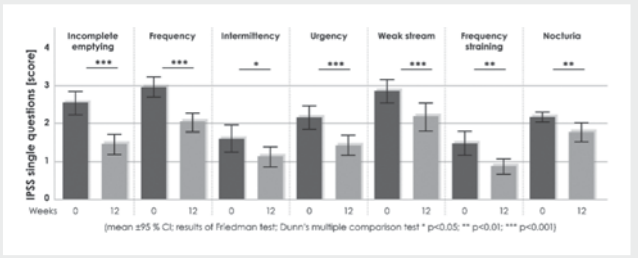
DOI 10.1055/s-0039-3399723

Benign prostate hyperplasia (BPH) is affecting numerous men, symptoms starting usually after the fourth decade. At an early stage of symptoms development related to BPH, pumpkin seed extracts are applied [1, 2]. Hence, the effects of a proprietary oil-free hydroethanolic pumpkin seed extract (EFLA® 940) prepared from seeds of the Styrian oil pumpkin, *Cucurbita pepo* L. *ssp. pepo var. styriaca* (Cucurbitaceae) and extraction with 60% ethanol/water (DER_{native} 15 – 25:1), were investigated in a single-arm, mono-center pilot study, on the symptoms of BPH [3]. In total, 60 men (95% confidence interval (CI): 60.3–64.3 years) took part in the pilot study. The extract was ingested as a tablet prepared with common excipients once a day. Assessment of changes in International Prostate Symptoms Score (IPSS) within the treatment period of 3 months was carried out, frequency of nocturia was recorded by bladder diary, postvoid residual urine volume was determined by ultrasound. As a major outcome of this pilot study, a significant symptom reduction of 30.1% at average was recognized for the total IPSS. According to IPSS questionnaire and bladder diary, nocturia significantly decreased over time ($P < .0001$) (▶**Fig. 1**). After 12 weeks of treatment, postvoid residual urine



▶**Fig. 1** Comparison of IPSS single question scores at timepoints 0 and 12 weeks of interference with a hydroalcoholic pumpkin seed extract.

volume was significantly reduced. After 8 and 12 weeks of intervention, the symptom alleviation also significantly (P < .001) improved QoL according to IPSS questionnaire.

The outcome of this pilot study indicates that the oil-free hydroethanolic pumpkin seed extract seems to provide significant health benefits in patients suffering from BPH related symptoms.

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SL K-02 Add-on therapy with EPs 7630 in patients with chronic obstructive pulmonary disease – an overview on clinical efficacy and safety

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The herbal drug preparation EPs® 7630 (Umckaloabo®; ISO Arzneimittel, Ettlingen, Germany) from the roots of Pelargonium sidoides has already been shown to be efficacious and safe in adults, adolescents and children from the age of one year on suffering from acute respiratory tract infections [1]. Recently, EPs® 7630 add-on therapy was also acknowledged in the Swiss National Chronic Obstructive Pulmonary Disease (COPD) Guidelines for pharmacological management of stable COPD [2]. Among the main goals of current pharmacotherapy in COPD as described by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) Report 2019 [3] are the reduction of frequency and severity of exacerbations and the improvement of health status. Based on this background, an overview on clinical efficacy and safety of EPs® 7630 treatment in COPD is presented. Study results show that add-on therapy with 3x30 drops/day EPs® 7630 over 24 weeks in addition to a standardized baseline treatment according to GOLD led to a significantly prolonged time to exacerbation, to fewer exacerbations, improved quality of life, higher patient satisfaction, less days of inability to work, and a reduction in antibiotic use in patients with moderate to severe COPD. The incidence of adverse events was comparable to placebo. In conclusion, these results demonstrate a statistically significant and clinically relevant superiority of EPs® 7630 add-on therapy compared to placebo and an excellent long-term tolerability and safety in the treatment of patients suffering from COPD.

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SL K-03 Efficacy and tolerability of EPs® 7630 for children and adolescents with acute bronchitis

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EPs® 7630 (Umckaloabo®; ISO Arzneimittel, Ettlingen, Germany) is a liquid herbal drug preparation from Pelargonium sidoides roots. EPs® 7630 shows antiviral, antibacterial and immunomodulatory properties which account for its therapeutic effects in respiratory tract infections such as acute bronchitis (AB).

We present data from three RCTs involving 820 patients (placebo: 307, EPs® 7630: 513) aged 1-18 years, diagnosed with AB.

In two clinical trials with 200 resp. 220 patients, changes in the total score of the BSS-ped, a short version of the physician-rated Bronchitis Severity Scale (BSS) were assessed. EPs® 7630 solution significantly reduced the total score of the BSS-ped including three typical AB symptoms, i.e. cough, dyspnoea, rales on day 7 (main outcome measure; placebo vs EPs® 7630: -1.2 vs -3.4 points resp. -2.9 vs -4.4 points; p<0.001). In a further study with 399 patients aged 6-18 years, EPs® 7630 film-coated tablets (10mg/20mg/30mg/placebo t.i.d.) significantly improved the BSS-ped between day 0 and day 7 in the two higher dosages (placebo vs EPs® 7630 (10/20/30): -3.3 vs -3.6/-4.4/-5.0 points; p<0.001 for placebo vs EPs® 7630 (20/30)).

The EPs® 7630 treatment group showed a significantly better outcome than placebo as assessed with the Integrative Medicine Outcome Scale (IMOS). These trials also confirm the good tolerability of EPs® 7630. Accordingly, patients treated with EPs® 7630 were significantly more satisfied with the treatment, as evidenced by the Integrated Medicine Patient Satisfaction Scale (IMPSS) (p<0.001).

EPs® 7630 is an efficacious and well-tolerated herbal medicine for the treatment of AB in children and adolescents.

SL K-04 Effect of the fixed combination of valerian, lemon balm, passionflower and butterbur extracts (Ze 185) on prescriptions of benzodiazepines

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Stress is an increasing problem that can result in various psychiatric and somatoform symptoms. Among others, benzodiazepines and valerian preparations are used to treat stress symptoms.

The aim of this study was to investigate whether the prescription of a fixed herbal extract combination of valerian, lemon balm, passionflower and butterbur (Ze 185)[1,2] changes the prescription pattern of benzodiazepines in hospitalised psychiatric patients. In a retrospective case-control study anonymised medical record data from 3252 psychiatric in-house patients were analysed over a 2.5-year period. Cases (n = 1548) with a prescription of Ze 185 and controls (n = 1704) were matched by age, gender, hospitalisation interval and main ICD-10 F-diagnoses. Primary objective was to investigate the effect of Ze 185 on the prescription pattern of benzodiazepines. Secondary objectives investigated the prescriptions of concomitant drugs and effectiveness of the hospital stay. Distribution of drug classes was analysed by the WHO's ATC code.

The data of this retrospective case-control study provide some evidence that Ze 185 in addition to standard therapy could reduce the need for benzodiazepines. Data showed that both therapeutic settings had a comparable clinical effectiveness but with significantly fewer prescriptions of benzodiazepines in the Ze 185 group (p = 0.006).

This is of clinical importance because suitable alternatives to benzodiazepines are desirable. To obtain more support for this hypothesis, a dedicated randomised, controlled clinical trial monitoring drug safety is required.

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Short Lectures L: Others

SL L-01 An imposter in the house? Best practices for prevention of adulteration in the U.S. botanicals and dietary supplements industry

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Substantial differences exist in regulatory frameworks governing manufacture of dietary supplements in the US and Europe, but critical economic and safety concerns about adulteration of medicinal plant ingredients and products are shared. A basic, global quality requirement is establishment of ingredients identity. How do supplement manufacturers assure accurate identification of botanicals, especially if dependent on global supply networks, and numerous geographic sources and vendors? Can adulteration be anticipated and avoided? How to recognize and avoid potential adulterants? Strategic tools for adulterant prevention in natural plant products are authenticated plant reference collections with selective emphasis: ingredients, their confounding species, and known or potential adulterants.

In 2013, Traditional Medicinals, Inc., a leading U.S. manufacturer of medicinal herbal teas, initiated an botanical identity program with several goals: 1) to build a collection with at least one representative whole plant voucher for each individual ingredient used in our products (>100 species), and confounding species; 2) to research, obtain known and potential adulterants; and 3) assemble strong comparative reference collections consisting of sample materials from suppliers and representative finished products. We proved the presence and identity of an intrusive high-pyrrolizidine contaminant in a passionflower shipment, demonstrating the utility risk-lowering value of standard reference collections.

Authenticated reference collections are used by both in-house chemistry and botany experts for baseline identity testing and comparison of results. The herbarium, reference collections and their applications have proven to be critical for proving compliance with CFR requirements, audits, and for influencing best practices in the industry.

SL L-02 Development of *Artemisia annua* essential oil liposomes with antifungal activity against *Candida* species

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Essential oils have been widely used for their antimicrobial and antifungal properties but their instability to light and high volatility can limit the clinical practice [1], and this problem can be overcome by drug delivery systems. The aim of the present study was to formulate, optimize and evaluate vesicles loaded by *Artemisia annua* essential oil (AEO). GC-MS analyses revealed that AEO main constituents were camphor, artemisia ketone, and 1,8-cineole. Loaded liposomes (100 µL/mL) were optimized for their size, polydispersity index (PDI), ζ-potential and morphology. Recovery, encapsulation efficiency (EE%), release and antifungal activity on *Candida* species were evaluated [2]. Quantitative analysis was carried out using a HPLC1100-DAD. The vesicles, stable over a one month period at 4 °C, exhibited a spherical shape, had average sizes about 250 nm, a ζ potential of approximately -10 mV and a PDI of 0.21. The EE% was around 75 % while the recovery was over 90 %. The drug release study showed that after 14 hours almost 100 % of AEO was released. The minimum fungicidal concentrations (MFCs) of AEO and EO-loaded liposomes was performed against 10 fungal strains of *Candida* using amphotericin B as positive control. Results showed that the MFC values ranged from 9.8 to 42 µL/mL of AEO and from 5 to 10 µL/mL of EO-loaded in liposomes. Significant difference by t student test was expressed as $p<0.01$. The findings suggested that loaded liposomes proved to be more effective against *Candida* species than the free essential oil. The formulation can decrease the volatility of AEO, optimize its biological properties and defeat fungal infections.

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SL L-03 Does quercetin intake alter the concentration of pesticides in honeybees?

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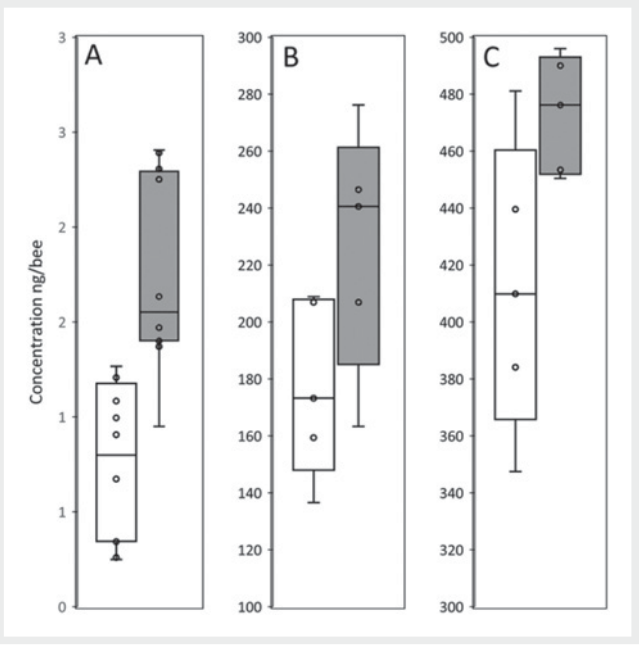
Honey bees regularly ingest quercetin, one of the most abundant phytochemicals in plants. Past research suggests that honey and pollen feeding upregulates the bees' detoxification system and that dietary quercetin reduces the toxicity of imidacloprid and tau-fluvalinate [1,2]. The aim of this study was to investigate the effect of dietary quercetin on the concentration of three pesticides present in honey bees. Honey bees (n = 600) were divided in five groups and fed either quercetin-sucrose paste or only sucrose for 72 h before being exposed to the neonicotinoid imidacloprid (oral exposure), the fungicide tebuconazole or the acaricide tau-fluvalinate (contact exposure). Bees were anesthetized with CO2, frozen, and extracted with a validated QuEChERS method. Finally, the concentrations of all three above-mentioned pesticides were determined by LC-MSMS. Quantification results of the three pesticides in honey bee samples (▶ **Fig. 1**) displayed that there was a significant difference in concentration of imidacloprid ($P=2^{x}$ 10-5) and tau-fluvalinate ($P=3^{x}$ 10-2) between the bees that were fed quercetin-sucrose paste and bees that were fed only sucrose. However, there was no significant difference in the level of tebuconazole in the group of bees that were fed quercetin-sucrose paste and bees that were fed only sucrose ($P=4.2^{x}$ 10-1). The results of this study demonstrate that honey bee intake of

quercetin leads to a reduction in the concentration of imidacloprid and tau-fluvalinate by up regulating the detoxification systems in bees. Hence, quercetin may be a promising bioactive compound in chemo-ecological aspects to counteract the hazard of pesticides.



▶ **Fig. 1** Concentration of imidacloprid (A), tau-fluvalinate (B) and tebuconazole (C) in honey bee samples when they were fed quercetin-sucrose paste (white plots) or only sucrose (shaded plots).

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SL L-04 The efficient isolation of secondary metabolites from Chilean native fruits by counter-current chromatography

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The analysis of secondary metabolites from natural products its not simple due to the high structural variety, large number of compounds and different physicochemical properties [1]. Hence, chromatography is a prerequisite for the isolation of compounds for complete structural characterization [1]. Counter-current chromatography (CCC) was developed as an alternative to conventional methods, with the high advantage of lack of solid support [2]. The solvent system can be adjusted according to the polarity of the target compounds to favor separation based upon their partition coefficients [3]. The aim of this work is to describe the isolation and structural characterization of secondary metabolites from Chilean fruits, including *Ribes* and *Gaultheria* berries, *Gevuina avellana* nuts, and the arils of *Prumnopitys andina*.

Four anthocyanins were isolated from the *Ribes* species, including delphinidin and cyanidin glycosides and rutinosides. From *Gaultheria* species, four anthocyanins were isolated and were identified as cyanidin and delphinidin arabinosides and galactosides. In addition, the arabinoside and rhamnoside of quercetin, as well as three monotropein-derivatives were obtained. From *Gevuina avellana*, two dipeptides were isolated from the roasted samples. In the arils of *Prumnopitys andina*, the steroid 20-hydroxyecdysone was found. All the isolated compounds were obtained with high purity and were structurally characterized by extensive NMR analysis, QTOF mass spectrometry, absorbance profile and IR spectrum. CCC proved to be a valuable tool for the isolation of main secondary metabolites in complex mixtures from Chilean native fruits. The isolated compounds are being assessed for the bioactivity and health-promoting properties of native Chilean food resources.

▶ **Fig. 1**

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Pre-Congress Posters

Animal Healthcare and Veterinary Phytotherapy

P-V-04 *In vitro* activity of essential oil mixture against *Staphylococcus aureus* isolated from cats with pyoderma

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Staphylococcus (S.) aureus is isolated in cats suffering from pyoderma. A shampoo containing 5% rosemary (Rosmarinus officinalis), 5% oregano (Origanum vulgare), and 2% Turkish thyme (Thymus serpyllum) essential oil (EO) was shown to have *in vivo* antifungal activity in cats with microsporiasis [1]. The treatment was well tolerated, without any adverse effects. Aim of this study was to investigate the *in vitro* efficacy of this EO treatment in feline *S. aureus*-associated pyoderma by testing the EO mixture as well as single components against *S. aureus* isolated from skin of pyodermic cats. A microdilution test using different EO

concentrations (rosemary, oregano: 0.625%, 1.25%; 2.5%, Turkish thyme: 0.25%,0.5%,1%, mixture: 0.75%, 1.5%, 3%, 6%) and three different incubation times (1, 2, 5 min) was performed, colony forming units (CFU) were determined. Oregano, Turkish thyme oil, and the mixture of EOs exhibited an *in vitro* antibacterial activity, while rosemary oil did not inhibit bacterial growth (Tab. 1). Higher concentration of EOs as well as longer incubation time led to reduction of CFU, even a total growth inhibition could be achieved. Overall, the EO mixture showed better antibacterial efficacy than single EOs. EO concentration has stronger impact than incubation time. Interestingly, comparison of single bacterial isolates reveals a heterogenous dose dependent reduction of CFU and different susceptibility patterns of *S. aureus* isolates have to be discussed. To conclude, the EO mixture is a promising candidate for treatment of *S. aureus*-associated pyoderma in cats. The antibacterial effect most likely originates from oregano and Turkish thyme oil.

Table 1

EO	Concentration of EO	CFU / 10 µl [mean, n = 5] Incubation time 1 min	2 min	5 min
Rosemary	0.625 %	> 800	> 800	> 800
	1.25 %	> 800	> 800	> 800
	2.50 %	> 800	> 800	> 800
Oregano	0.625 %	> 800	> 800	> 800
	1.25 %	625.4	546.6	464.2
	2.50 %	21	4.2	0.0
Turkish thyme	0.25 %	733.6	715.4	691.2
	0.50 %	645.2	581	517.6
	1.00 %	300.4	230.2	115.6
EO mixture	0.75 %	675.6	602.8	545.8
	1.50 %	425.4	343.2	204.0
	3.00 %	238.2	150.4	63.2
	6.00 %	35	7.2	0.4

> 800 CFU / 10 µl represents confluent growth

▶ **Tab. 1**

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P-V-05 *In vitro* biological activities of some South African Syzygium and Eugenia (Myrtaceae) species with potential as phytogenic feed additives

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Antibiotic use in animals, especially as growth promoters in livestock feeds, is a risk factor for the development of antibiotic resistance in humans and contamination of the environment, constituting a one health challenge. Several alternatives including phytogenic feed additives may be potential substitutes. Plants are potential sources of compounds with antimicrobial properties.

Antiquorum sensing, anti-adherence, cytotoxicity, phytochemistry and ultrastructural effects of four South African plant crude acetone extracts; *Eugenia zeyheri, E. erythrophylla, Syzygium legatii* and *S. gerrardii* were determined. Anti-quorum sensing activity was determined by inhibition of quorum sensing (QS)-controlled violacein pigment production in *Chromobacterium violaceum.* Inhibition of adherence of *E. coli* to intestinal cells by extracts was evaluated using a Caco-2 cell enterocyte anti-adhesion model. Ultrastructural effects of *E. zeyheri* and *S. legatii* extracts on *E. coli* were assessed using electron microscopy. Phytochemical analysis was done using Gas Chromatography-Mass Spectrometry.

Minimum quorum sensing inhibitory concentration (MQSIC) and minimum bactericidal concentration (MBC) ranged between 0.08 and 0.16 mg/ml and 0.08 and 0.31 mg/ml respectively. Apart from *S. legatii*, other plant extracts and vanillin (positive control) had good antiquorum sensing activity. *Eugenia zeyheri* and *S. gerrardii* significantly reduced adhesion of *E. coli* to Caco-2 intestinal cells. The extracts of *E. zeyheri* and *S. legatii* damaged the cytoplasmic membrane and inner structure of *E. coli*. α-Amyrin, friedelan-3-one, lupeol, and β-sitosterol were abundant in the extracts.

The plant extracts have potential as phytogenic feed additives although animal trials are needed. Compounds in the extracts may be drug templates to develop novel antimicrobial agents.

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P-V-08 Evaluation of the traditional use of some Yemeni plants for the treatment of some livestock diseases

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Livestock is an integral component of agriculture production in Yemen. The aim of our work resides in the scientific substantiation of the ethnoveterinary use of some plants [1] based on the evaluation of their bioactivities and toxicological properties. Searching the scientific literature has revealed various pharmacological activities that may support the claimed healing activities of eleven (▶ **Tab. 1**) out of fourteen remedies for some of their ethnoveterinary utilization. Moreover, three remedies were found to demonstrate toxic effects in experimental studies (▶ **Tab. 1**). It can be concluded that our work has provided valuable scientific information on some Yemeni plants that could be utilized for the benefit of farmers to rational the use of plants.

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Table 1: The ethnoveterinary uses supported by scientific studies, and the toxicological properties of some plants used in ethnoveterinary medicine in Yemen.

Species/ Family	Part used	Indication	Toxic effects
Socotran Aloe species/ Asphodelaceae	Fresh latex	Constipation, colic and worms (internal use). Boils, suppurating abscess, large fresh lacerations and infected bites of ticks, lice or flies (external use).	Genotoxicity, mutagenicity and carcinogenicity of some constituents of Aloe such as aloe-emodin [3].
Boswellia sacra Flueck./ Burseraceae	Underbark	Infected wounds (external use).	
	Oleo-gum resin	Mastitis (external use).	
Cissus rotundifolia Vahl/ Vitaceae	Unspecified part	Stomach pain (internal use).	
Cyphostemma digitatum (Forssk.)Desc./ Vitaceae	Leaves	Appetite stimulation (internal use).	
Psiadia punctulate (DC.) Vatke / Asteraceae	Unspecified part	Bone fracture (External use).	
Pulicaria undulata (L.) C.A. Mey./ Asteraceae	Unspecified part	Insect repellent.	
Rumex nervosus Vahl / Polygonaceae	Unspecified part	Pimples in the eye, mixed with salt.	
Aristolochia bracteolate Lam./ Aristolochiaceae	Unspecified part	Bloating by giving *A. bracteolate* with *S. bicolor* grains (internal use).	Mutagenicity, carcinogenicity and nephropathy due to aristolochic acid I and II [4].
Sorghum bicolor (Poaceae)	Grains		
Trigonella foenum-graecum L. (Fabaceae)	Seeds	Constipation by giving the fenugreek seeds together with barley grains (Internal use).	Controversial results on the toxicity of fenugreek seeds such as antifertility, abortifacient, abnormal foetal development, and other cellular and molecular alterations [5].
Hordeum vulgare (Poaceae)	Grains		

▶ **Tab. 1**

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P-V-09 Novel herbal veterinary narcotics for aquacultures of *Clarias gariepinus*

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Japanese Kampo medicine uses plant extracts as analgesic and natural narcotics. Herbal fishing narcotics have been documented in numerous cultures [1]. The catfish *Clarias gariepinus* is a species favorable for food production in aquacultures. EU laws forbid slaughtering animals without narcosis. *C. gariepinus* has proven resistant to all licensed narcotic agents for fish. The active constituents of most surveyed plants are haemolytic saponins which lead to a loss of consciousness in the fish via hypoxia. Only *Zanthoxylum piperitum* and *Barringtonia asiatica* were not described as saponin drugs and acted via neurological narcosis in preliminary experiments. In Kampo *Z. piperitum* is well known for its use as an analgesic, e. g. as the main component of the health insurance covered prescription Daikenchuto in Japan [2], where it was also used as an effective fishing narcotic. Additionally, *Cissus quadrangularis* was included in this study as it is used in West Africa as an enhancer of the narcotic effects of other fishing poisons [3]. As expected, the positive control, (MS222), had the weakest narcotic effect. Both *Z. piperitum* and *B. asiatica* achieved full narcosis after 15 min. The combination of all three plant extracts provided the quickest and fullest sedation after 10 min, indicated by absence of operculum movement, loss of equilibrium, no response to handling and no adverse reactions. All groups made a full recovery after a few minutes in fresh water without adverse effects. Meat samples of euthanized test animals were fried for organoleptic testing. No difference in consistence or taste could be detected.

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P-V-10 Can plants with good anthelmintic activity against free-living and animal parasitic nematodes be effective against plant parasitic nematodes?

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Root-knot nematodes (*Meloidogyne* spp.) are important pests in agricultural crops, causing more than 95% infestation in crops while livestock losses due to the animal parasitic nematode *Haemonchus contortus* cost tens of billions of dollars annually. Synthetic chemicals are highly effective, however increasing parasite resistance to anthelmintic drugs, environmental and health concerns have awakened interest in alternative methods of nematode control. Plants were selected on the basis of published activity against free-living (*Caenorhabditis elegans*) and animal parasitic (*Haemonchus contortus*) nematodes. Extracts of *Lantana rugosa, Leonotis leonurus* and *Clasusena anisata* were screened for corresponding efficacy against root-knot nematodes *Meloidogyne incognita C. elegans* and *H. contortus L. leonurus* and *C. anisata* acetone extracts had the best activity at 1 mg/mL with mortality of 100 and 92% on *M. incognita* juveniles after 48 hours of incubation respectively. The water extract of *L. leonurus* had the highest mortality of 99% on *C. elegans* after 24 hours of incubation but exposure indicated weaker activity. The dichloromethane/methanol extract of *L. leonurus* inhibited 100% of *H. contortus* eggs from hatching at 0.104 mg/mL, followed by acetone extracts of *L. rugosa* (96%) and *L. leonurus* (77%). Solvent-solvent fractions of *L. rugosa* and *C. anisata* had good activity against *C. elegans* while those of *L. leonurus* inhibited *H. contortus* eggs from hatching. In the present study, there was good translation of plant extract activity against animal and plant nematodes. Further investigations aim to identify active compounds and mechanism of action of the most promising extracts.

P-V-11 Effects of phytogenic feed additives on mortality and growth performance of weaned piglets

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Weaning is a stressful period in the life of pigs coming along with increased susceptibility to environmental and pathogenic challenges and resulting in decreased growth performance [1]. Phytogenic feed additives (PFA) have the potential to influence palatability of the feed and support health and well-being of animals [2]. Consequently, the current study aimed to evaluate the benefits of a PFA on growth-performance in piglets post-weaning.

A total of 192 piglets (each 96 m and f) weaned at day 23.4 ± 1.21 were distributed to 24 pens with 8 piglets. 12 pens each were randomly assigned to either an unsupplemented control (NC), or a diet supplemented with a PFA for a 35-day feeding trial. Body weight (BW) and feed intake were recorded after weeks 1, 2, 3 and 4. In group NC, 7 piglets died or had to be removed during the trial,

whereas no piglets were removed in the PFA group. Withdrawn animals had low BW and were greatly affected the mean BW of NC compared to PFA. As a result, no significant difference on growth performance was observed, although PFA animals showed numerically higher ADG in each period (up to +6.8% from day 21-35). Considering mortality and numerically increased ADG, there was a tendency for increased total BW gain with the PFA compared to the NC (+11.4%, P=0.083).

In conclusion, the applied PFA was suitable to support the animals through the post-weaning period, resulting in decreased mortality and numerically improved BW gain.

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P-V-12 Effects of a phytogenic feed additive in growing-finishing pigs

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Digestarom® DC Power is a new generation phytogenic feed additive. It contains the BIOMIN® Duplex capsule technology, which combines mixtures of essential oils matrix-encapsulated in a core and coat surrounded by extracts and herbs. This enables a slow and continuous release of ingredients along the digestive system to enhance gut health and the performance of pigs. In our study, we separated 240 pigs (LW x LR X PI) at the age of 9 - 10 weeks in two different groups in 24 pens. For the following period of 14 weeks, control group was fed a basal diet and treatment group was fed a basal diet supplemented with Digestarom® DC Power (100 g/t). The study was carried out in IFIP- National Experimental Station, France. The final body weight and the average daily gain were numerically increased in the Digestarom® group. In addition, the feed conversion rate (FCR) was numerically decreased in the treatment group. The fat and lean content of the carcass of one pig per pen (24 pigs in total, 12 per group) was determined by CT scan. The lean content in Digestarom® DC Power fed pigs was increased by 3.6% ($p<0.1$), whereas the fat content was decreased by 8.7% ($p<0.1$). Thus, Digestarom® DC Power results in an optimized FCR and a better weight performance numerically, but with no statistical significance. Furthermore, pig fed the feed additive showed an increase of lean content and a reduction of fat content. Those facts promise a higher economic benefit.

P-V-13 The *in vitro* antibacterial activity and safety of *Morinda lucida* leaf extracts against *Salmonella* serovars relevant in livestock infections

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Salmonella infections are of great importance in human and animal health. Infections caused by non-typhoidal *Salmonella* are either non-invasive or invasive systemic infections that require effective antimicrobial therapy. The aim of this study was to investigate the antibacterial activity, safety and anti-biofilm potential of leaf extracts of *Morinda lucida* against eight *Salmonella* serovars. *M. lucida* is used in traditional treatment of human typhoid and malaria fever. Acetone and aqueous leaf extracts of *M. lucida* were screened for antibacterial activity against several serovars of *Salmonella enterica* subsp. *enterica* including *S. enterica* serovar Gallinarum (birds), Dublin (birds and ruminants), Choleraesuis (pigs), Braenderup (birds), Idikan (humans and birds), Kottbus (birds), Typhimurium (birds and ruminants) and Enteritidis (birds and humans) using a serial microdilution assay. The cytotoxic and anti-biofilm potential of the acetone and aqueous extracts were also determined against human colon cancer (Caco-2) cells and *Salmonella* biofilm formation respectively.

The minimum inhibitory concentration (MIC) of the extracts ranged from 0.09 to 1.87 mg/ml. The LC_{50} values of the acetone and aqueous extracts against the Caco-2 cells were 0.46 and 0.33 mg/ml respectively. The acetone extract had the strongest anti-biofilm activity against *S. Enteritidis*. The range of selectivity index (SI) values of the acetone and aqueous extracts was 1.00 to 6.57 and 0.23 to 8.28 respectively. The potential usefulness of this plant species as an alternative for treatment of human and animal salmonellosis supported by these results. However, *in vivo* data is necessary to further investigate this claim.

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P-V-14 Use of garlic in parasite management: results of in vitro examinations

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Due to the increase in parasite resistance, research on the utility of herbs for parasite regulation was conducted [1]. Garlic is used in many herb mixtures. Anthelmintic effects of garlic have been described [2]. The aim of this study was to evaluate the effect of two garlic formulations, i.e. garlic dragee (GD) and fresh garlic extract (GE), at three dilution levels (0%, 10%, 20%) on the exsheathment of parasitic third stage larvae. GDs were sourced from the local pharmacy. The content of one GD was solved in 10 mg water. GE was prepared by cooking 75 g of crushed fresh garlic with 125 g water over 5 minutes (the content of GD corresponded to 6 g of fresh garlic according to the leaflet information). The parasitic larvae were extracted from faeces of pastured goats after incubation of the feces at 28 °C for 10 days. Purified third larvae were incubated with GD, GE, tetramisole hydrochloride (positive control) and water (negative control) for 3 hours at room temperature. The rates of exsheathment were counted 20, 40, 60 and 80 minutes after starting the exsheathment process with diluted sodium hypochlorite. Significantly lower exsheathment rates were registered for GD and GE in the 0% (5,7; 1,1) and 10% (55,8; 6,2) but not in the 20% dilution (57,5; 19,5). Although the *in vitro* examination showed dilution dependent effects of garlic solutions on the exsheathment of parasite larvae, further studies are necessary to find effective dosages when feeding herbal mixtures in small ruminants.

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P-V-15 Use of grapefruit seed extract in parasite management: first results of in vitro examinations

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DOI 10.1055/s-0039-3399741

Increased resistancies of parasites are a challenge in parasite management worldwide. During the last decade a lot of research about secondary plant ingredients were conducted [1,2,3]. But farmers are still missing solutions in alternative parasite management. The aim of this study was to investigate the influence of grapefruit seed extract (GSE) on the exsheathment rate of parasitic third larvae in vitro. GSE was sourced from the local pharmacy. The content of bioflavonoids (naringin, hesperidin, néohepéridine, narirutine) was according to the certificate at 8 mg/ml. The parasitic larvae were extracted from faeces of pastured goats of the station after incubation of the feces at 28 °C for 10 days. The purified larvae were incubated with two differently concentrated GSE dilutions(GSE1: 1,6 mg/ml; GSE2: 3,2 mg/ml), with tetramishole hydrochloride (positiv control) and water (negativ control) for 3 hours at 22 °C. The proportion of exsheathment was counted 20, 40 and 60 minutes after starting the exsheathment process with diluted sodium hypochlorite solution. After 60 minutes the exsheathment rates of the negative control, positive control, GSE1 and GSE2 were 96,7 %, 4,2 %, 6,4 % and 15,1 %, respectively. The exsheathment rate of the positive control reflects the situation of resistancy in parasites of goats of the station. Although the in vitro examination in GSE showed comparable results with the positive control further investigations are necessary to verify these effects under agricultural field conditions.

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P-V-16 *In vitro* study of the *Plumbago indica* root extract on *Fasciola gigantica*

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The triclabendazole-resistant Fasciola spp. populations have been reported in a number of developing countries, therefore the drug discovery has been required. The objective of this study was to evaluate the trematodicidal action of *Plumbago indica* (PI) root extract on newly excysted juvenile (NEJs) and 4 weeks of *Fasciola gigantica* after incubating the parasites in M-199 medium containing the PI at increasing concentrations (0.1, 1, 10, and 100 µg/ml) when compared with triclabendazole (TCZ) using relative mortality (RM) index, survival index (SI) ratios, and percentage of larvae migration inhibition (%LMI) assays. The percentage of LMI in NEJs incubated with PI extract showed lesser efficacy than TCZ at the low concentrations but when NEJs incubated with PI at 10 µg/mL showed 87.3% LMI while flukes incubated with TCZ was 83.3% LMI. The RM and SI values of NEJs and PI-treated 4 weeks flukes showed progressively decreased when increasing incubation time and dosages. Flukes treated with PI at 100 µg/ml show 50% RM in early 1 h after incubation and were completely immobile at 24 h. In contrast, flukes incubated with TCZ showed active movement until 12 h, they had 90% RM. The flukes incubated with PI at 100 µg/ml showed less than 50% SI after 6 h and were completely dead at 24 h. The swelling and blebbing tegumental surface of *F.gigantica* occurred after incubation, but their tegument did not peel off. These results suggest that PI extract could be against the motility of NEJs and 4-weeks stage of *F.gigantica.*

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P-V-17 The anthelmintic effects of *Artocarpus lakoocha* stem extract on *Fasciola gigantica*

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Triclabendazole is currently used for chemotherapeutic treatment of fascioliasis in the cattle; however, the parasites' eggs have been found in the gallbladder after treatment. *Artocarpus lakoocha* Roxb. has been used to cure parasitic infections in human in the Southeast. Therefore, the efficacy of *Artocarpus lakoocha* stem (AL) extract on newly excysted juveniles and adult of *Fasciola gigantica* was evaluated after incubating the parasites in M-199 medium containing 250, 500, 750 and 1000 µg/ml of the crude extract, or triclabendazole (TCZ) at the concentrations of 80 µg/ml as the positive control using percentage of larvae migration inhibition (LMI) assay and observation by Transmission electron microscope (TEM). The percentage of LMI were 74% and 76% when flukes treated with AL extract at 250 and 500 µg/ml, and flukes treated with extract at 750 and 1000 µg/ml were 100% of LMI. The ultrastructure of tegument showed numerous granules type I and II that migrated up to the apical surface. The swelling and blebbing of the apical plasma membrane as well as swollen mitochondria in the syncytium and tegumental cells which exploded. The serve swollen mitochondria and vacuole were observed in the muscle bundle. The necrotic substances were found in the tegumental cells, muscle bundles, parenchymal cell, and vitelline cells after 12 h. Hence, *in vitro* studies should be performed to evaluate whether the AL extract may act as a potential anthelmintic drug for treatment fascioliasis.

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P-V-18 Effects of a standardized natural yeast-thiamine-complex in 34 dogs with tick infestation

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Ticks play an essential role in the spread of vector-borne diseases herefore tick control becomes even more important [1]. The resistance of ticks, climatic changes with long-term effects, as well as increasing global movement of animals and goods have favored development and spread of ticks and their diseases [2]. The aim of this study was to investigate the effects of the natural yeast-thiamine-complex (Formel-Z®) in dogs with tick infestation within a one-year tick season.

he average amount of free and attached ticks was counted before and after the beginning of daily administration of the natural complex (167.85 mg/kg bw/day the product specification). A total of 34 dogs of different breed (with fur of moderate hair length) included in study based on a dog-owner questionnaire.

The number of free ticks on the animals was significant reduced by 74.43% (M ±SD before feeding: 2.84±2.06, M±SD after feeding: 0.73±0.45) after the administration of the standardized yeast-thiamine-complex. the number of attached ticks showed a decrease (M±SD before feeding: 2.13±1.93, M±SD after feeding: 0.69±0.51) by 67.62%. In addition, none of the dog-owners noticed an increase in dead ticks, while some of them reported a visible improvement of fur and skin properties of their animals.

In conclusion, the results provide a first evidence for the efficacy of a standardized natural yeast-thiamine-complex for the prevention of tick infestation through a potential repellent effect. Thus, the standardized natural product a good to reduce tick infestation.

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P-V-19 Standardization of an *in vitro* spectrophotometric method for the evaluation of the ammonia-binding properties of plant extracts rich in saponins

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Saponins have been described to play an essential role in gas reduction from the intensive farming industry due to their potential lowering ammonia monogastric animals[1,2]. Thus, saponins are a natural way to decrease odors from pigs and poultry wastes, resulting in improved animal welfare and productivity [3]. To monitor their activity, ABC50 method (for Ammonia-Binding Capacity). allows defin of a product necessary to reduce 50% of the free ammoniac in solution (ABC50 value). The lower the ABC50 value, the more the tested product or molecule is efficient bind ammonia. However, many studies have shown that close control of pH value, as well as several parameters, r a standardized and reliable method[3-5]. The objective of this study was improve the method by developing a standardized protocol to neutralize bias and allow studying complex plant extracts rich in ammonia binding molecules such as saponins. The optimized method was carried out on a yucca extract, showing stable results, with the mean intra-day ABC50 value 2.80±2.38, and inter-day ABC50 value of 2.70±0.07, a good of the method. It was also tested on a complex mixture of saponin-rich plants mean intra-day ABC50 value 1.60±0.06 and inter-day ABC50 value of 1.60±0.06. his work resulted in the standardization of a method useful to evaluate ammonia-binding capacity of natural products such as saponin-rich plant extracts.

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P-V-20 Polyphenolics from Romanian native medicinal plants and used in veterinary medicine

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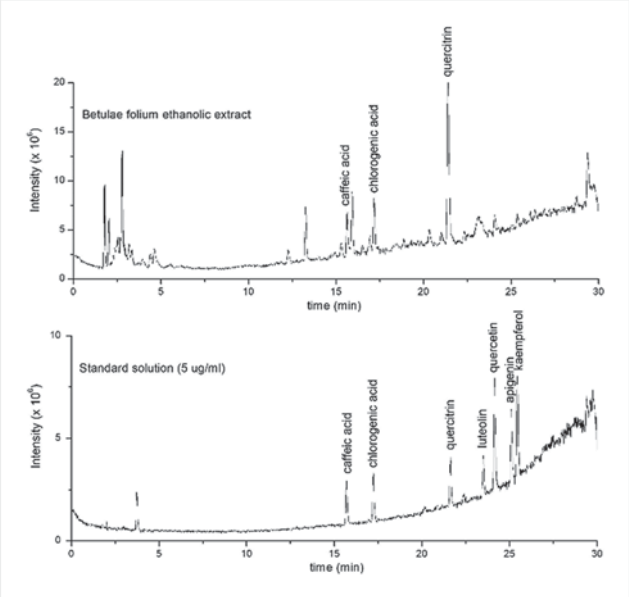
Flavonoids and polyphenols are widely distributed in medicinal plants and have positive health effects. The aim of this study were to determine the flavonoids and polyphenols with a spectrophotometrical and by an UHPLC-LC-MS/MS method [1].



▶ **Fig. 1**

Eighteen widely used medicinal plants were studied. Total flavonoids and total polyphenolics content were determined quantitatively by spectrophotometrical method from the methanolic and ethanolic (70⁰) extracts of plants. Additionally an UHPLC-LC-MS/MS method was developed for determination of seven standards from extracts. Detection was carried out in SRM mode (chlorogenic acid, caffeic acid, quercitrin, luteolin, quercetin, apigenin, kaempferol)l.

Medicinal plants are a rich source of flavonoids and polyphenolics. The seven standard were detectabled in high concentration from Hyperici herba, Betulae folium and Potentillae herba (Fig.1.).

There are a wide variety of phenolics and flavonoids and the obtained antioxidant values confirms a high antioxidant capacities of extracts. For the determination of polyphenolic profile the developed UHPLC-DAD-QTOF-MS/MS method is fast and acceptable. Polyphenols may be developed in veterinary products and used for animals.

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P-V-21 Effects of garlic (*Allium sativum* L.) in postweaning pigs – a placebo controlled study

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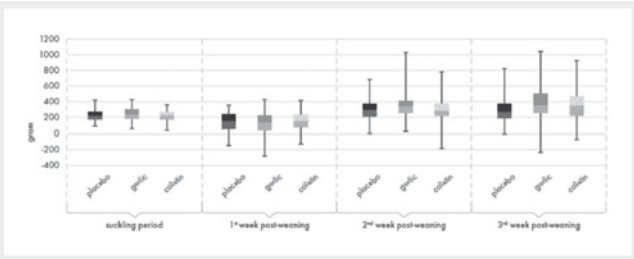
Postweaning diarrhoea (PWD) is one of the most serious problems in pig production, leading to a high antimicrobial use. Preventive oral administration of *Allium sativum* L. (AS, garlic) represents an option to reduce antimicrobial medication by improving health and performance in postweaning pigs. A trial was conducted on a commercial Swiss farm [1]. Six-hundred piglets were randomly assigned to three treatment groups. For the first two weeks post weaning, the piglets received orally 0.3 g dried AS-powder/kg body weight/day, 6 mg colistin-sulphate/kg body weight /day or a placebo (PL). Piglets were observed until the end of the third week post weaning. For the time of the study, daily weight gain (DWG) was measured weekly on individual basis. Data were analysed using generalized mixed effect models.

DWG of placebo-treated piglets was significantly lower compared to AS (61 g; p=0.008) and colistin-treated piglets (61 g; p=0.001) on the third week post weaning (▶**Fig. 1**). Due to severe diarrhoea, three out of nine (33%) of AS and the placebo pens were treated with antibiotics. In conclusion, oral administration of dried AS-powder does not prevent severe PWD but improves the growing performance compared to placebo. Thus, AS contributes to limiting the use of antimicrobials to strictly therapeutic indications.



▶**Fig. 1** Daily weight gain (in game per day) per treatment group from weaning until third week post-weaning.

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P-V-22 Ethnoveterinary use of medicinal plants in the treatment of claw diseases – a survey with farmers from 20 Swiss cantons

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DOI 10.1055/s-0039-3399748

Claw diseases and lameness constitute one of the most severe challenges in livestock production, both for animal welfare and economic reasons. From 2011 to 2014 we conducted five ethnoveterinary surveys in 19 German speaking Swiss cantons, and in the Italian speaking canton of Ticino. A total of 208 interviews were carried out with 273 farmers, 1671 use reports (UR) were recorded, comprising detailed information about plant species, plant part used, the manufacturing process for the end-product, dosing, administration, and therapeutic intention. Among them, 89 UR with 22 different plant species were specifically linked to claw diseases of cattle (80), goats (6), sheep (2) and pigs(1). For seven species more than five UR were reported. We determined the concentration of dry plant material in the final product (g herb/100g product; median, minimum-maximum): *Malva* ssp (22 UR (21 herb, 1 flower); 0.24g/100g; 0.002-1.82), *Matricaria chamomilla* L. (11 UR (10 flower, 1 herb); 0.46g/100g; 0.04-2.5), *Picea abies* (L.) H. KARST. (10 UR (resin); 24.7g/100g; 9.9-100), *Calendula officinalis* L. (7 UR (flower); 0.2g/100g; 0.0003-0.82), *Thymus vulgaris* L. (6 UR (herb); 0.02g/100g; 0.015-0.43), *Senecio ovatus* (P. GAERTN., B.MEY. & SCHERB.) WILLD. (6 UR (herb); 0.37g/100g; 0.07-0.57), and *Sanicula europaea* L. (6 UR (herb); 0.17g/100g; 0.07-0.27). *Senecio ovatus* could not be recommended due to the content of pyrrolizidine alkaloids. Besides, insufficient pharmacological data are available for *Sanicula europaea*. For the remaining five species antimicrobial, anti-inflammatory and wound healing properties are well known, and clinical data with extracts of these plants have been published before.

Main Congress Poster

Poster Session 1

P-001 A validated UHPLC-CAD method for quantitative determination of Astragaloside VII

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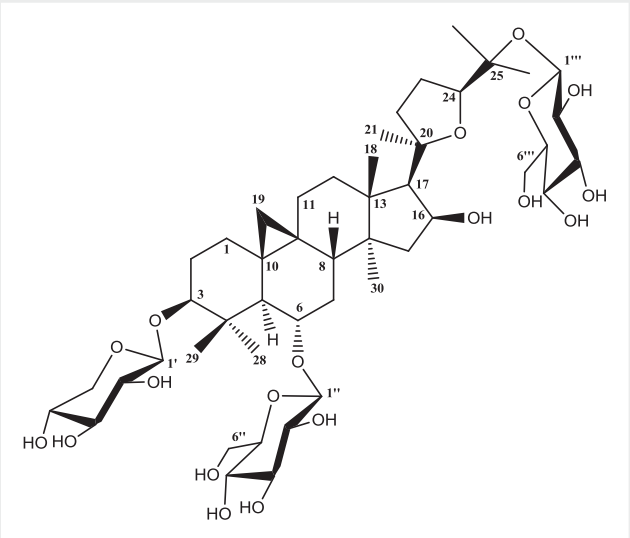
DOI 10.1055/s-0039-3399749

Astragaloside VII (AST VII) (▶**Fig 1**), the first tridesmosidic saponin identified in nature [1], possesses potent immunostimulatory/adjuvant effects [2]. Based on the promising adjuvant properties comparable to current adjuvants (i.e. Alum and QS-21), our team has decided to carry out further studies on AST VII including semi-synthesis studies to discover and develop new human/animal vaccine adjuvants [2–5].

Since more than 450 *Astragalus* species grow wildly in Turkish flora, one of the first challenges of this adjuvant development project is to examine these species by efficient analytical methods to find AST VII rich plant materials and select the rich species for possible cultivation and/or pilot production studies. Thus, aim of this study was to develop a UHPLC method coupled with the Charged Aerosol Detector (CAD) in order to determine AST VII content simultaneously, precisely and sensitively in *Astragalus* samples. A fifteen minutes method was developed using C18 (100 mm x 4 mm x 3 µm) column, eluting with gradient Water:Acetonitrile mixtures at 0.75 mL/min flow rate. The linear regression analysis of calibration plots showed good linear relationship with r^2=0.9995 in concentrations ranging from 52 to 208 µg/mL. The method was validated for its calibration curve, specificity, precision and robustness. The recovery was found to be in the range of 98.17 to 101.86%. As a conclusion, for the first time, a UHPLC method was validated to quantify AST VII utilizing CAD for its detection.



▶**Fig. 1** Structure of AST VII.

Acknowledgement This study was supported by TÜBİTAK (Project Number: 116Z958).

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P-002 Analysis of Herbofix® herbal infusions and comparative study with traditional infusions employing HPTLC, HPLC-DAD and UPLC-MS

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DOI 10.1055/s-0039-3399750

Herbofix® is a new line of herbal infusions in the form of capsules, creating an instant beverage with irresistible flavor and beneficial effects. The herbs used are carefully selected and harvested from the Mediterranean basin and elsewhere, e. g. ginseng, rosemary, yerba mate, lemongrass, hops, lemon balm, elderberry, peppermint, elecampane, turmeric, fennel and lemon verbena. The capsules are compatible with Nespresso® machines making the procedure simple and practical [1, 2]. The recognized properties of the botanicals provide an effective solution to everyday problems: energy, relaxation, respiratory and digestion. In the current study, the four different Herbofix® products were analyzed with HPTLC, HPLC-DAD and UPLC-MS. Characteristic peaks corresponding to the main compounds of the contained herbs were detected. Minor compounds were also identified. Furthermore, the commercial products were compared with infusions prepared with the traditional way (infusion for 1 min and 5 min) in order to detect similarities and differences between them. According to the results the yield of the extraction procedure is highest when the Nespresso machine is used compared to traditional infusion. The produced extracts were rich in bioactive compounds while differences could be detected depending on the type of the extraction.

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P-003 Aquaphotomics study of a resurrection plant *Haberlea rhodopensis*

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Haberlea rhodopensis is a resurrection plant with many applications in pharmacology, medicine and cosmetics[1]. Metabolic profiling of this plant revealed numerous bioactive components with many health benefits [2]. This vast richness of protective compounds actually enables it to survive long periods in completely desiccated state, and quickly and fully

recover upon rewatering [3]. However, the mechanism of how these components act together to protect the plant is not fully understood and is a subject of research.

Aquaphotomics is a novel scientific discipline, concerned with studying water, its structure and roles in biological systems[4]. It uses light-water interaction as a source of information and thus allows completely non-destructive, rapid, real-time measurements. Numerous aquaphotomics studies on plants revealed interesting roles of water molecular species in plant tissues which provided rationale to apply aquaphotomics methodology to study the water in leaves in *H. rhodopensis* plants during desiccation and subsequent rehydration in order to understand the mechanism of protection of its tissues against the damage and survival in the dry state[5].

The study discovered that together with rapid loss of water this plant performs dynamic control of water molecular structure keeping it in certain state. In the completely dried state *H. rhodopensis* plant drastically diminished free water molecules, cutting down the possibility for biochemical reactions, and accumulated water dimers and water molecules with 4 hydrogen bonds. In summary, what was concluded is that the protective compounds act synergistically to shape the water in plant tissues in a way which enables “drying without dying”.

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P-004 Chemical profiling of *Croton gratissimus* Burch

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Croton gratissimus is widely used in traditional medicine to treat a range of conditions, including hypertension, diabetes, arthritis, malaria and urinary tract infections. The aim of this study was to isolate and identify chemical markers for this species and to determine their concentrations in different populations.

Aerial parts of *C. gratissimus* (n =72) were collected in the Gauteng, Limpopo and Mpumalanga provinces of South Africa. Liquid chromatography coulped to mass spectrometry (LC-MS) method was developped and three major compounds were targetted as chemical markers. Preparative high performance liquid chromatography (prep-HPLC) system was used to isolate marker compounds (major peaks). Method development and validation was carried out using Waters Xevo G2-XS QTof quadrupole Time-of-Flight mass spectrometry. High performance thin layer chromatography (HPTLC) profiling was carried out using a CAMAG HPTLC instrument.

Three flavonoids were isolated and identified using nuclear magnetic resonance (NMR) spectroscopy as iso-orientin [1], trans-tiliroside [2] and 6′-O-(4″-methoxy-trans-cinnamoyl)-kaempferol-3-β-D-glucopyranoside [3]. The coefficients of determination (R2) were above 0.999. The limits of detection varied from 0.18-0.48 µg/g, the limit of quantification from 0.16-0.53 µg/g) and an intraday precision of 0.6-2.2% was obtained. The concentrations of the three compounds in the extracts ranged from 0.90-2.27mg/g dry mass [1], 1.68-4.11 (mg/g) for [2] and 0.08-0.39 mg/g [3] which indicates significant variation. The HPTLC fingerprint revealed the presence of the three biomarkers in all the samples investigated.

The methods developed contributes significantly towards the quality control of *C. gratissimus* samples.

P-006 Determination of perillaldehyde in perilla herbs based on relative molar sensitivity (RMS) using a combination of ^{1}H-quantitative NMR and HPLC/UV

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^{1}H-quantitative NMR (^{1}H-qNMR) has become a powerful tool for producing absolutely pure reference standards (RSs) for HPLC. However, when the RS is unstable, the purity gradually declines before HPLC is performed and creates problems in generating reliable quantitative data. To address this, a method using a stable RS with relative molar sensitivity (RMS) to analyte has been reported [1]. In this study, we used a stable RS for the determination of the unstable compound perillaldehyde in perilla herbs, which is listed in the Japanese Pharmacopoeia (JP). We applied an off-line combination of ^{1}H-qNMR and HPLC/UV to estimate the RMS of the analyte (perillaldehyde) to the RS. Diphenyl sulfone (DS), an inexpensive and stable reagent with a high purity, was selected as the RS. The response ratio (Rr) of the analyte to the RS, obtained by HPLC/UV, was corrected using the molar ratio (Rn) obtained by ^{1}H-qNMR. The experiment was validated using the same sample in five NMR and seven HPLC instruments at five institutes. The average RMS (Rr/Rn) was 0.983 and the reproducibility relative standard deviation (RSD) was 1.027%. The perillaldehyde content of perilla herbs determined based on RMS was identical to that determined by the assay listed in JP. These results revealed that the quantitative assay for perillaldehyde in perilla herbs did not require an easily decomposable perillaldehyde reagent and that the method using the RMS of perillaldehyde to DS was thought to be acceptable to JP.

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P-007 Development of a fast and efficient separation method of secondary metabolites from *Rhodiola rosea* roots by high performance countercurrent chromatography

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Extracts of the dried roots of *Rhodiola rosea* L. (rose root) are traditionally used for their adaptogenic properties, more precisely for their antidepressive, anti-fatigue, anxiolytic, cardioprotective, CNS stimulating, neuroprotective, and nootropic effects [1,2]. *Rhodiola rosea* has also been shown to increase the lifespan of model organisms such as *Drosophila melanogaster* [3].

Avoiding the problem of irreversible adsorption to stationary phases, high performance counter current chromatography (HPCCC) is a highly suited separation method providing higher resolution rates compared to conventional chromatographic techniques [4]. To gain access to the full spectrum of a wide range of diverse metabolites in a standardized rose root dry extract obtained by 70% ethanol extraction, a fast and efficient HPCCC method was developed. For the newly established normal phase HPCCC method a two-phase solvent system consisting of ethyl acetate, *n*-butanol and water with a gradient ranging from 27.26/20.69/52.05 to 29.01/19.19/51.80, respectively, was used. Thirteen fractions were obtained based on their TLC pattern. Their dereplication was performed by UPLC-ESI-MS analysis, which led to the identification of phenylethanoids, phenylpropanoids, several phenolic compounds and their glycosides in enrichments ranging from ~50 to 70% within one fractionation step out of the subjected crude extract.

The herein established HPCCC-based fractionation method enabled the preparative enrichment of major and minor constituents in one separation step, which was used for efficient further orthogonal chromatographic isolation of eight rose root metabolites for the determination of their not fully captured bioactivity spectrum.

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P-008 Development of an HPLC-MS/MS multi-method for the detection of sesquiterpene lactones and polyphenols occurring in Arnica plant materials

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Arnica montana is a flowering plant native to Central, Eastern and Northern Europe, for which Germany bears a special responsibility within the EU program "Natura 2000" [1] and the German program "Bundesprogramm Biologische Vielfalt – Verantwortungsarten" [2, 3]. In recent years, populations of this important medicinal plant in Germany and throughout Europe have collapsed dramatically, especially in lowland locations. Within the *Arnica montana* population there are different genetic varieties which have to be considered for the conservation of biodiversity and for the maintenance of the reproductive capacity of the plant. An open question is whether these genetic varieties also differ in their secondary metabolite profiles.

The aim of this study is to develop an analytical method for the detection (contents and profiles) of sesquiterpene lactones in particular as well as some representative flavonoids and caffeic acids in flowers and leaves of *Arnica montana*.

For this purpose, first an in-house database of sesquiterpene lactones, flavonoids and caffeic acids present in *Arnica montana* was established on the basis of literature data. Subsequently, fragmentation patterns of precursor ions of ingredients were measured in product ion scan mode from extracts of *Arnica montana* plant material (flowers and leaves) in positive and negative ionization mode. From the spectra, characteristic fragment ions were selected for the development of an HPLC-MS/MS multi-method. HPLC gradient conditions and MRM-MS parameters were optimized. The methodology for the quality controlled quantification of major constituents and identification of suitable reference compounds as biomarkers for high throughput analysis of *Arnica montana* varieties is in progress.

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P-009 DNA barcoding analysis: quality control of published DNA sequences

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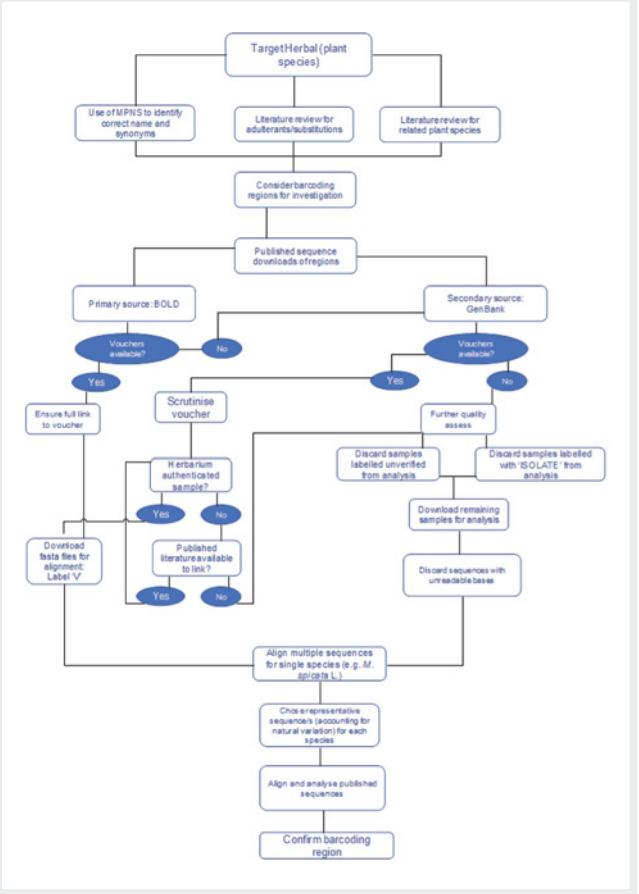
DNA barcoding methods are an important addition to the identification and quality testing of herbal medicines when applied correctly [1].

A robust and complementary method in identifying plant materials is the use of DNA barcoding which, when applied within a quality framework [2], forms a key component of the successful authentication of herbal drugs [3, 4]. However, as with any methodology, high quality reference information is required. The open access nature of DNA databases has provided an abundance of sequence data but has also created the issue of incorrectly labelled sequences and unverified data. In this



▶ **Fig. 1** Quality control method used to assess publicly available DNA sequence data.

study these issues are highlighted via an investigation of published *Mentha spicata* L. sequences, and a guideline for the quality assessment of sequence data introduced.

Data were collected from two online databases relating to *M. spicata* and two barcode regions, the ITS2 and *trnH-psbA*. As would be expected, variation was found between the published sequences. However, several sequences appeared to be mislabelled, and on investigation of some accompanying manuscripts a link to unverified material was shown.

To quality control this issue, several steps were taken to critically evaluate sequence data. This was then further developed into a guideline for the assessment of published sequences (▶**Fig. 1**) which can be used for any investigation.

It is clear that publicly available sequences should be subject to such scrutiny and quality control for best practice. Utilising this method, an operator can gain a higher level of confidence in the published sequences used and a harmonised approach during data analysis.

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P-010 Effect of electromagnetic radiation on the phenolic content of mexican arnica (*Heterotheca inuloides* Cass.)

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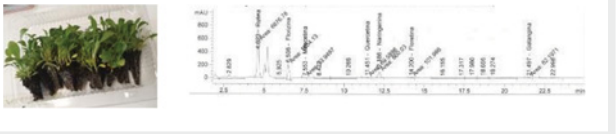
Mexican medicinal and aromatic plants have potential for their different uses and applications. Such is the case of Mexican arnica, which has reported different biological activities [1,2]. This attributed to its chemical composition, mainly the phenolic compounds. The study of the application of biophysical methods in the agricultural sector has increased. In particular, treatments using electromagnetic field [3]. There is a history that the quality of electromagnetic radiation can affect pigment biosynthesis [4]. With that background the objective of this work was to apply electromagnetic field to plants of Mexican arnica (*Heterotheca inuloides* Cass.) and evaluate their impact on their content of phenolic compounds. Arnica seedlings obtained in Ozumba State of Mexico, were subjected to electromagnetic field treatments during eight exposure times: 30 sec 1, 5, 10, 15, 20, 30, 45 minutes and a control treatment. Three applications of the treatments were made every 15 days. The seedlings were transplanted in the field at room temperature. The content of phenolic acids and flavonoids were obtained by HPLC (▶**Fig. 1**). There were significant differences between treatments, the highest accumulation of gallic acid (20 min exposition; 0.79 µg/mL), chlorogenic acid (45 min exposition; 222 µg/mL), syringic acid (30 min;1.61 µg/mL), vanillic acid (20 min exposicion;1.35 µg/mL), caffeic acid (30 min exposicion;1.53 µg/mL), rutin (10 min exposition; 918 µg/mL), phloridzine (10 min exposition; 5845 µg/mL), miricetin (10 min exposition;25.35 µg/mL), quercetin (5 min of exposition, 11.82 µg/mL).



▶**Fig. 1** Chromatogram for the identification of Mexican arnica flavonoids by HPLC.

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P-011 High-performance liquid chromatography for analysis of corosolic acid in *Lagerstroemia* species and their hypoglycemic activities

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Metabolic syndromes are one of the global health problems. Functional foods with α-glucosidase inhibitory properties is a complementary choice for diabetes patients. *Lagerstroemia speciosa* has been applied traditionally for an antihyperglycemic purpose in traditional medicines. Corosolic acid is considered as bioactive constituents for glucose lowering effect of *L. peciosa* [1, 2]. In the present study, we aimed to determine corosolic acid in *Lagerstroemia* species using the developed HPLC-UV method and their α-glucosidase inhibitory potency. In addition, we developed the new source of corosolic acid using the plant tissue culture technique. According to analytical performance including precision, sensitivity, and accuracy, the developed HPLC-UV method is reliable and applicable for corosolic acid determination of the *Lagerstroemia* species. The compound exists in varied amount in different *Lagerstroemia* species (0.21 ± 0.01 to 3.77 ± 0.17 mg/g dry wt.). The mature leaves composed the highest content. Although standardized extract usually prepared using *L. speciosa*, our results revealed that *L. macrocarpa* and *L. loudonii* contained much higher amounts of corosolic acid. In addition, the callus culture of *L. speciosa* also produced high content of corosolic acid. The corosolic contents of investigated *Lagerstroemia* species with α-glucosidase inhibitory activity. Therefore, this method is worth for antidiabetic standardization of *Lagerstroemia* derived materials.

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P-012 *Hypericum perforatum* quality of raw material: coming full-circle

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Saint John's wort (*H. perforatum* L., SJW) products are widely used in different forms and regulated by different legislations. Previous studies highlighted the significant variability in the quality of marketed products of different provenance [1, 2]. In addition to extremely low concentrations of hypericins and the detection of dyes, a number of samples were found to showcase chemical fingerprints distinct from the pharmacopoeial description. This project aims to establish baseline standards for high quality materia prima including an understanding of the natural variability. Therefore, a collection of raw materials from 14 countries was analysed using NMR-based metabolomics profiling and HPTLC. A number of samples' heavy metal content was determined. The analysis of the raw materials showed some degree of variability, which is non-significant compared to the marketed products' variation. The chemical differences noticed where identified, disambiguated and preliminarily assigned to 3 fingerprint types. The existence of four geographically distinct subspecies of SJW [3] was investigated as a potential factor behind these differences and an attempt to build a statistical model to predict the provenance of the samples based on NMR results was made. The difference between the legal limits for cadmium prescribed by the European Pharmacopoeia and WHO, respectively, highlights the need for regulatory concordance. Understanding the materia prima remains a challenge due to the numerous factors involved. Therefore, regulatory agencies need to consider and assess this complexity, in order to resolve quality challenges in the first step of the value chain.

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P-013 Integrated approach for the extraction and quality assessment of fibre-type *Cannabis sativa* L. based on UPLC-PDA and HPTLC

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Recent research has emphasised the promising therapeutic potential of fibre-type *Cannabis sativa* L. (hemp) and related phytocannabinoids [1]. Among the most abundant and valuable non-psychoactive cannabinoids are cannabidiol (CBD) and cannabidiolic acid (CBDA), both exhibiting a wide spectrum of pharmacological activities [2]. Efficient analytical methodologies for cannabinoids determination are thus crucial for hemp quality testing and utilisation [3]. In this context, the present study aimed to develop an integrated procedure for the effective extraction and quantitative analysis of the major cannabinoids in hemp.

Ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and dynamic maceration (DM) were examined using EtOH as reference solvent [4]. UAE displayed superior performance and was further optimised through Design of Experiments (DoE), maximising the recovery of cannabinoids. Rapid and reliable quantification of principal cannabinoids was achieved by means of UPLC-PDA. The method was developed and validated utilising highly-pure cannabinoids isolated by centrifugal partition chromatography (CPC) [5]. Several extraction solvents were also evaluated using the optimised UAE protocol and UPLC-PDA. HPTLC densitometry combined with chemometrics was implemented for classification of the extracts obtained with different solvent types to ensure a broad range of bioactive compounds.

In this work, a highly-efficient UPLC-PDA method coupled with a simplified UAE-based extraction protocol is presented for the quantification of the principal cannabinoids in hemp. The proposed HPTLC methodology enables the rapid and cost-effective fingerprint analysis for solvent selection.

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P-014 Integrated NMR-based profiling and HPLC-DAD analysis for Extra Virgin Olive Oil (EVOO) authentication assessment

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Extra Virgin Olive Oil (EVOO) possesses a high-value rank in the food industry, thus making it a common target for adulteration [1]. Therefore, several methods have been made available over the years. However, the issue of authentication remains unresolved with several national food safety organizations struggling to choose the most reliable one. Over the course of this study, the aim was to determine the origin of EVOOs suggesting a fast method that could easily be adopted. An established HPLC-DAD method focusing on EVOO polyphenols was used to reinforce the NMR based metabolic profiling.

Samples from three Mediterranean countries, Spain, Italy and Greece, as well as blended samples were analyzed with HR-NMR spectroscopy and with HPLC-DAD after hydrolysis aiming to quantitate both free and bound forms of hydroxytyrosol (HT) and tyrosol (T). The NMR spectra were recorded, preprocessed and underwent multivariate statistical analysis leading to the discovery of certain biomarkers related to the classification of the samples based on their geographic origin. In addition to the exploitation of the entire oil's composition, statistical correlations were made between the NMR metabolite profiling and HPLC

quantitative data of HT and T which are also related to the health claim issued by EFSA [2].

In conclusion, EVOO was classified according to its origin using NMR spectroscopy and was combined for the first time with a more established method in food analysis, that is HPLC-DAD. Finally, specific biomarkers were identified (i.e. aldehydic derivatives of the hydroxytyrosol and tyrosol moiety, like oleacein and oleocanthal, and triterpenoids such as cycloartenol), which are characteristic of each geographic origin.

S. Beteinakis wishes to thank the IKY foundation. The authors are grateful to the EU Programme 'Olive-Net'.

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P-017 Matrix free laser desorption ionization as a versatile tool for the chemical characterization of complex mixtures of phenolic compounds

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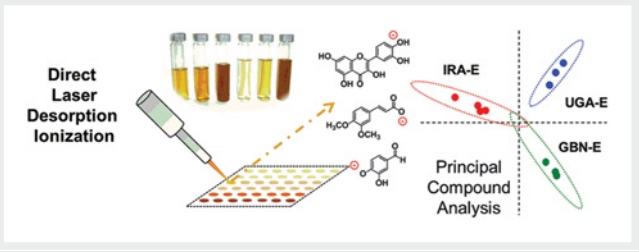
The rapid and precise chemical characterization of complex mixtures of small molecules is essential for contemporary natural products' (Nps) research, as well as for the quality control of herbal medicines and dietary supplements. While LC-MS is generally considered as method of first choice to meet these analytical challenges, the current work will present matrix free laser desorption ionization (LDI) as a versatile supplement or potential alternative to this approach [1, 2].

Among others, particularly phenolic NPs exhibit close structural similarities to matrices used in matrix assisted laser desorption ionization. Consequently, these compounds may be ionized by laser irradiation without matrix support. This concept is being outlined by two examples:

The first focuses on the chemical profiling of propolis obtained from different global regions, where high resolution LDI-MS permitted the identification of 34 compounds (mostly cinnamic acid and flavonoid derivatives) from a crude extract of French propolis. A contemporaneously performed LC-ESI-MS experiment solely detected 23 of these compounds. Moreover, LDI-MS facilitated the differentiation of propolis samples according to their origin by principal compound analysis (PCA). The second example discusses the LDI-monitoring of the metabolic profiles of apple seedlings (*Malus x domestica*) after being treated with various elicitors. Selected plants were further infected by *Erwinia amylovora*, the causative agent of fire blight. Again LDI-MS permitted the detection of key-metabolites (e.g. caffeic acid, phloridzin, and quercitrine) and also facilitated PCA grouping according to the applied treatment.

These results highlight the interesting prospective of LDI-MS as powerful analytical tool and supplement to existing methods.



▶ **Fig. 1**

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P-018 Metabolomics approach for discrimination of water extracts of Citrus-type crude drugs using NMR and HR-LC-MS

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A metabolic study on the differentiation of Citrus-type crude drugs (CCDs) was carried out for quality evaluation. Recently, we reported approximately five possible classifications for methanol extracts of CCDs based on metabolomics using NMR [1], and compared with HR-LC-MS based on metabolomics [2]. In this study, we analyzed water extracts of CCDs using NMR and HR-LC-MS for metabolic discrimination, and compared their contributors with that of methanol extracts and their discrimination because CCDs are usually used as the ingredient of decoction in the traditional Japanese medicine. The water extracts of four CCDs (33 samples); Kijitsu, Tohi, Chimpi and Kippi, were analyzed using ^{1}H- and ^{13}C-NMR and HR-LC-ESI-MS. These data were processed using different analysis software and the resulting data sets were then imported into a multivariate statistical analysis software. The PCA score plots indicated the discrimination of the water extracts of CCDs into the same three groups in both NMR and LC-MS analyses. This discrimination was the same as the results of methanol extracts. In the loading plots, naringin and neohesperidin were identified as contributors for discrimination by both NMR and LC-MS analyses. α- and β-Glucose and sucrose were identified as contributors by the NMR only. Limonene, identified as a contributor in the methanol extracts of CCDs was not identified as a contributor in the water extracts. In contrast, four flavonoids and two coumarins were identified as contributors by the LC-MS only. These results suggested that the combined NMR- and LC-MS-based metabolomics are applicable to discriminate water extracts of CCDs.

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P-019 NIR spectroscopy in simulation – a new way for augmenting near-infrared phytoanalysis

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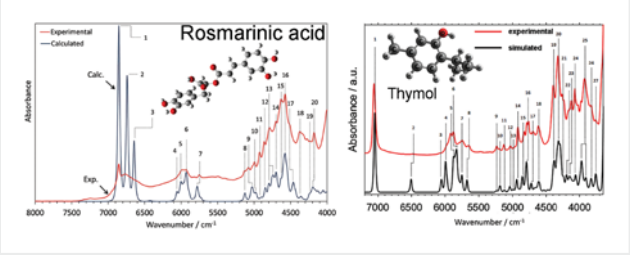
Near-infrared (NIR) spectroscopy is a powerful tool for qualitative and quantitative phytoanalysis. The backbone of NIR spectroscopy, multi-variate data analysis, provides no physical insight into the molecular system. In our research [1] we employ the methods of computational chemistry to unveil the origins of NIR bands, and to establish the basic relationship between the wavenumbers influential in quantitative models, and the underlying molecular background. Examples of substances with importance in phytoanalysis are presented, rosmarinic acid [2,3] and thymol [4], as prototypic polyphenol and monoterpene.

NIR spectra simulations not only provide deep understanding of the spectral bands. In addition, the features of quantitative models obtained in analytical routines may be interpreted. Fundamental relationships with the basic factors may be established, e.g. how the sensitivity of the molecule to its chemical environment is reflected in the models, and thus an understanding of how these factors affect the analytical spectroscopy is obtained.

This work was supported by the Austrian Science Fund (FWF), M2729-N28.



▶**Fig. 1**

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P-020 Online supercritical fluid extraction – supercritical fluid chromatography – diode-array detection: an interesting approach for investigation of milk thistle (*Silybum marianum*) preparations

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Online supercritical fluid extraction – supercritical fluid chromatography – diode-array detection (SFE-SFC-DAD) is a new and innovative analytical tool that allows fully automated sample preparation followed by chromatographic separation in a single flow path. Among its ecofriendly character, rapid extraction and analysis, as well as reduced artificial operation errors make it to an interesting approach for natural product analysis [1, 2]. The purpose of this study was to investigate its suitability for rapid quality control of commercially available milk thistle preparations. These preparations are widely used due to hepatoprotective abilities credited to the contained silymarin, an isomeric mixture of flavolignans [3]. During method development, a multitude of parameters associated with the online coupling of extraction and analysis had to be investigated. A chiral amylose carbamate column was identified as ideal stationary phase, as it traps the analytes at the head of the column until the end of the extraction and subsequently enables rapid chromatographic separation of the selected marker compounds (silydianin, silychristin, silybin A, silybin B, isosilybin A, isosilybin B). Suited extraction parameters had to be conducted based on their extraction efficiency and their influence on the chromatographic resolution. Best results were obtained by using 20% ethanol as modifier for 15 min, at a temperature of 40°C and a pressure of 150 bar. Finally, the developed online SFE-SFC-DAD method had been applied to the qualitative analysis of different milk thistle preparations. With a total runtime of 30 min, including extraction as well as analysis, the assay provides an interesting approach for further investigations.

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P-021 Optimization of extraction condition for flavonoids in Genkwa Flos using response surface methodology

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Genkwa Flos is the flower buds of *Daphne genkwa* Sieb. et Zucc. (Thymelaeaceae)a traditional medicine in Korea and China [1, 2]. enkwanin is characterized as the reference phytochemical for the quality control of this herbal medicine in Chinses and Taiwan Pharmacopoeia. ive flavonoids including genkwanin in the Genkwa Flos using ultra-performance liquid chromatography coupled with a photodiode array detector. o optimize the condition of extraction, which mapped out a mixture matrix design (MMD) for extract solvent and three-level full factorial design with regression analysis [3]. Water, methanol, and acetone were as the extraction solvents for MMD and the extraction method. Following multiple regression analyses of, 3D response surface and the contour plots to determine the optimal conditions. extractsolvent44% water, 56% acetone, and 0% methanol12 hours150 rpm70℃. This work proposed a method for the optimal extraction of flavonoids in Genkwa Flos and

the determination of five flavonoids apigenin and genkwanin by UPLC-DAD.

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P-022 Phytoanalytical profiling of *Cassia auriculata* by LC-PDA-ESI-MS / MS and HPTLC supporting its metabolic claims

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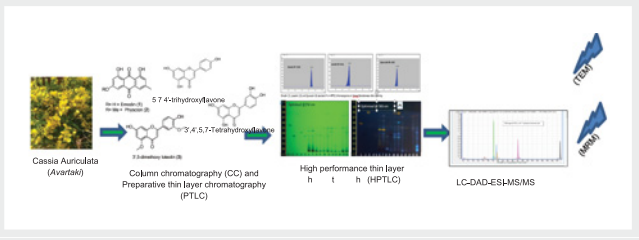
DOI 10.1055/s-0039-3399767

Cassia auriculata Linn (CA) (*Avartaki*) is a traditional Indian Ayurvedic medicine used in the treatment of Diabetes mellitus (DM). Several preclinical studies have reported anti-hyperglycaemic, cardioprotective, anti-hyperlipidaemic, and anti-inflammatory activities suggesting potential of CA in diseases associated with metabolic syndrome.

This study provides important clues on the presence of possible compounds responsible for the pharmacological activity of the seeds.

With the help of column chromatography (CC) and Preparative thin layer chromatography (PTLC) 13 compounds isolated from CA, these have been found single and synergistic mild to potent antidiabetic in nature. The isolation and quantitation showed the presence of anthraquinones like 6-methyl-1,3,8-trihydroxyanthraquinone,1,8-Dihydroxy-3-methoxy-6-methyl-9,10 anthraquinone and flavonoids like 3′,4′,5,7-Tetrahydroxyflavone, 5,7,4′-trihydroxyflavone with quercetin, quercetin-3-O-rutinoside with common phenolics in CA.

High performance thin layer chromatography (HPTLC) studies used to differentiate CA from other plants like *Cassia alata, Cassia occidentalis, Cassia siamea, Cassia angustifolia* and *Cassia fistula*. The marker compounds showed good separation and resolution with distinct pattern of each variety noted for addressing adulteration with quality control. We also identified 39 compounds first time in CA by target extraction mass spectrometry (TEM) technique in LC-MS-MS. The optimised Multiple reaction monitoring (MRM) technique by LC- MS MS developed for 7 phytochemicals isolated from CA. Compounds detected in samples confirmed by qualifier ions and the ion ratio, with fragmentation pattern. With isolation and identification compounds, we have generated data about chemistry of drug. Using LC-PDA-MS-MS, the presence of bioactive markers has established in this drug in a quantitative validated way



▶ **Fig. 1**

P-023 Qualitative and quantitative LC-MS analysis of different Rhodiola rosea rhizome extracts

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Rhodiola rosea L. (Sedum roseum (L.) Scop.) is an herbaceous plant in the family Crassulaceae, forming thick rhizomes. Ethanolic macerates of its underground parts have been used in folk medicine for a long time [1]. Adaptogenic properties were confirmed in rats and rabbits in vivo [2-4]. Main compounds thought to be responsible for its activities are phenylethanoid glycoside salidroside and a group of cinnamyl alcohol glycosides, rosarin, rosavin and rosin [5].

Rhodiola rosea rhizome batches collected at two mountainous habitats in Carinthia (Austria) on three time points over the vegetation period of 2015 were prepared as ethanolic macerates (38, 70, 96% V/V) and 85% methanolic ASE extracts. They were qualitatively and quantitatively analyzed by UHPLC-PDA-ESI-MS, using a C-18 column (Agilent ZORBAX SB-C18, 2.1x100mm, 1.8µm) and gradient elution with acetonitrile/water + 0.1% formic acid. It was aimed to compare characteristics and effectivity of different extraction methods.

18 compounds were identified in methanolic ASE extracts of Rhodiola rosea. Methanolic ASE of freeze-dried plant material produced highest yield in salidroside, cinnamyl alcohol glycosides and flavonols. Among macerates of fresh plant material, 96% ethanol was the preferable solvent. Macerates of higher ethanol content showed increased yield and lowered hydrolysis of glycosides during extraction.

Rhodiola rosea is a great source for different substances of potential pharmacological relevance. Rhizomes of wild origins seemed to underly great variability in chemical composition dependent on growth site.

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P-024 Quality of cranberry-derived products: one HPTLC method for identification and detection of adulterants

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In the past decade Cranberry (*Vaccinium macrocarpon* Aiton) products became one of the most popular herbal dietary supplements for treatment of urinary tract infections. This popularity has resulted in numerous cases of adulteration, particularly with low-cost sources of anthocyanins, flavonoids and proanthocyanidins [1].

The aim of this study was to establish an HPTLC method for identification of cranberry-derived ingredients and detection of different known adulterants, based on multiple derivatization steps and detection modes.

A new HPTLC method was developed. A SPE cartridge was used to remove sugars and increase the detectability of flavonoids, catechins and anthocyanins. The new method, featuring a different mobile phase, detection modes and 2-derivatization steps, was tested with 52 cranberry-derived ingredients. Seven known adulterants were included in the analysis: bilberry and blueberry fruits, hibiscus flower, grape seed and skin, peanut skin, and pine bark extracts. Most of the samples show a fingerprint similar to that of cranberry juice. Nine samples of both, pomace and fruit, show no zone due to anthocyanidins. The method was capable of distinguishing all adulterants. Two commercial extracts presented zones characteristic for peanut skin and bilberry fruit and lacked cranberry zones. Those adulterations were not detected by HPLC.

The use of multiple detection in the HPTLC method allows verifying the identity of cranberry ingredients and also the detection of several types of adulteration, without additional chromatography. The novel method was proposed to the US pharmacopoeia to be included as identification method of cranberry-derived ingredients.

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P-025 A plant used in Mexican traditional medicine improves colitis due to regulation of antioxidant mechanisms

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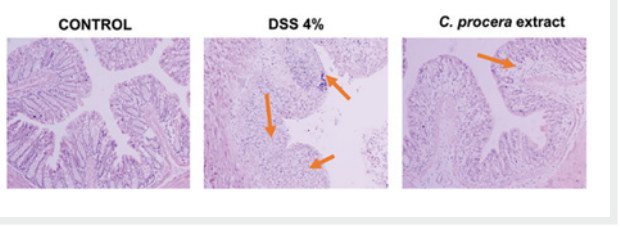
Cyrtocarpa procera, known as "Chupandilla" is a plant used for gastrointestinal problems in Mexican traditional medicine[1, 2] Not only ethnobotanical use of this plant has been reported, but antibacterial, antifungal and antioxidant properties had been described for Chupandilla's extract [1]. It´s well known that oxidative stress is one of the most important factors for both progression and severity of colitis, due to an impairment of antioxidant defenses [3]. Here, we evaluated the antioxidant effect of Chupandilla's methanolic extract in a DSS-induced colitis.

DSS dissolved in drinking water was used for colitis induction on female BALB/c mice.

Compared to untreated mice with colitis, mice with Chupandilla's extract presented an increase in survival rate, less severe colitis symptoms like diarrhea, bleeding and weight-loss.

At microscopical level, Chupandilla's extract greatly reduced loss of crypts architecture and cellular organization and inflammatory infiltrate.

In terms of oxidative stress, treated group with extract also showimprovements; antioxidant enzymes likeno loss of activity in colon, while mice presented considerable low activities. Chupandilla's extract prevented activity dual role in both inflammatory and oxidative stress processes during colitis.



▶ **Fig. 1**

C. procera extract, presented good *in vitro* antioxidant capacity, probably due to the presence of polyphenolic compounds, in particular flavonoids, which had been reported anti-inflammatory and antioxidant activities. Crhysin, naringenin, kaempferol and catequin were the flavonoids identified in *C. procera* extract. Thus, the improvement of UCcould be mechanisms of compounds.

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P-026 Quantification of Silymarin in *Silybum marianum* with near-infrared spectroscopy: a comparison of benchtop vs. handheld devices

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Silybum marianum is part of the Asteraceae family and widely used for its regenerative effect on toxic liver diseases for which silymarin, a sum parameter of six flavanonol derivatives, has the main responsibility [1]. While the European Pharmacopeia suggests a Soxhlet method for silymarin extraction, the required 16 hours are excessively time-consuming [2].

As near-infrared spectroscopy (NIRS) is known for its fast and non-invasive measurements [3, 4], the aim of this study is the development of a time-saving and cost-efficient alternative for quantification of silymarin and its flavanonol derivatives in milk thistle seeds. Additionally, the quality of a handheld compared to benchtop NIR spectrometer is investigated.

Milk thistle seeds were measured in milled and ground state with one benchtop and two handheld spectrometers. Using chemometric pre-treatment partial least square regression (PLSR) models were calculated and quantified with cross validation (CV). In the example of silymarin the benchtop device NIRFlex N-500 (Büchi, Flawil, Switzerland) gave the best results both for milled and ground samples (RMSECV between 0.01 and 0.17%), though the MicroNIR 2200 (Viavi Solutions, Milpitas, USA) provided a similar performance (RMSECV between 0.01 and 0.18%). Resembling results were gained by the microPhazir (Thermo Fisher Scientific, Waltham, USA) (RMSECV between 0.01 and 0.23%), only the ground samples gave no satisfactory output.

This study proves that NIRS offers an alternative for the quantification of silymarin and its flavanonol derivatives. Soxhlet extraction cannot only be replaced by measurements with benchtop devices but even handheld spectrometers with their possibility of on-field measurements offer a good choice.

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P-027 Quantification of small quantities of a standardized Dry Grape Extract in complete feed using UHPLC-MS/MS

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A specific and sensitive method for quantification of small quantities of a standardized Dry Grape Extract in complete feed based on UHPLC-MS/MS has been developed and validated. The standardized Dry Grape Extract studied is a natural commercial feed additive (Nor-Grape®, Nor-Feed, France) used in animal nutrition, consisting of a complex mixture of grape seed extracts and grape skin extracts in which malvidin-3-O-glucoside (M3OG) has been identified and quantified. M3OG was used as phytomarker for the quantification of the additive in feed. Key steps in the preparation of the sample are the use of several successive and selective extractions allowing firstly to eliminate a large number of undesirable molecules and in a second time to recover and concentrate the anthocyanins of the additive present in the sample. The use of an internal standard (cyanidin-3-O-sambubioside) makes it possible to compensate for the losses of the analyte during sample work-up. M3OG quantification is performed by the standard addition method to compensate **ion suppression during HPLC-MS/MS analysis.** The method was developed and validated in-house in accordance with the guidelines recommended by IUPAC (selectivity, calibration and linearity, trueness, precision, recovery, limit of quantification, measurement uncertainty) [1] for the quantification of a small dose of the standardized Dry Grape Extract in complete feed (30ppm). This patented method of analysis has all the prerequisites to be used by European authorities as part of the registration of a feed additive [2].

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P-028 Quantitative determination of fatty acid content in *Sclerocarya birrea* A.Rich.Hochst (Marula) seed oil using MIR calibration models

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The quality control of seed oils is an important aspect to consider. Natural seed oils have received much attention as cosmetic ingredients, due to the purported folkloric benefits to the skin. Marula oil is a cold-pressed light-nutty oil included in cosmetics due to the abundance of fatty acids. The commercialisation of Marula seed oil as a natural ingredient in cosmetic products necessitates the need for quality control procedures to ensure the supply of good efficacious oil. There are currently no standards available for the quality control of Marula seed oils. Hence, the study was undertaken to provide some insight into the chemical variation and to investigate the feasibility of using mid-infrared (MIR) spectroscopy as a simple and non-destructive technique for the quantification of the major fatty acids in Marula seed oils. A comparative study was conducted using 1D and 2D chromatography as reference methods. Spectra of the oil were acquired using a Bruker® Alpha-P MIR spectrometer. Partial least squares (PLS) regression models were developed based on MIR data. The calibration models revealed good correlation between the MIR data and 1D-GC values (R^2 > 0.80). The predictive ability of the models (Q^2_{cum}) was greater than 0.50. In contrast, the correlation between 2D-GC and the MIR data was low, with R^2 values ranging between 0.17 and 0.30. The predictive ability of the models was low (Q^2_{cum} < 0.50). In this study, MIR spectroscopy was identified as a good alternative quality control method, since the technique yielded promising results.

P-029 Quantitative metabolomics of Iranian saffron based on their HPLC-DAD and MALDI-TOF-MS

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More than 85% of saffron (Crocus *sativus* L.) is producing in 22 region of Iran [1]. The quality control and its geographical effects is the main concern behind it. In this study, we developed a chromatographic fingerprint of 22 type saffron from 22 regions of Iran. Multivariate clustering methods of PCA and HCA are used for finding similarities and dissimilarities in three wavelengths 440, 308, 254 lead to the samples divided in different class base on their HPLC fingerprints. Then as a reliable, sensitive and fast method of analysis, MALDI-TOF-MS introduced for simultaneous determination of crocins, safranal and picrocrocin. Eventually and according to International Committee of Harmonization (ICH) regulation for the validation of analytical methods parameters including linearity range for crocins, picrocrocin and safranal was obtained at the range of 22-200, 32-200 and 22-100 ng/spot respectively. Also limit of quantification (LOQ) for crocins, picrocrocin and safranal were 7.18, 10.71 and 6.76 ng/spot respectively. Finally, this work is introducing MADLI-TOF as a very fast and reliable method for quantitative metabolites profiling of medicinal plant.

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P-030 Simultaneous determination of three canthin-6-one alkaloids in different extracts of *Eurycoma longifolia* and *Eurycoma harmandiana* using HPLC-UV

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Eurycoma longifolia Jack. and *Eurycoma harmandiana* Pierre are well-known herbal medicine in Thailand and neighboring countries. For several decades, *E. longifolia* has been studied for various pharmacological properties. Several compounds have been discovered in these plants, among these canthin-6-one alkaloids have been the outstanding compounds likely to be active ingredients. However, analytical method in separation and quatification of canthin-6-one alkaloids are limited. Analytical separation and qualification were achieved using high-performance liquid chromatography (HPLC) with UV detection at 354 nm. The samples were analyzed using Lichrospher® 100 RP18 with mobile

phase consists of 0.1% acetic acid: acetonitrile (65:35 v/v). Linearity was observed in the range between 0.39-50 µg/mL with correlation coefficient of 0.9996-0.9998. Limit of detections were 2.52-2.88 µg/mL, and limit of qualifications were 7.63-8.73 µg/ml. Recovery values were in an acceptable range. The result revealed high contents of total canthin-6-one alkaloids in *E. harmandiana* (1.72±0.06 mg/g DW) compared to *E. longifolia* (0.16±0.01 mg/g DW). Moreover, different extracts of *Eurycoma* spp., (methanol, hydro-methanol (50%), ethanol, and hydro-ethanol (50%)), were prepared and tested of canthin-6-one alkaloids (9-hydroxycanthin-6-one, canthin-6-one and 9-methoxycanthin-6-one). The maximum of canthin-6-one content was observed from 50% methanolic extract followed by 50% ethanolic, methanol and ethanol extracts, respectively. In conclusion, A HPLC procedure for determination of canthin-6-one alkaloids is developed. The HPLC method was shown to be simple, accurate and selective for separation and quantification of three known canthin-6-one alkaloids in the root extracts of both *E. longifolia* and *E. harmandiana*.

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P-031 Solvent-free microwave extraction of essential oil of *Cinnamomum* species

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In recent years, natural extracts obtained by various extraction methods and have been widely used as additives in health food and cosmetics, and even as drug sources for many diseases in eastern and western countries. In addition to conventional hydrodistillation and solvent extraction methods, there is a green and eco-friendlier method, namely as solvent-free microwave extraction (SFME), can be used for the extraction of aroma plants [1, 2]. The principle of SFME is that microwave can resonate with *in-situ* water of plant causing tissues to swell and burst without adding any water or solvent and release the essential oil [3]. In this study, the leaves and barks of different *Cinnamomum* species were extracted by SFME to obtain the essential oils (CBEOs), and the optimal extraction methods were also discussed. The results showed that the maximum yield of CBEO was 0.75 % at irradiation power 600 W and the extraction time of 30 min. The main volatile compounds of CBEO were analyzed and identified by the electronic nose and compared with authentic standard compounds. The major compounds of CBEO were limonene (16-20 %), α-pinene (11-15 %), myrcene (9-10 %) and 1,8-cineole (7-9 %), respectively. Meanwhile, principal component analysis (PCA) combined with E-nose fingerprint could clearly discriminate samples from different *Cinnamomum* species.

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P-032 The quality variation of Danshen – an interdisciplinary approach to studying herbal medicine

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In 2015, danshen, a Chinese medicinal plant used in circulatory and pain-related diseases, had a market value of around £54 million with ten thousand tonnes of the crude drugs being sold. Its cultivation sites are scattered throughout China and other Asian countries. Processing varies from site to site. [1]
Utilise metabolomic approaches alongside value chain analysis this project aims at understanding the quality of danshen and the interrelationship among its chemistry and pharmacology along with its value chain.
The result shows 4 out of 15 Vietnamese market danshen samples and 6 out of 16 Chinese online store samples, at the concentration of 100 ug/ml, cause cytotoxicity ($P<0.05$) in RAW 264.7 Six samples from Vietnam exhibit NO inhibitory effects (10.28% to 26.17%). Seventeen authenticated samples were tested but only two samples showed significant inhibition (25.38% and 13.23%) without cytotoxicity. With regards to heavy metal concentrations, all 24 finished products were below with threshold of the Chinese Pharmacopeia, but also show no activity. Two samples from Chinese online stores exceed acceptable cadmium levels (0.3 and 0.67 mg/kg^{-1}).
HPTLC and NMR results suggest that drying straight after harvesting results in higher levels of tanshinones and salvianolic acids compared to the traditional processing which includes fermentation in the dark for several days prior to sun-drying.
In conclusion, this is the first study on danshen quality control to use interdisciplinary metabolomic approach including NMR, HPTLC combined with a pharmacological assay. Processing causes huge quality variation among market samples, especially in tanshinones, resulting in significant variation of the biological activity of Danshen.

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P-033 Using ion mobility 2D separation and specificity as a routine strategy to enhance profiling of complex medicinal plant extracts

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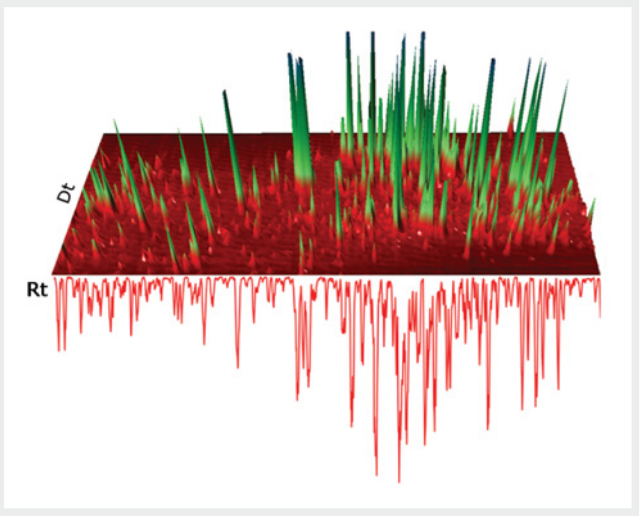
Several *Passiflora* (Passifloraceae) species are utilized as phytomedicines (sedative/tranquillising), due to their flavonoids, mainly *C*-glycosylflavones (apigenin and luteolin derivatives; frequently occurring as isomers), but only a few species are commercially exploited [1]. A non targeted screening strategy comprising the combined peak capacity of UPLC/ion mobility and collision cross section (CCS) measurements has been investigated as a strategy to produce routine unequivocal identification of marker flavonoid isomers in complex medicinal plant extracts. The phytochemical screening of *Passiflora alata, P. edulis, P. incarnata* and *P. caerulea* leaf extracts using ion mobility mass spectrometry has enabled generation of a "known" and "known unknowns" reference CCS speciation finger print profile, which has been incorporated into a MS/CCS library. Utilising standards, reference CCS of 6-*C*/8-*C*-glycosylflavone isomer pairs orientin/

isoorientin (187.7 Å^2/198.1 Å^2) and vitexin/isovitexin (188.8 Å^2/195.5Å^2) have been generated. The isomer CCS specificity has enabled unique deconvoluted isomeric quantitation of chromatographically coeluting isomers to be performed. The individual calculated concentrations of coeluting isoorientin and orientin in *Passiflora* extracts have been determined and compared to a conventional mass spectrometry approach. The enhanced peak capacity (▶**Fig. 1**) enabled more information to be extracted from fragmentation studies and the individual fragmentation spectra have been obtained for flavonoid isomers which are co-eluting with structurally related compounds.

The screening approach investigated illustrates the potential to enhance specificity when profiling phytochemical make-up in medicinal plants, where CCS values for knowns/ known unknowns and highly specific ion mobility product ions can be generated for all analytes in a single acquistion.



▶**Fig.1** Retention time (Rt) vs ion mobility separation (Dt)

Acknowledgments CNPq, FAPESP (Brazil)

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P-034 Validated HPLC-DAD method for resveratrol determination during identification in raw materials and stability, preformulation, permeability studies

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Resveratrol (3,4′,5- trihydroxystilbene) is a nutraceutical that has exerted anti-inflammatory and antioxidant activity [1]. It has been found also as anticancer agent, and substance with effect on neurological diseases and other maladies[2].

The aim of this study was to develop an HPLC-DAD method suitable for resveratrol determination during identification in raw materials and its stability, preformulation and permeability studies.

The HPLC method with UV detection with the Kinetex C18 column (100 × 2.1 mm, 5 µm) was used. The mobile phase consisted of ACN/0.5% acetic acid in ration 20/80. The detection wavelength was 306 nm, the column temperature was set at 40°C. The method meets all required validation parameters (selectivity, linearity, precision, accuracy). The changes of resveratrol permeability was investigated by using PAMPA test.

The proposed RP-HPLC method is a suitable technique for the determination of resveratrol in different approaches such as preformulation, stability and permeability studies. Resveratrol permeability coefficient (pH 7.4) was 74.89 ±27.28x10^{-6} cm/s, what classified drug as well-permeable.

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P-035 Age-related variation in polyphenol content and expression of phenylpropanoid biosynthetic genes in a medicinal and aromatic perennial *Agastache rugosa*

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Agastache rugosa (Fisch. & C.A.Mey.) Kuntze is an East Asian medicinal and aromatic herb rich in bioactive polyphenols, but its phytochemical composition is highly variable.

To elucidate potential factors which may cause the variability of the profile of pharmacologically useful phenylpropanoids, we studied the phytochemical profile and gene expression in leaves of one-, two- and three-year-old field grown *A. rugosa* and their mother plants. Using UHPLC –qTOF-MS thirty six different polyphenolic compounds were detected in *A. rugosa* leaves, out of which twelve were quantified. The quantities of the three major compounds (rosmarinic acid - RA, apigenin glucoside, chlorogenic acid) differed significantly between plant groups of different age and different age of a mother plant as confirmed by the PCA and PLS analyses. There was also a close relationship between one-year old plant groups and their respective progenitors which suggests an existence of a transgenerational phenomenon.

Quantitative real-time PCR analysis showed that phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), 4-coumarate:CoA ligase (*4CL*), tyrosine aminotransferase (*TAT*), hydroxyphenylpyruvate reductase (*HPPR*), chalcone synthase (*CHS*) and chalcone isomerase (*CHI*) transcript levels do not reflect end-product concentrations. Among the analyzed phenylpropanoid biosynthetic genes *ArRAS* (encoding rosmarinic acid synthase) was identified to be the rate-limiting step for RA biosynthesis in field grown *A. rugosa*. Thus, we identified *ArRAS* as a putative functional candidate for further characterisation via i.e. reverse genetic approaches to practically control rosmarinic acid production.

P-037 Expression profile of three genes involved in terpene biosynthesis in *Lavandula angustifolia* cultivars

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The *Lavandula* species are economically important plants cultivated for their essential oils with many therapeutic properties resulting from the biological activity of certain oil constituents.

The study to characterize the 1-Deoxy-D-xylulose-5-phosphate synthase (DXS) which catalyzes the first step of the plastidial methylerythritol phosphate (MEP) pathway for the production of most essential oil constituents and two other genes such as borneol dehydrogenase (BDH) which generates camphor through the oxidation of borneol and linalool synthase (LINS) that produces linalool by expression as genes involved in major terpene biosynthesis in four different lavender cultivars ('Provence Blue', 'Sevtopolis', 'Vera' and 'Codreanca') of the *Lavandula angustifolia* species by quantitative Real-Time PCR.

Total RNA was isolated from leaf tissue using SV Total RNA Isolation System kit (Promega). The transcriptional activity of BDH, DXS and LINS in leaf tissue was analyzed by absolute quantification, based on "in house" standards previously amplified using GoTaq G2 Green Master Mix (Promega) and specific primers targeting a 120–300 bp fragment size. The quantitative RT-PCR was performed in a Rotor-Gene 6000 5 Plex HRM Real-Time PCR system (Corbett) using a GoTaq 1-Step RT-qPCR kit (Promega).

The results show that while DXS was barely detected, BDH was heavily expressed in leaf tissue for all lavender cultivars and the linalool synthase was strongly expressed in the `Provence Blue`(PB) cultivar. The raw data does not indicate a direct correlation between the expression of the analyzed genes and further studies will be performed to elucidate the activation mechanism of terpene biosynthesis.

P-038 Genetic diversity and phytochemical characterisation of *Sideritis scardica* populations from Greece

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Sideritis scardica (Lamiaceae), commonly known as Mountain tea is endemic to Balkan countries and is an important medicinal and aromatic species. Lately, environmental and management factors resulted on decreasing of natural populations of *Sideritis*. Considering the risk of extinction of the species but also the demands of growers and consumers for high quality herbal products the present study aimed to evaluate the genetic and chemotypic diversity of *S. scardica* populations native to Greece. For this reason five populations (n = 20 to 25 individuals per population) were collected in 2018, from mountainous areas of N. Greece during the flowering period. After the evaluation of population size *in situ*, intra- and inter-population genetic diversity was determined in individual plants using SCoT molecular markers. High genetic diversity was detected within the studied populations. Additionally, the populations were highly differentiated based on SCoT markers. The qualitative volatile profile of the populations was developed through GC-MS analysis after simultaneous steam distillation and solvent **extraction** of composite herbal samples. Phytochemical analysis determined the presence of hydrocarbon, oxygenated monoterpenes and sesquiterpenes with most predominant: α- and β-pinene, α-campholenal, tr-pinocarveol, tr-verbenol, myrtenal, carvacrol, β-caryophyllene and caryophyllene oxide. Their quantitative composition varied among the populations. Our findings revealed that such diversity could substitute a great genetic pool for the selection of superior germplasm native to Greece for further exploitation in domestication and breeding programs of *Sideritis* species.

P-039 Abstract see SL YRW-06

Abstract see on page 1397

P-040 Induction of secondary metabolism of marine derived *Streptomyces cacaoi*

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Microbial natural products have an adaptive role as signal molecules or defense tools in ecological interactions [1]. Biosynthesis of these molecules is suppressed in standard laboratory conditions where there are no ecological triggers. Thus, only a portion of the chemical diversity of a microbial strain is discovered by standard fermentation protocols. However, using different fermentation conditions or different approaches such as co-culture, biosynthesis of these suppressed molecules can be triggered, and new natural products can be isolated [2,3].

In our previous studies, it was demonstrated that marine derived *Streptomyces cacaoi* had a potent antimicrobial effect against *Enterecoccus feacium* and *MRSA* [4]. A comprehensive statistical optimization of this promising strain was aimed to enrich the chemical diversity of the ethyl acetate extract and to increase its bioactivity. Response surface methodology was used for statistical optimization of some fermentation paramaters. As responses, the chemical diversities of the ethyl acetate extracts were monitored by HPLC-DAD system, and the antimicrobial effects were determined by disc diffusion assay on *Bacillus subtilis*. As a result, the diameter of the inhibition zones (150 µg extract) varied between 6 to 25.5 mm against *Bacillus subtilis*. Also, chemical profiles of the extracts varied even in the major compounds. Consequently, this study demonstrated the importance of optimization protocols for the discovey of new/novel molecules in microbial natural product research.

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P-041 Abstract see SL YRW-08

Abstract see on page 1398

P-042 Orchids and their mycorrhizal fungi: an insufficiently explored relationship

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Orchids are associated with diverse fungal taxa, including non-mycorrhizal endophytic fungi and mycorrhizas. Orchid mycorrhiza (OM) symbiosis is an excellent model for investigating the biological interactions between plants and fungi due to their high dependency on these symbionts for growth and survival. To capture the complexity of OM interactions, significant genomic, numerous transcriptomic, and proteomics studies have been performed, unravelling partly the role of each partner. In this review, the orchid and mycorrhizal fungus relationship will be described summarizing the recent published literature on OM with special attention to the nutrient exchange model, the correlation on fitness and distribution of orchid populations, and finally the chemical communication and defense mechanisms. Based on the recent finding on orchids endophytes, OM relationship, arbuscular mycorrhiza (AM) and OM similarities [1, 2], a putative model representing the different strategies that OM fungi might employ to establish this symbiosis is proposed. It is hypothesized here that (i) orchids would excrete signaling molecules such as strigolactones and flavonoids to facilitate the establishment of the symbiosis. In response, (ii) OM fungi would secrete mycorrhizal (Myc) factors to activate the common symbiosis genes, (iii) evade the pathogen associated molecular patterns triggered immunity and secrete effectors to overcome the defense mechanism (iv) and finally secrete phytohormones to help the colonization or disrupt the crosstalk of plant defense phytohormones. To challenge this supposed model, metabolomics studies with special attention to each partner contribution are encouraged and some technical approaches are proposed.

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P-044 Rediscovering traditional vegetables to enhance food and environmental sustainability of Sub-Saharan agriculture

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Traditional vegetables are valuable but underutilized crops in Sub-Saharan agriculture due to the spread of staple crops (e.g., common bean and common spinach). However, staple crops are highly water demanding and usually require tilling and chemical treatments to grow. Furthermore, they rarely show a balanced equilibrium between macro and micronutrients, thus impoverishing people diet.

This study focused on the evaluation of the impact of no-tillage practices and water scarcity on the nutritional profile of cowpea (*Vigna unguiculata* L. Walp.) and jute mallow (*Corcorhus olitorius* L.) edible portions.

After harvesting, cowpea seeds and jute mallow leaves were boiled, dried and grinded.

Cowpea seeds were evaluated for their total starch, proteins and aminoacidic content. Jute mallow leaves were studied for their polyphenolic composition and folates profile.

These species proved to be an extraordinary source of healthy and nutritionally valuable compounds. For instance, HPLC analyses on cowpea seeds showed a relevant amount of aminoacids (4–33.67 $mg^{*}g^{-1}$), many of which essential (e.g., threonine and tryptophan). Jute mallow leaves revealed a huge variety of polyphenols, among which cryptogenic acid (50–185 $\mu g^{*}g^{-1}$), neochlorogenic acid (57–186 $\mu g^{*}g^{-1}$) and many quercetin glycosides (99–683 $\mu g^{*}g^{-1}$), as shown by mass spectrometry analyses.

Notably, these phytochemicals were not influenced by stress conditions, thus confirming the capability of such species of providing healthy diet also if cultivated under conservative agriculture managements. Therefore, these traditional vegetables are good candidates to promote and couple food and environmental sustainability of Sub-Saharan agroecosystems.

P-045 Variation in the composition and antioxidant activity of *Vaccinium myrtillus* populations collected in Croatia and Montenegro

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Vaccinium myrtillus L., Ericaceae, (bilberry) leaves are traditionally used in southeastern Europe for the treatment of diabetes. In the present study, the variation of the phytochemical composition, as well as antiradical and tyrosinase-inhibiting activity of 11 bilberry populations from Croatia (5) and Montenegro (6) was investigated, considering their use for cosmetic purposes. Content of phenols, flavonoids and phenolic acids, as well as antiradical and enzyme inhibiting activity of leaf extracts were determined spectrophotometrically. The content of the most abundant flavonoid, hyperoside, was determined using HPLC with DAD detection. Furthermore, the content of selected metals (K, Ca, Mn, Fe, Cu, Zn, Br, Rb, Sr) was determined using TXRF. A significant quantitative variability of total phenols (386.8–133.9 mg/g), flavonoids (83.1–12.0 mg/g) and phenolic acids 112.4–38.0 mg/g) was recorded. The extracts displayed excellent antiradical activity (IC_{50} between 120.8–11.1 mg/g) and somewhat weaker antityrosinase activity (IC_{50} between 400.3–170.9 mg/g). The hyperoside content in the extracts varied between 73.1 and 5.7 mg/g. There was no statistically significant correlation between the content and area of origin. However, the chromatograms of extracts from Montenegro could be easily distinguished from the Croatian ones by the presence of an additional peak in chromatogram, which, according to its UV spectrum, represents a quercetin derivative. The observed activities of bilberry leaf extracts suggest that they might be used in cosmetic products. However, before the development of such products, a detailed analysis of plant material is advisable in order to select the populations with the highest activity.

P-046 Assembly of a fungal macrocyclic polylactone is catalyzed by two iterative polyketide synthases

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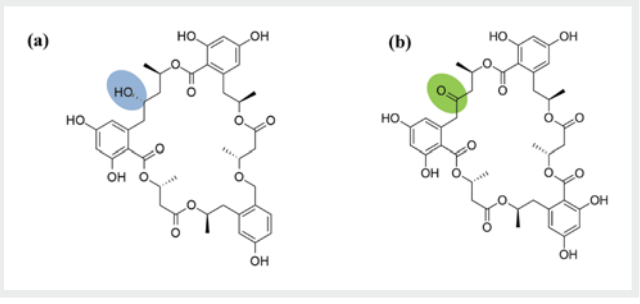
Menisporopsin A is a bioactive macrocyclic polylactone produced by the fungus *Menisporopsis theobromae* BCC 4162. With the transcriptomic results during the production phase of menisporopsin A, non-reducing polyketide synthase (NR-PKS) gene namely *men 1* and reducing polyketide synthase (R-PKS) gene namely *men 2* are believed to participate in the menisporopsin A biosynthesis. Both genes were cloned into vectors for heterologous expression in *Aspergillus oryzae* NSAR1 and the transformants were analyzed using HPLC and metabolites produced were identified by NMR, HR-MS and their optical activities. Three major metabolites produced by transformants are (-)-orthosporin, (-)-6-hydroxymellein and structural derivative of menisporopsin A, ascotrichalactone A, which differs from menisporopsin A only in the presence of a keto group as highlighted in ▶**Fig. 1**. Based on these results, we can conclude that the formation of macrocyclic polylactone requires only R and NR-PKSs and *trans*-acting ketoreductase is needed for the final step of menisporopsin A biosynthesis [1].



▶**Fig. 1** Structures of **(a)** menisporopsin A and **(b)** ascotrichalactone A.

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P-047 BAHD-like malonyltransferase genes from *Digitalis lanata* and *Arabidopsis thaliana* and their putative role in cardenolide biosynthesis

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Cardenolides, which are used for the treatment of congestive heart failure, are still produced by extraction from plants. A more efficient way to produce cardenolides could be achieved by a yeast-based metabolic engineering approach [1]. However, several steps in cardenolide biosynthesis still need to be elucidated.

Malonyl-coenzymeA: 21-hydroxypregnane 21-*O*-Malonyltransferase (21MaT) catalyzes the formation of pregnane-21-*O*-malonyl hemiesters from pregnane precursors. Even though a 21MaT from *D. purpurea* has already been characterized [2], no 21MaT gene could be identified as yet.

Only recently, the BAHD-type malonyltransferase pMaT1, an enzyme from *Arabidopsis thaliana*, was found to possess 21MaT activity *in vitro*. Initially, the identification of an *At*pMaT1 homologue in *Digitalis* failed. Therefore, a function-based search for malonyltransferases was performed. One sequence from the *D. purpurea* transcriptome [3], annotated as quercetin 3-*O*-glucoside-6″-*O*-malonyltransferase (*Dp*QGMaT1), appeared to be a promising candidate. Primers were deduced and *D. lanata* mRNA was used as a template to isolate the respective *D. lanata* homologues. The attempt yielded a putative *Dl*21MaT1 sequence which was 94% identical to *Dp*QGMaT1. Meanwhile, we identified and cloned three more promising candidate genes from *D. lanata*. Two recombinant *Dl*21MaTs were already tested for enzyme activity, using various pregnane substrates, such as 3β-*O*-acetyl-5β-pregnane-14β,21-diol-20-one, 5β-pregnane-21-ol,3,20-dione, and 21-hydroxyprogesterone. All substrates were accepted. However, enzyme activity was very low (1 %) compared to the recombinant *At*pMaT1. Since 21MaT activity is higher in crude protein extracts from leaves of *D. lanata* than in *A. thaliana,* we cloned two more *Dl*21MaT genes that will now be characterized with regard to their substrate preferences.

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P-048 Biotransformations with the enzymatic secretome of *Botrytis cinerea* combined with organic solvents for the generation of novel complex stilbene derivatives

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The enzymatic biotransformation of natural products (NPs) using the *Botrytis cinerea* enzyme secretome has proven to be an innovative and effective way to produce original derivatives [1]. Such reactions from simple natural stilbenes such as resveratrol and pterostilbene have led to various dimerization products, some with interesting biological activities. One of the difficulties of the approach is that the reactions take place in water, which is limiting because of the low solubility of NPs in this type of medium. This may explain the low yields obtained by some reactions. In order to overcome this problem, organic solvents were added to the reaction medium. Several organic solvents such as methanol, isopropanol, butanol and isobutanol were examined. The reactions were carried out at the analytical scale using different amounts of each organic solvent. UHPLC-PDA-ELSD-MS profiling of the crude reaction mixtures revealed the presence of compounds with different molecular weights as a function of the organic solvent used. In order to isolate these unusual compounds, the reactions were optimized, scaled-up and fractionated by semi-preparatory HPLC with a new dry load injection method to favor high resolution fractionation [2]. This approach has enabled the isolation of more than 60 pure compounds fully characterized by HRMS and NMR spectroscopic methods. Surprisingly, some have adopted a solvent molecule into their structures, which explains the unusual chemical profiles observed. This fortuitous discovery opened the door to obtaining series of derivatives of NPs with great value for biological screening campaigns.

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P-049 De novo transcriptomic analysis and establishment of cell suspension culture system to improve saponin production in Oplopanax elatus

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Oplopanax elatus, commonly called Asian devil's club, is a valuable medicinal plant in the family Araliaceae that recommends itself as source of herbal preparations. Although triterpenoid saponins make up the major bioactive component of O. elatus, nothing is known about the genes that are involved in the biosynthesis of these complex compounds. To analyze the triterpenoid saponin biosynthetic pathway in O. elatus, we firstly generated a transcriptome library using Illumina HiSeq™2500 sequencing platform, and identified 122 unigenes encoding 47 putative enzymes in pathways for terpenoid backbone (MEP and MVA pathways) and triterpenoid saponin biosynthesis. The tissue-specific expression of selected genes suggests that the leaves of O. elatus are the main site of triterpenoid saponin biosynthesis. In addition, we established cell suspension culture of O. elatus to develop a sustainable source of naturaceuticals. The total saponin production in the cell suspension of O. elatus was significantly increased by melatonin at 195% at a dose of 100 μM compared with the mock-treated control. The expression analysis suggests that the enhanced production of saponins in melatonin-treated O. elatus is mediated by the increased transcription of genes involved in triterpenoid saponin biosynthesis pathway. Taken together, the transcriptome dataset generated in this study will serve as a valuable resource for accelerating genomic and functional genomic research in O. elatus, and our results provide valuable information for improving the production yield of saponins in plant suspension cultures.

P-050 Effect of illumination on growth and phytochemical profile of *Salvia yangii* grown *in vitro*

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Plant *in vitro* cultures are a feasible system for testing influence of single environmental factor on biosynthesis of medicinally relevant compounds. Light spectrum and intensity can be simple means to stimulate production of specialized metabolites. Our aim was to examine the influence of different types of light on the morphogenetic response and the production of terpenoids and phenylpropanoids in *Salvia yangii* B.T.Drew (syn. *Perovskia atriplicifolia* Benth.) *in vitro* cultures. Shoots were illuminated with white, PAR (photosynthetic active radiance), red, blue, and mixed red/blue LED, as well as cool-fluorescent light of different intensities. Phytochemical analysis was performed using HSPME-GC-MS for volatile compounds and LC-MS for polyphenols and abietane diterpenoids. Among volatiles, hydrocarbon monoterpenes (camphene, 3-carene, limonene, α-pinene), oxidized monoterpenes (eucalyptol, camphor, borneol), monoterpene ester (bornyl acetate) and sesquiterpenes (β-caryophyllene, isocaryophyllene), and from the non-volatile compounds, rosmarinic and carnosic acids were examined. Higher light intensity corresponded to differences in the chlorophyll and polyphenol content. The largest differences in the essential oils constituents were between PAR and red light. In case of rosmarinic and carnosic acids, the red light was most efficient. Light used in controlled conditions of *in vitro* cultures modified morphogenesis and influenced the content of selected metabolites. This can be used obtaining pharmacologically active compounds, such as carnosic acid, which in nature is produced in relatively small amounts.

P-051 Enhanced carbazole alkaloid and antioxidant capacity in callus culture of medicinal plant *Clausena harmandiana* by Bacilius subtilis elicitor

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Clausena harmandiana (Pierre) Guillaumin (Rutaceae), is a medicinal plant native to Thailand. Its major active compounds are carbazole alkaloids, which possessed various pharmacological activities and antioxidant activity [1,2]. Two carbazole alkaloids, clausine K and 7-methoxymukonal, are the major compounds in the callus culture of *C. harmandiana* [3]. Enhancing the productivity of callus culture can provide an alternative for carbazole alkaloids production and minimize destructive harvest of wild-plant materials.

To improve the productivity, we tested the effects of two biotic elicitors, *Bacillus subtilis* and *Trichoderma harzianum*, on accumulation of two carbazole alkaloids in *C. harmandiana* callus culture. Antioxidant activity of the callus extracts were also assessed by DPPH and FRAP assays along with total phenolic contents.

Callus was induced from *C. harmandiana* leaf explants cultured on Murashige and Skoog medium supplemented with 0.1 mg/L thidiazuron, 1 mg/L naphthaleneacetic acid, and 5% sucrose. A living *B. subtilis* culture at 0.1 and 1% (V/V) significantly enhanced clausine K level in callus up to 5-fold. However, *T. harzianum* did not affect carbazole alkaloids accumulation. In addition, 1% (V/V) *B. subtilis* and 1% (V/V) *T. harzianum* significantly promoted total phenolic contents in callus up to 2.5-fold. DPPH and FRAP assays indicated significant enhancement of antioxidant activities in callus treated with various concentration of *B. subtilis* and *T. harzianum*, which highly correlated to phenolic contents. The established *C. harmandiana* callus culture and the used of *B. subtilis* as elicitor is an efficient alternative and sustainable source for production of clausine K with improved antioxidant activities.

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P-052 Establishment of callus and cell suspension culture of *Momordica charantia* L. and their phytochemicals

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Momordica charantia L. is an edible and medicinal plant of the family Cucurbitaceae with reported wide range of biological activities. In this study, effect of Murashige and Skoog (MS) medium [1] supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin (KN), 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) on callus formation of leaf, stem, and root explants of *M. charantia* were studied. After that, cell suspension cultures were initiated on liquid MS medium supplemented with plant growth regulators that gave the best callus response. Cells were harvested every week for determination of growth curve and phytochemicals accumulation. The experiments were performed in triplicate. The results showed that the highest callus formation frequency and callus quality score were obtained from leaf and stem explants cultured on MS medium supplemented with 2.0 mg/L 2,4-D and 0.5 mg/L KN for two weeks. Growth of cell suspension culture rapidly reach log phase from the first week. The results from Folin-Ciocalteu and DPPH assays showed that extracts of 2-week-old cell suspension cultures of *M. charantia* had highest total phenolic contents (equivalent to 14.23±6.37 mg gallic acid/g DW) and antioxidative activity (equivalent to 0.62±0.04 mg ascorbic acid/g DW) which were significantly higher than results from extracts of cells harvested at different times of culture, and were not statistically different from the results from extracts of intact fruits of *M. charantia*. Phytochemical profiles are under investigation.

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P-053 Genetic features of lycopene content associated with flesh color in watermelon

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Recent interest has focused on different flesh colors in watermelon due to one of rich resources for the antioxidant cis-isomeric lycopene, one of the carotenoids. The formation of lycopene is a major step in carotenoid biosynthesis, in which lycopene β-cyclase (LCY-B) and lycopene ε-cyclase (LYC-E) enzymes are involved in the formation of lycopene, and LYC-B is involved in the formation of β-carotene. This study is to elucidate genetic relationship among watermelon genotypes with red, yellow or orange flesh color from the whole genome resequencing data. An elevated level of lycopene was noted in all red flesh watermelon lines ranging from 333 to 477 μg/g, while orange-fleshed watermelons have previously been reported to contain mainly β-carotene (91~171 μg/g), with traces of lycopene and phytoene. In the present study, we selected 2369 SNPs with lower PIC values (0.1-0.38) to discriminate 24 genotypes that exhibited different flesh colors. A cluster analysis indicated that red flesh genotypes with a high lycopene content was separated from the non-red flesh inbred lines, such as yellow or orange with a low lycopene content. We randomly selected several SNPs on protein coding genes that presented polymorphism between red flesh and non-red flesh types. Results revealed that these SNP-carrying genes presented preferential and stage-specific expression between red and yellow genotypes. The selected SNP-linked to red flesh loci were further validated, and those SNPs were converted into cleavage amplified polymorphic sequence (CAPS) markers which allows marker-assisted selection of watermelons with high lycopene content.

P-054 How light photoperiod and medium composition could increase the production of a potent anticancer metabolite by Nostoc

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Cryptophycin-1 is the first circular depsipeptide which was isolated in 1990 from Nostoc sp. as a tubulin-inhibitor with potential anticancer activity. The total synthesis of cruptophycin faces stereoselctivity issues and its yield is only moderate (<13%), and unsuitable for the industrial production [1].

These considerations provide a rationale to investigate alternative solutions, like stressing the environmental conditions of the culture (ATCC53789) in order to find the optimal for the metabolite's over-production (more than 0.52mg/L of culture) [2]. Here, we examine the effect of light photoperiod and media's composition. In light photoperiod we tested 24:0,16:8,12:12,8:16 (Light:Dark). In medium composition, Nitrogen's concentration was examined to test the implication of heterocysts, a N-fixing cell type. The culture was cultivated in a light-box, the biomass of a 20ml-sample was extracted and then metabolite's concentration was calculated through HPLC. Lastly, the "growth/time" and "metabolite's concentration/time" curves were drawn.

Remarkably, photoperiod has an opposite effect on the two curves. Regarding the growth, the optimal period was 16:8>12:12>8:16>24:0 (Light:Dark), however the inverse happens for the cryptophycin's production. It is shown that even if the presence of non-light is needed for the cell's growth, the metabolite's production is higher under constant light conditions.

Regarding the Nitrogen concentration, and so the implication of heterocysts, it was noticed that they were not involved in the depsipeptide's expression process.

These stress-tests' results contribute on the formation of the optimal culture conditions for the metabolite's overproduction. That would progress the research and development of the novel potential anticancer drug.

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P-055 Abstract see SL YRW-10

Abstract see on page 1398

P-056 Polyketide-related biosynthesis of plant anthranoids

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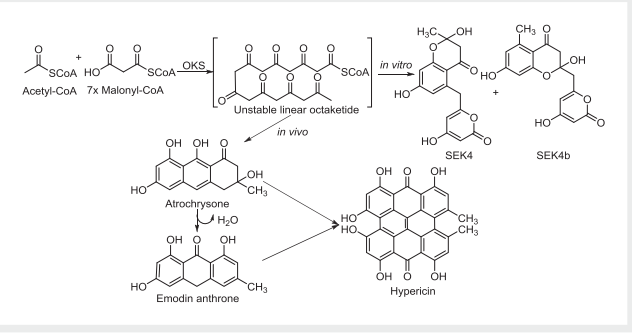
Hypericin, a naphthodianthrone present in the medicinal plant *Hypericum perforatum* (Hypericaceae), was proposed to be biosynthesized via the acetate-malonate (polyketide) pathway. One molecule of acetyl-CoA condenses with seven molecules of malonyl-CoA, yielding a linear unstable octaketide, which undergoes cyclization and decarboxylation to produce

atrochrysone and/or emodin anthrone; hypericin precursors. This reaction is believed to be catalyzed *in vivo* by an octaketide synthase (OKS) either alone or together with tailoring enzyme/s, as octaketide cyclase (OKC). OKS-encoding transcripts from *H. perforatum* [1], *Aloe arborescens* [2] and *Polygonum cuspidatum* (unpublished) were cloned and heterologously expressed in *Escherichia coli*. All three recombinant OKSs formed a linear octaketide which, was incorrectly folded and cyclized to give two aromatic octaketides (SEK4 and SEK4b) under *in vitro* conditions (Fig. **1**). Conversely, we detected the functional biosynthesis of anthranoids in cell-free protein extracts from yeast-extract-treated *Cassia bicapsularis* (Fabaceae) cell cultures without shunt products formation [3].

Our aim is to study at the biochemical and molecular biological levels the OKS enzyme and its interaction with accessory factor/s (tailoring enzyme/s) in the anthranoid scaffold biosynthesis, as exemplified by cannabinoid biosynthesis [4].

We transiently expressed *H. perforatum* OKS in *Nicotiana benthamiana*. Furthermore, a number of putative tailoring enzymes required for anthranoid scaffold formation were cloned from *H. perforatum*. Co-expression of HpOKS and these recently cloned enzymes in *N. benthamiana* is under investigation. Using three publicly available *Cassia* transcriptomes, we were able to identify a number of PKSs that are undergoing cloning and functional analysis to test them as potentially anthranoid-forming enzymes.



▶ **Fig. 1** *In vitro* and *in vivo* products resulting from the OKS reaction.

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P-057 Production of depsides and other phenolic acids in agitated cultures of black and red aronias after feeding with caffeic acid

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Depsides and other phenolic acids (PhAs) are a subgroups of polyphenolics with antioxidant activity very important in phytotherapy and also in cosmetology.

Our earlier investigations with in vitro cultures of black aronia (Aronia melanocarpa) and red aronia (A. arbutifolia) confirmed especially high production of depsides [1,2,3].

The aim of the present studies were the investigations on PhAs production after feeding of culture media with caffeic acid (CafA) in agitated cultures of both aronias.

The culture were maintained for 20 days (3-series) on Murashige & Skoog medium [4] (BAP – 1 mg/l, NAA – 1 mg/l) without and with addition of CafA (0.1; 0.5; 1.5; 10 mmol/l).

In the methanolic extracts from biomasses the estimation of 22 PhAs were performed using HPLC method [5].

The presence of seven PhAs were confirmed. The main metabolites in biomass (extracts) of both aronias were depsides – chlorogenic, isochlorogenic and neochlorogenic acids (black aronia) and chlorogenic, isochlorogenic and cryptochlorogenic acids (red aronia). The total amounts of PhAs ranged from 290 to 661 mg/100gDW and from 194 to 967 mg/100gDW, in black and red aronias, respectively. The highest amounts of PhAs were documented after addition of 1 mmol/l and 5 mmol/l of CafA, in black and red aronias, respectively.

The obtained results documented that the feeding of culture media with CafA stimulate the high production of depsides with important antioxidant activity.

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P-058 Production of phenolic acids in shoot cultures of black aronia (*Aronia melanocarpa*) cultivated in RITA bioreactors

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Background: Black aronia (*Aronia melanocarpa*) is plant species of North-American origin cultivated in Poland. Fruits of this plant contain some subgroups of antioxidants – phenolic acids and anthocyanins important in phytotherapy and also cosmetology [1].

Our previous studies with different types of in vitro cultures of black aronia (agar and agitated cultures) documented the high biosynthetic potential of the cells for production of phenolic acids [2,3].

Aim: The aim of the present study was the establishment of shoot cultures in temporary immersion system – in RITA bioreactors and analysis of phenolic acids in cultured in vitro biomass and media. The cultures were maintained on Murashige and Skoog medium [4] enriched in 1 mg/l BAP and 1 mg/l NAA, for 4 and 8 weeks (3 series). In methanolic extracts of collected biomasses and culture media samples the LC-DAD analysis of 26 phenolic acids was performed [5].

Results: In biomasses extracts the presence of 11 phenolic acids was confirmed. The quantitatively dominant compounds were: isochlorogenic acid, cryptochlorogenic acid, 3,4-dihydroxyphenylacetic acid and 3-phenylacetic acid (max. 236.16; 153.96; 151.80 and 101.04 mg/100gDW, respectively). Extracts from the culture media were found to contain no metabolites. Higher total content of phenolic acids was confirmed offer 4-week growth cycles (786.88 mg/100gDW and 380.66 mg/100gDW, respectively).

Conclusion: The obtained results documented for the first time the satisfactory production of some phenolic acids in shoot cultures of black aronia cultivated in RITA bioreactors. The obtained total content of phenolic acids are interesting from practical point of view.

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P-059 Regulation of histone 3 acetylation for increasing ginsenoside production in adventitious root cultures of *Panax ginseng*

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Histone acetyltransferase (HAT) is known as an epigenetic enzyme that acetylates specific lysine residues on the histone tail to promote chromatin dynamics and gene expression. In contrast, histone deacetylase (HDAC) removes the acetyl functional groups from the lysine residues of histone tails. This indicates that HATs and HDACs play a role as transcriptional activators or repressors involved in multiple biological processes. Despite the knowledge concerning HATs and HDACs, evolutionary and functional information regarding HATs and HDACs in Panax ginseng are still unknown. In this study, using comprehensive bioinformatic analyses, we identified 13 HATs (PgHATs) and 26 HDACs (PgHDACs) in the P. ginseng genome. The expression analysis using qRT-PCR revealed spatial variations in the expression of PgHATs and PgHDACs in different organs. Methyl jasmonate (MeJA), a vital plant hormone essential for plant defense responses and developmental processes, is an effective elicitor of ginsenoside biosynthesis in cultured cells and adventitious roots of P. ginseng [1]. To investigate the role of histone 3 (H3) acetylation on the MeJA-induced ginsenoside biosynthesis, ginseng adventitious roots were co-treated with MeJA and histone deacetylase inhibitors sodium butyrate and vorinostat. The expression of ginsenoside biosynthesis-related genes and the production of ginsenosides were significantly increased by MeJA and inhibitor co-treatment compared with the MeJA-treated samples. In addition, the analysis of histone H3 acetylation using immunoblotting suggested that histone deacetylase inhibitors enhanced MeJA-induced acetylation of histone H3 lysine 14, 18 and 27, indicating that histone H3 acetylation is required for stimuli-induced expression of ginsenoside biosynthesis-related genes.

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P-060 RNAi mediated knock down of 3β-hydroxysteroid dehydrogenases in *Digitalis lanata* shoot cultures

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Cardenolides are well-established substances for the treatment of atrial fibrillation or cardiac insufficiency. Latest research suggests that cardenolides could also be used in the treatment of various types of cancer [1].

Cardiac glycosides are still isolated after extraction from plants. For achieving higher amounts, e.g., *in planta* it is fundamental to understand cardenolide biosynthesis. We here focused on the investigation of 3β-hydroxysteroid dehydrogenase (3β-HSD) that is supposed to be involved in cardenolide biosynthesis. We screened *D. lanata* for the occurrence of 3β-HSD genes, determined their expression and established RNAi-mediated knock-down mutants. 3β-HSD knock-down mutants will be further investigated for a direct connection between 3β-HSD expression and cardenolide content.

Up to now, two 3β-HSDs (*Dl*HSD1 [2] and *Dl*HSD2), that share an amino acid sequence identity of 68.9% and a nucleotide sequence identity of 71.5% were identified. *Dl*HSD2 was recently found by a function-based search in the transcriptome database of *D. purpurea* [3]. *Dl*HSD2 is isolated, cloned and expressed in *E. coli* and 3βHSD (oxidation and reduction) activity with pregnenolone and 5β-pregane-3,20-dion is demonstrated. RNAi mediated knock down of the respective two *D. lanata* 3β-HSDs is verified by qPCR and stable transformed shoot cultures are now about to be characterized with regard to their cardenolide contents.

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P-061 Sodium nitroprusside triggers mitragynine biosynthesis in Kratom

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Kratom or *Mitragyna speciosa* (Korth.)Havil. belongs to the Rubiaceae family. The psychoactive compounds, accumulated in this plant, such as mitragynine and its derivative like 7-hydroxy-7H-mitragynine are opioid agonists [1]. It exhibited numerous pharmacological activities e.g. analgesic, antidiarrheal, antidepressant, and muscle relaxant, for instance [2]. For this reason, mitragynine is a molecule of interest and being a candidate for an oral analgesic drug. Elicitation is a powerful technique to increase secondary metabolite in the plants. We aimed to study the effect of sodium nitroprusside (SNP) on mitragynine

production in kratom. The present study, we elicited the in vitro kratom culture with SNP, a nitric oxide (NO) donor. Plant elicited with 1 mM SNP for 48 h increased secologanin production, not alter mitragynine production significantly. Treatment plants with SNP together with CPTIO, a NO scavenger revealed the suppression of secologanin biosynthesis. In addition, treatment with 1 mM nifedipine (a calcium channel blocker) inhibited secologanin synthesis significantly. This evidence suggested that calcium channel involved in the NO response. Transcription profile analysis of gene-associated with mitragynine biosynthesis indicated that the mRNA levels were increased when elicited with SNP and reduced in plant treated with SNP and CPTIO or nifedipine. The reason that SNP did not increase the amount of mitragynine because of the limitation of tryptamine in the plant [3]. In conclusion, NO released from SNP could promote mitragynine biosynthesis by triggering their biosynthetic genes and increase secologanin production.

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P-062 Telomerase activators derived from *Astragalus* sapogenins via biotransformation with the recently discovered endophytic fungus *Camarosporium laburnicola*

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Telomeres are nucleotide sequences that are located at the end of chromosomes shortening with each cell division. Telomerase is a reverse transcriptase enzyme, and it helps to replenish telomere ends that are truncated by aging and stress factors [1].

Telomere shortening is considered not only a result of the biological aging process but also a risk factor for many disorders such as neurodegeneration, macular degeneration, coronary artery diseases, hypertension, diabetes, pulmonary fibrosis. Thus, activation of telomerase enzyme introduces a great promise for the treatment of many chronic and degenerative diseases. Cycloastragenol, isolated from *Astragalus* species, is the only natural product as a telomerase activator in the dietary supplement market. The initiation of clinical studies with Cycloastragenol derivatives in Alzheimer's disease and metabolic syndrome is a sign of their potential [2,3,4].

Aims of this study were: i) to carry out microbial transformation studies on *Astragalus* sapogenins using endophytic fungi (*Alternaria eureka, Neosartorya hiratsukae, Penicillum roseopurpureum, Camarosporium laburnicola*) isolated from the tissues of *Astragalus* species ii) to expand our chemical library with new/novel metabolites; iii) to investigate effects of the metabolites on telomerase activation.

As a result, 42 metabolites were identified [5] and screened in Telomerase PCR ELISA Assay using HEKn cell line. Sixteen compounds increased telomerase activation ranging from 1.2 to 11.3-fold between doses of 0.5 to 300 nM compared to the control (DMSO). The most potent molecules were found to be A-ring modified cycloastragenol derivatives obtained with the fungus *Camarosporium laburnicola*.

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P-064 Tissue cultures of *Gypsophila elegans* as means for production of pharmacologically active triterpenoids

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Due to the chemical features of saponins, the compounds are considered valuable in phytotherapy. They present expectorant, exudative or anti- inflammatory activity, and recently gained considerable attention as aid in the delivery of biopharmaceuticals into target cells [1]. Aiming at overcoming problems with cultivation and processing of saponin rich plants, we developed the tissue culture method of *Gypsophila elegans* M. Bieb. Temporary immersion system (TIS), and polyurethane foam platforms were used for growing of *G. elegans* roots, callus tissues and cell suspensions. Differences in the biomass production of cell cultures were observed depending on the medium and supplementation with growth regulators. Both solid and liquid media were found to be effective for the cell and roots biomass growth. The highest biomass increase of callus cells was recorded on a MS medium supplemented with plant growth regulators such as kinetin (1μM), naphthaleneacetic acid (0.5 μM), and 2,4-dichlorophenoxyacetic acid (2.5 μM). In case of organ cultures, the largest increase in roots growth was observed on the MS liquid medium with a reduced of macro-, and microelements concentration (1/2 MS). Extracts obtained from the in vitro plant material were examined for the content of triterpene saponins and their derivatives. The content of several gypsogenin saponosides was confirmed using LC-MS.

The applied techniques of *G. elegans in vitro* cultures were effective in terms of cell and organ multiplication allowing for obtaining a substantial amount of biomass. The obtained plant material can be considered as a convenient source of medicinally relevant triterpenoids.

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P-065 Use of *Botrytis cinerea* enzymatic secretome to generate original phenylpropanoids derivatives having Wnt inhibition on triple negative breast cancer cells

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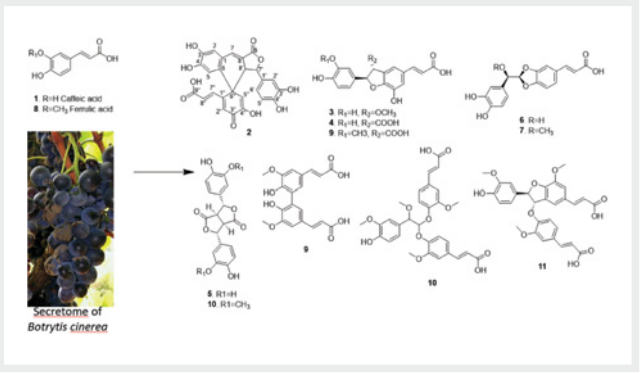
The biotransformation of natural products using the *Botrytis cinerea* enzymatic secretome has proven to be an innovative and effective way to produce original derivatives [1]. Such reactions produce very complex compounds from simple natural product scaffolds, some of which have interesting biological activities. In the present work, the biotransformation of two 4-hydroxy-cinnamic acid derivatives was investigated. Caffeic acid and ferulic acid were successively incubated with the crude enzymatic extract (secretome) of *B. cinerea*. These biotransformations, were first carried out at the analytical scale for 24 hours. They were monitored by UHPLC-high resolution mass spectrometry (HRMS) to verify the complete transformation of starting substrates and the production of related derivatives. The reactions were then scaled-up with 200 mg of the starting compounds, controlled by HPLC-PDA-ELSD and purified by high-resolution semi-preparative HPLC. The same selectivity and a precise prediction of the separation between the analytical and semi-preparative scale were obtained using geometrical chromatography transfer methods [2]. The isolated compounds were fully characterized by NMR and HRMS. Using this approach eleven dimeric or trimeric phenylpropanoids analogues were obtained. Among these compounds, three are described here for the first time and some of them presented Wnt inhibition activities in breast cancer cells.



▶Fig.1

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P-066 Variation of phenolic compounds and expression of phenylpropanoid biosynthetic genes in two medicinal and aromatic species of *Salvia* subg. *Perovskia*

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Salvia yangii B.T.Drew (*Perovskia atriplicifolia*) and *S. abrotanoides* (Kar.) Sytsma (*P. abrotanoides*) are both rich in bioactive polyphenols and terpenoids and thus widely used in traditional medicine in Iran, Afghanistan and Pakistan. Targeted comparison of metabolic profiles of these two species revealed significant differences despite their close taxonomic relationship.

To get a better understanding into mechanisms regulating the biosynthesis of phenolics, roots and leaves of *S. yangii* and *S. abrotanoides* collected in three growth stages during the vegetative season were analyzed for non-volatile phenolics profile and key biosynthetic gene expression. The LC- MS analysis showed that in leaves non-volatile phenylpropanoids were exclusively represented by rosmarinic acid while in roots RA and its two derivatives: salviaflaside and salvianolic acid L were found. The accumulation of RA and its derivatives was observed in both – leaf and root tissue, during vegetation season.

To enable monitoring the expression of phenylpropanoid biosynthetic genes, partial cds of phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), 4-coumarate:CoA ligase (*4CL*), tyrosine aminotransferase (*TAT*), hydroxyphenylpyruvate reductase (*HPPR*) and two isoforms of rosmarinic acid synthase (*RAS1 and RAS2*) in *S. yangii* and *S. abrotanoides* were amplified and sequenced. Quantitative real-time PCR analysis was used to investigate the expression pattern of amplified biosynthetic genes. We used a comparative analysis of phytochemical and gene expression profiling to elucidate transcriptional mechanisms underpinning biosynthesis of non-volatile phenylpropanoids in *S. yangii* and *S. abrotanoides* during a vegetative season.

P-067 Assessing the role of the gut microbiome for the mode of action of the fixed herbal combination STW-5

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STW-5 is a fixed combination of nine medicinal plants, that is used for the treatment of functional gastrointestinal disorders.

An *in-vitro* platform was used to simulate the digestion of STW-5 in the upper intestinal tract, followed by microbial fermentation with human faecal

samples to assess its possible impact on human gut microbiome. STW-5 was predigested by means of the static *in-vitro* digestion model InfoGest [1] to mimic the conditions in the upper gastrointestinal tract. Thereafter, the predigested STW-5 was incubated with 10% stool suspension obtained from 10 healthy donors under anoxic conditions at 37°C (physiological conditions). Samples were drawn after 30min, 4h and 24h of incubation, in order to investigate the changes of the metabolic profiles caused by gut microbial metabolization of STW-5 by UHPLC-HRMS metabolomics, and to detect shifts in the microbial community composition occurring upon incubation with STW-5 by Illumina 16S-rDNA Next Generation Sequencing [2].

In-vitro predigestion did not significantly alter the composition of STW-5. While phenolic constituents like liquiritin were completely degraded after 30min by the human stool slurries, residual amounts of the triterpene glycoside glycyrrhizic acid (2-13% of initial levels) were still detectable in 4 of 10 samples after 24h. Metabolites like davidigenin became detectable after 30min or 4h and decreased again in 7 samples after 24h, indicating their further metabolization. The microbiome composition of the 10 samples was very heterogenous, indicating that microbiome shifts caused by STW-5 depend on the individual donor's gut microbiome.

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P-068 Development of escin-based nanovesicles loaded with berberine chloride and percutaneous permeation study

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Escin (ESN) is the major active constituent isolated from the *Aesculus hippocastanum*. ESN is clinically used as anti-inflammatory and anti-oedema agent, as well as to increase venous wall tone [1]. Due to its chemical structure, it was selected in order to study its ability in replacing cholesterol inside the liposome bilayer. The obtained new liposome was tested for its carrier properties, loading a natural active molecule, berberine chloride (BRB HCl). BRB is an isoquinoline alkaloid used in the Traditional Chinese Medicine from the ancient times, because of its several therapeutic properties [2]. Different liposomes made of ESN and loaded with BRB HCl were prepared according to the thin-layer evaporation method. All liposomes showed an high ζ-potential, a low polydispersity index and spherical shape, by light scattering analysis and microscope observation, respectively. The dialysis bag method, followed by HPLC-DAD analysis, was used in order to determine the Encapsulation Efficiency (EE) and to investigate the in vitro release of BRB HCl and ESN, with the following results: the EE was approximately 67% for BRB HCl and 94% for ESN. Liposomes chemical-physical stability was monitored for a month, at 4°C and the deformability was evaluated by extrusion. Successively, the cutaneous absorption was studied by skin-PAMPA (parallel artificial membrane permeation assay) and the *in vitro* permeation assay on rabbit ear skin, using vertical diffusion Franz cells. The passive transport through the skin was comparable for the ESN liposome and the conventional cholesterol liposome. Moreover liposome loaded with ESN and BRB HCl showed an higher permeation for ESN and BRB HCl, compared to the free molecules. Finally, the *in vivo* irritation test on rats proved the suitability of this co-delivering formulation for dermatological applications.

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P-069 Development of lipid-based nanocarriers for increasing gastro-intestinal absorption of lupinifolin extracted from *Albizia myriophylla* Benth.

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Lupinifolin (L), a plant flavonoid, has been reported to possess various pharmacological effects [1–3]. It is most likely to exert low oral bioavailability because of poor water solubility [4]. The objective of this study was to develop lipid nanocarriers as drug delivery systems to increase gastro-intestinal absorption of lupinifolin extracted from *Albizia myriophylla* Benth. Three types of nanocarriers, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and nanoemulsions (NE) were prepared by emulsification-sonification technique. All three types of nanocarriers loaded with lupinifolin, LSLN, LNLC, and LNE, were successfully synthesized. The lipid components chosen to formulate nanacarriers were Dynasan®116 and/or medium chain triglyceride. Physicochemical characterizations along with releasing profiles of lupinifolin-loaded lipid nanocarriers were compared. It was found that the best lipid nanocarriers for lupinifolin was LNLC, which demonstrated the particle size of 151.5 ± 0.1 nm, monodispersity distribution with PdI of 0.24, negative surface charge at − 41.2 ± 0.7 mV, high encapsulation (99.3%), and high loading capacity (5.0%). The obtained LNLC exhibited prolonged release in simulated circulatory system, but produced low release in gastro-intestinal condition (3.7%). Intestinal permeability of the nanocarriers was further evaluated in everted intestinal sacs method. The results from the ex vivo study indicated that LNLC significantly increased the absorption, compared to native lupinifolin. Lupinifolin absorption through LNLC was about 16 times higher than the native form. In conclusion, lupinifolin-loaded lipid nanocarriers were successfully formulated as delivery systems to enhance its oral bioavailability. Further in vivo experiments are needed to validate the results from this study.

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P-072 Liposomal incorporation of *Thymus* essential oils and *in vitro* antibacterial activity

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Essential oils (EOs) of *Thymus* species have been known to possess antimicrobial properties and therefore are of great interest in pharmaceutical, food and cosmetic industry. However, they are poorly soluble in water, unstable and susceptible to degradation. Encapsulation of EOs in different nanocarriers represents a valid strategy to overcome these limitations as well as to enhance their biological activity [1, 2]. The present study aimed to evaluate the antibacterial potential of liposomally-encapsulated phenol rich EOs from *T. longicaulis* C. Presl, *T. pulegioides* L. and *T. vulgaris* L. originating from Croatia. The commercial *T. vulgaris* EO was used for the optimization of liposomes' composition and preparation procedure. Based on the high encapsulation of EO and satisfied physical properties achieved, liposomes containing 20 mg/mL of soy phosphatidylcholine, 2 mg/mL of cholesterol and 5 mg/mL of EO were selected as optimal for testing the antibacterial activities against *S. aureus*, *E. faecalis* and *E. coli*. The mean diameters of liposomes were 187–216 nm with polydispersity indexes from 0.43 to 0.53, while encapsulation efficiency varied between 51% and 57%. The obtained minimal inhibitory concentrations (MICs) were in the range 0.25–2 mg/mL. Encapsulation of the *Thymus* EOs in liposomes improved their solubility, stability and enhanced their antibacterial activities exhibiting MICs two-fold lower than the MICs of the corresponding pure EOs against *S. aureus* and *E. coli*. Liposomally-encapsulated *T. longicaulis* EO demonstrated the strongest antibacterial effect showing four-fold increase of activity against *E. faecalis*, thus indicating its potential use as biopreservative and natural remedy.

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P-073 Abstract see SL YRW-05

Abstract see on page 1396

P-074 Novel nanocarriers for the bioactive natural products alkannins for topical use

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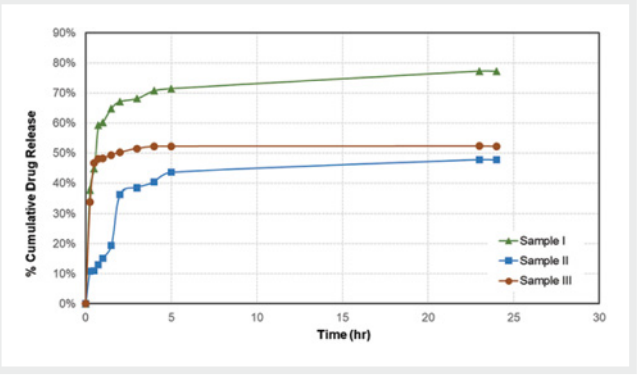
Nanocarriers, such as liposomes, niosomes and nanoemulsions are extensively investigated drug delivery systems (DDS) for lipophilic and hydrophilic drugs [1]. Alkannins and shikonins (A/S) are naturally occurring hydroxynaphthoquinones with a well-established spectrum of wound healing, regenerative, antimicrobial, anti-inflammatory, antioxidant and antitumor activities [2]. Our group has successfully developed and characterized liposomal DDS of A/S using several phospholipids [3]. The aim of the present study was to prepare and characterize novel nanocarriers containing a mixture of alkannin and its derivatives (API) isolated as in [4] from the hexane extract of *Alkanna tinctoria* roots, using PHOSAL® 40IP (kindly donated by Lipoid GmbH, Ludwigshafen, Germany), by the reverse phase evaporation technique [5]. PHOSAL® contain soybean phosphatidylcholine in sunflower oil and can be used as carriers for lipophilic and amphiphilic APIs. In order to optimize the formulation, different ratios of PHOSAL:cholesterol (10:1, 6.5:1 and 3:1 w/w) and PHOSAL:drug (40:1, 22.5:1 and 5:1 w/w) were examined, monitoring the particle size distribution, ζ-potential values, entrapment efficiency and drug release. The prepared formulations exhibited mean particle size from 120–200 nm, PDI values ranging between 0.117–0.269 and ζ-potential ones ranging from − 11 to − 18 mV. Furthermore, the API loading ranged between 3.12–5.98%, while entrapment efficiency reached 70%. The in vitro release was measured at phosphate buffer saline (pH 5.5 with 1% SDS) using UV spectrophotometry at 519 nm, as shown in ▶**Fig. 1**. These findings are considered promising and could be used for further design of dermal nanocarriers using PHOSAL® 40 IP.



▶**Fig. 1** Release profile of APIs from different samples (519 nm).

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P-075 Preparation, characterization and *in vitro* evaluation of novel silymarin-loaded nanomicelles

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Silymarin (SLM) is a mixture of flavonolignans extracted from fruits of *Silybum marianum* L. Gaertn. (Asteraceae). SLM has been used from the ancient times in the treatment of liver and gallbladder diseases and its efficacy in many pathologies is reported [1]. However, the low aqueous solubility and poor oral bioavailability limit its clinical use. To overcome these drawbacks, two novel nanomicellar formulations were developed. Polymeric nanomicelles made of Soluplus and mixed nanomicelles combining Soluplus with Vitamin E TPGS were obtained by the thin film method. The solubility of silymarin increased by ~ 6-fold when loaded into nanomicelles. Furthermore, both formulations were chemically and physically characterized in terms of average diameter, homogeneity, zeta potential, morphology, encapsulation efficiency, drug loading, critical micellar concentration and cloud point. Nanomicelles showed sizes of ~ 60 nm and polydispersity index 0.1. The encapsulation efficiency was 92% indicating the high affinity between silymarin and the core of the nanomicelles. The physical and chemical parameters of SLM-loaded formulations stored at room temperature and in refrigerated conditions were monitored over three months. Stability and release studies in media mimicking physiological conditions were conducted. A DPPH assay was performed to evaluate the antioxidant properties of SLM. Preliminary studies with artificial membranes indicated that nanomicelles increased the intestinal absorption of SLM. Subsequently, transport studies employing Caco-2 cells demonstrated that mixed nanomicelles statistically enhanced the permeability of SLM with respect to polymeric nanomicelles and unformulated extract. Finally, uptake studies indicated that both nanomicellar formulations entered into Caco-2 cells via energy-dependent mechanisms.

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P-076 Study of disturbed metabolism correction in tumor cells treated by combination of iron oxide nanoparticles and *Limonium* extract

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It is known that metabolic pathways are tightly connected with key signs of malignant tumor: heterogeneity, metastasis, reprogramming of the cellular environment. The aim of the work was to study the correction of metabolic disorders of the experimental tumor (sarcoma 45) with the help of the extract from the *Limonium gmelinii* roots [1]. As previously shown, iron oxide nanoparticles are able to reactivate immune cells [2], so, we synthesized them in our extract.

Growth of sarcoma 45 was accompanied by a sharp increase in the content of lactic acid (LA) in almost all analyzed organs. Significant decrease from 17.04 to 0.3 mmol/g after Limonidin application was found in the kidneys, 1.5 times decrease noted in the liver and 2–3 times decrease in the lungs, heart and skeletal muscle. Limonidin contributed to the restoration of normal LA content in most of the internal organs, especially in the parenchymal organs – in the kidneys, liver and spleen. Tumor growth was characterized by a decrease in the content of pyruvic acid by 50-60% in the liver and heart of tumor carriers, and in the kidneys and lungs its content decreased by 30-50% with insignificant fluctuations in the spleen. It was noted that Limonidin contributes to an increase in the content of pyruvic acid in the liver, spleen and muscles by 30-40%, in the tumor – by 70%, in the lungs, heart and kidneys – by 2–4.2 times. Based on our results, we may say that the drug corrects the disturbed metabolism of tumor cells.

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P-077 Application of matrix solid-phase dispersion for HPLC analysis of polyphenol profile in 50-years old herbarium specimens of *Polygonum aviculare*

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Voucher specimens stored in herbaria are valuable for comparative studies in chemotaxonomy and diversity of medicinal plants. They aid in proper identification of unknown material and plant samples stored in various institutions. However, herbaria specimens are under special protection and often phytochemists are not allowed to take samples for chemical analysis Therefore, only little is known about the stability of polyphenol compounds in plant material stored for decades or even centuries

In this study, we employed Matrix Solid-Phase Dispersion (MSPD) procedures [1] to prepare extracts for HPLC analysis using as little as 100 mg of plant material from up to 50-years old samples of *Polygonum aviculare*.

The one step extraction with C18 silica gel as dispersing phase in proportion of 1:4 of herbal material eluted with methanol was sufficient to obtain repeatable profiles comparable to classical solvent extraction. The major flavonoids such as quercetin-, myricetin- and kaempferol-glycosides were well preserved in most of the 20 analyzed herbarium samples as compared to the freshly prepared material from validated *Polygoni avicularis herba*. 100 mg of plant material was enough for extraction and did not provoke significant damage to the specimens. The samples despite differences in collection years and classification to various subspecific taxons were all very similar in terms of the TLC and HPLC profiles

and content of major flavonoids. The tested method proves the concept of using MSPD as convenient approach for extraction of unique historical samples or when only small amounts are available.

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P-078 Authenticity assessment and detection of adulteration in Bulgarian rose (*Rosa damascena* Mill.) essential oils

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Rose (*Rosa damascena* Mill.) oil is one of the most expensive essential oils and therefore often subject to adulteration.

Common cases of adulteration include the admixture of fatty oils or high-boiling glycols, the addition of specific compounds or of (fractions of) similar but cheaper essential oils [1]. Physicochemical properties like relative density, refractive index and optical rotation outside certain limits for rose oil specified in ISO 9842 indicate crude adulterations.

Invisible under GC conditions usually applied for rose oil volatiles, the admixture of fatty oils or other non-volatiles is uncovered by GC/MS after transesterification, high-temperature GC using dimethyl polysiloxane as stationary phase and TLC on silica gel developed with isopropyl ether:acetic acid (94:6) and light petroleum:diethyl ether:acetic acid (90:10:1), respectively. Since enantiomeric ratios of chiral compounds are highly specific for an essential oil, chirality evaluation of *linalool, β-citronellol,* and *cis/trans rose oxide* is also used as a powerful tool in authenticity control of rose oil [2], and the ratio of *R/S* linalool in the present study was found to be 1.2, whereas *β-citronellol* is solely found in its *S*-configuration.

Most significant, the ratios of major constituents in rose oil and typical minor compounds (not or hardly available as isolates or individual compounds) proved to be an appropriate authenticity criterion, and uncommon values were found to be diagnostic for adulteration with major constituents.

Finally, NIR spectroscopy combined with chemometrics using cluster analysis covering data from in-house quality control of >100 batches provides an additional tool for genuineness and purity control purposes.

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P-079 Comparison of oil cakes from *Camellia oleifera* and *Camellia sinensis* by LC-DAD-MS^n and LC-ESLD

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Camellia oleifera and *Camellia sinensis* belonging to the Theaceae are widely cultivated in eastern and southern Asia [1]. The core of both plants´ seeds contains a large amount of oil that is extracted to produce tea oil. Tea oil is the main edible oil in China's southern provinces. Besides it is used to produce soap, margarine, hair oil and other cosmetics [2]. After extraction of the oil, the residual oil cakes are commonly used to formulate natural bioactive specialities [3]. Studies have shown that these by-products have many applications for animal nutrition (growth performance, improving immune function, enhancing antibacterial and antiviral activities) [4,5]. Cultivation of *Camellia sinensis* is pursued mainly for producing tea from buds and leaves and most seeds are not exploited for their oil. *Camellia oleifera* is exclusively used for the production of tea oil, oil cakes are available in abundance. [1,3]. Due to the lower availability of *Camellia sinensis* oil cakes, a comparison of the metabolites present in oil cakes of both species was performed by LC-DAD-MS^n and LC-ESLD. The results show a similarity in the distribution of flavonols and triterpenoid saponins. This study suggests that cake oils from *Camellia oleifera* and *Camellia sinensis* are comparable so that both may serve as source for animal nutrition depending on their availability.

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P-080 Composition of rose (*Rosa damascena* Mill.) essential oils from various geographic origins

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Rosa damascena Mill. (Rosaceae) requires temperate climate as well as alkaline soil pH during flowering to attain high oil contents. It is usually cultivated at an altitude between 300 – 1500 m ASL. Having favorable edaphic and climatic conditions, Turkey and Bulgaria are the main rose oil producers in the world, followed by Morocco, Iran and a couple of a few other countries producing smaller quantities [1].

Different agrometeorological and technological conditions in these countries result in varying chemical compositions of the respective rose oils [2]. In the present study the chemical composition of 154 batches, originating from Turkey, Bulgaria, Iran, Ethiopia and Afghanistan, were evaluated. Essential oil composition was analyzed by GC-FID using surface-bonded polyethylene glycol as stationary phase.

Major constituents of the eleoptene fraction were found to be the acyclic monoterpene alcohols *β- citronellol, geraniol* and *nerol*. While *β-citronellol* content was similar in all the oils investigated (30.3%-32.6%), *geraniol* and *nerol* contents were highest in oils from Afghanistan (23.7% and 12.7%, respectively) but lowest in Ethiopian oil (16.6% and 7.2%, respectively). The stearoptene fraction predominantly consisting of the aliphatic hydrocarbons *nonadecane* (C_{19}) and *heneicosane* (C_{21}) was highest in Ethiopian rose oils (19.8% for C_{19} + C_{21}), followed by rose oils from Iran (14.4% for C_{19} + C_{21}). Since the roses in Ethiopia and Iran grow at much higher altitudes the higher stearoptene content in these oils could be explained by a thicker cuticular wax layer preventing rose petals from evaporation of water and volatiles upon solar radiation.

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P-081 HPTLC identity testing for Rhamni purshianae cortex and isolation of Cascarosides B, C and D as reference compounds

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Rhamni purshianae cortex is the herbal drug from the bark of *Frangula purshiana* Cooper, which is native to the Pacific coast of North America and has been traditionally used for treatment of constipation. The characteristic constituents of this laxative herbal drug are anthraquinones, oxanthrones, anthranols and anthrones . The herbal drug should not contain less than 8% hydroxyanthracene glycosides [1], of which 60-70% are cascarosides, mixed anthrone-C- and O-glycosides [2].

The aim of this study was to develop a suitable HPTLC method for revision of identity testing in the Cascara monograph of the European Pharmacopoeia [1].

The mobile phase water:methanol:ethyl acetate:acetic acid (19:21:90:2 v/v) was found optimal among twelve test solvents on normal phase HPTLC to get sharp bands of cascarosides A, B, C and D and a System Suitability Test with 50 g/l KOH in ethanol 50% v/v as derivatizing agent. Selectivity of the method for Rhamni purshianae cortex in comparison to *Frangula alnus* Mill., as a closely related herbal drug is demonstrated. Adulteration is not reported as being common. Since only cascaroside A is commercially available, cascarosides B, C and D were isolated. DryLab4 software (Molnar Institute Berlin) was used to develop an UHPLC -DAD-MS method for identification of cascarosides through their UV and mass spectra by comparison with data in [3].

The study demonstrates a method, which is proposed for adoption into the Ph. Eur. and provides for separation and location of cascarosides A-D on the HPTLC fingerprint.

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P-082 Identification and quantification of polymethoxylated flavonoids in different *Citrus* species using UPLC-QTOF-MS/MS and HPLC-DAD

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Many species from the genus *Citrus* (Rutaceae) are used in traditional medicine and contain the well-studied group of polymethoxylated flavonoids (PMFs). PMFs appear to have a broad spectrum of activities, including anti-inflammatory and chemopreventive activities. Consequently, they have big potential to be developed as therapeutic agents or dietary supplements [1-3].

The profile and yield of PMFs is different for each *Citrus* species. Seven different species were profiled using UPLC-QTOF-MS/MS. The generated data were analyzed using GNPS Molecular Networking [4]. At least 30 different PMFs were identified.

As the yield of PMFs is different for every *Citrus* species, a generally applicable HPLC-DAD method was developed and validated according to the ICH guidelines [5] to quantify the amount of nobiletin and the total amount of PMFs. A calibration model of the external standard nobiletin (1.51 - 150.90 µg/mL) showed a good linear response (R^2 > 0.999). The method displayed a good intermediate precision on time and concentration levels, for both nobiletin as the total amount of PMFs. The recovery of the method was 99.3% for nobiletin.

Analysis of the seven samples showed qualitative and quantitative differences in the composition of PMFs in the respective species. While *C. limon* contained the lowest amount of total PMFs, *C. depressa* contained the highest amount. Qualitative analysis revealed the most broad variety of different PMFs in *C. depressa*, as well as in *C. reticulata* and *C. reticulata x C. sinensis*. This makes them interesting sources of PMFs for future development of therapeutic agents or dietary supplements.

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P-083 Identification of radical scavenging ability and active ingredient of the hot water extract of *Forsythia viridissima*

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Elimination of oxidizing agents is an effective way to reduce oxidative stress levels. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay is generally used to determine antioxidant activity.

The aims of the present study were to evaluate the antioxidant activity of hot water extract of *Forsythia viridissima* dried fruit by DPPH assay and to identify the potential active antioxidant ingredient.

The DPPH radical scavenging activity of the extract was compared to that of L-ascorbic acid (standard). The RC_{50} values (concentration required for a 50% reduction of radicals) of the water extract and ascorbic acid were 556.7±13.0 µg/mL and 103.4±2.6 µg/mL, respectively. The analysis of the extract using ultra-high–performance liquid chromatography-quadrupole-time-of-flight mass spectrometry (UPLC-ESI-Q-TOF/MS) revealed eight compounds. Among them, only the peak area of quercetin-3-O-rhamnoside was found to decrease in a concentration-dependent manner following the DPPP and FVe reaction. Mechanism has not been elucidated yet, but there are many reports that the reaction of DPPH with antioxidants leads to a decrease in the HPLC peak. In addition, we also confirmed the decrease in HPLC peak after reaction

with DPPH using L-ascorbic acid and caffeic acid. Thus, accumulated evidence by in vitro test suggests that peak decrease is associated with radical scavenging activity in the presence of quercetin-3-O-rhamnoside.

In this study, the potential antioxidant activity of was confirmed by DPPH assay and quercetin-3-O-rhamnoside was found to be highly related to DPPH radical scavenging activity. An antioxidant study of quercetin-3-O-rhamnoside against oxidative damage of DNA, proteins, and lipids has been planned for the future.

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P-084 Identification of some *Gnetum spp.* (Gnetaceae) in Southern Vietnamese by DNA barcode

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Gnetum is a tropical forest gymnosperm genus with about 47 species [1] are accepted by 'The Plant list', [1] and distribute in tropical forest around the world. In Vietnam, there are about 9 species and varieties are recorded. However, some of them were not included in 'The Plant List'. Some are medicinally used in Vietnamese traditional medicine. Recent chemical and bioactivity studies on some of them revealed interesting results for future applications in health care. In Southern Vietnam, *Gnetum gnemon* L. var *griffithii* Markgr., *Gnetum gnemon* L. var *tenerum* Markgr.,*Gnetum macrostachyum* Hook.f., *Gnetum leptostachyum* Blume var. *elongatum* Markgr., *Gnetum leptostachyum* Blume var. *latifolium* Markgr., *Gnetum latifolium* Blume var. *funiculare* (Bl,) Markgr., *Gnetum macrostachyum* Hook f., *Gnetum montanum Gnetum montanum* Makrg. var *macrocarpum* Makgr. are recorded [2].

Due to the lack of detail information for plant identification, it is difficult to distinguish between species based only on their morphological characteristics. This study was conducted to use DNA barcodes as additional information to accurately identify *Gnetum* species in Southern Vietnam. 4 *Gnetum* samples, collected in Hon Son island, Kien Giang province (GL1, GL2, GL3) and Cu Chi district, Ho Chi Minh City (GL4) were used for morphological, anatomical investigation and DNA barcode determination. Results revealed that 4 samples GL1, GL2, GL3 and GL4 are *Gnetum parvifolium* (Warb.) W.C.Cheng, *Gnetum latifolium* Blume, *Gnetum ula Brongn*.and *Gnetum montanum* Markgr., respectively, with DNA uniformity rate up to 99%. This is the first time Vietnamese *Gnetum ula* has been recorded in Vietnam.

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P-085 LC-TOF-MS-based metabolomic fingerprinting of *Rumex* species

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The genus *Rumex*, belonging to the Polygonaceae family, comprises 183 accepted species including subspecies [1], about 20 of them being native to Central Europe. Some species are commonly used as vegetable but are also relevant in traditional medicine and modern phytotherapy. However, macroscopic differentiation of species proves to be difficult due to high similarities in morphological features, needing a combination of traits from basal leaves and mature fruits for the unambiguous identification.

This study aims to simplify the discrimination of *Rumex* species by liquid chromatography time-of-flight mass spectrometry (LC/TOF/MS) based metabolomic fingerprinting approach.

Accordingly, plant extracts of various species (*R. acetosa, R. acetosella, R. alpestris, R. alpinus, R. conglomeratus, R. sanguineus, R. scutatus, R. thyrsiflorus,* and *R. patientia*) and different geographic locations in Germany and Austria were analysed. The principal component analysis on a unit variance (UV) scaled dataset revealed clustering of samples according to their respective traditionally morphology-based subgenera. Orthogonal partial least squares discriminant analysis (OPLS-DA) models were used to identify specific discriminative MS-features as putative markers for all species, especially for three morphologically almost identical species *R. acetosa, R. alpestris* and *R. thyrsiflorus* of subgenus *Acetosa*. In contrast to a recent phylogenetic study [2] which proposed to combine *Rumex* subgenera *Acetosella* and *Acetosa,* our results show a distinct grouping of all species of subgenus *Acetosa*, whereas the cluster of *R. acetosella* samples is isolated, suggesting that *R. acetosella* is a separate subgenus at a chemotaxonomic level.

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P-087 ^{13}C-NMR dereplication of medicinal plant extracts using a home-made software

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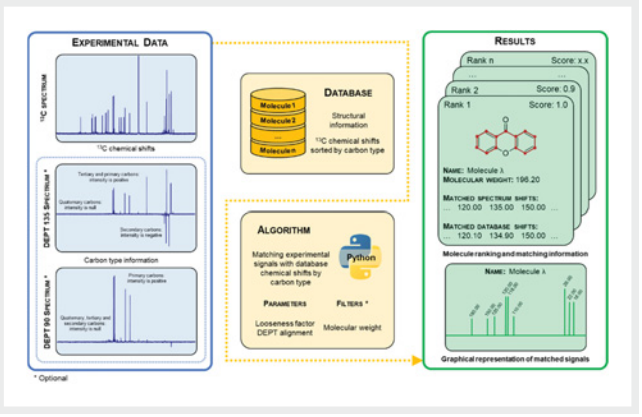
In natural products research, modern dereplication techniques reduce the usually time-consuming process of known compound isolation. Most commonly reported analytical tools during dereplication analysis are mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy [1–2]. Though low sensitive,^{13}C-NMR has the advantage of being appropriate to (complex) mixtures whereas diastereomers can easily be discriminated. Since NMR spectrometers nowadays provide useful dataset in a reasonable time frame, we have embarked on writing a software dedicated to ^{13}C-NMR dereplication. This (soon) freely available software, based on Python 3.5, processes ^{13}C as well as DEPT 135 and 90 data, optionally allowing a carbon-type (*i.e.* CH_3, CH_2, CH and C) as well as a MW filtering. It requires predicted or experimental carbon chemical shifts $\delta_{(C)}$ databases (DB) [3] and displays interactive results that can be refined based on the user's phytochemical knowledge (Fig. 1)

As a proof of concept, the present work presents the results obtained on two different medicinal plant extracts: *Rosmarinus officinalis* E392, used as an antioxidant food additive [4], and *Mentha piperita* essential oil, traditionally used in aromatherapy [5]. Here the predicted δ_C DB was built from 980 structures reported in Lamiaceae (SciFinder®) using ACD/Labs® software [6]. Interlocking ^{13}C and DEPT information, our program managed to successfully identify all the major compounds in both extracts.



▶ **Fig. 1** General process of the dereplication program.

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P-088 A high-throughput multivariate statistics platform for the discovery of tyrosinase inhibitors

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The implementation of high-throughput screening (HTS) technologies has become an indispensable tool for the detection of bioactive constituents, avoiding re-isolation of known compounds, reducing workload and cost. The aim of our study was the establishment of an integrated high-throughput multivariate statistics platform, relying on FCPC, HPTLC, and NMR, for the direct detection and identification of natural compounds with skin whitening properties prior to any isolation. Greek flora - due to its high biodiversity - was used as a source for the collection of selected plant material. Previous *in vitro* investigation of plant extracts against their tyrosinase inhibition activity revealed nine extracts as the most promising. The selected extracts were fractionated by FCPC using a certain protocol and each fraction was split in 3 equal parts for: i) HPTLC profiling and bioautography, ii) NMR profiling iii) *in vitro* assay. An integrated HPTLC-based procedure for the tracing of compounds that contributed to tyrosinase inhibitory effect in active fractions was established with the use of multivariate data analysis [1]. Additionally, NMR spectral data were correlated with the results of tyrosinase activity resulting in the identification of bioactive compounds through the combination of the Heterocovariance approach (HetCA) [2] and the statistical total correlation spectroscopy (STOCSY) [3]. The combined data deriving from NMR and HPTLC correlated to the biological activity by the statistically driven approach, revealed flavans, flavonols, phenolic compounds and stilbenoids between the most promising whitening agents, providing a major reduction in workload by direct use of routine information.

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P-089 A holistic UPLC-HRMS-based approach exploring the phytochemistry, bioactivity and metabolome impact of *Cichorium spinosum*' s decoction in *Drosophila melanogaster*

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Edible greens ("chórta") comprise an integral part of Greek tradition and they have been used both as nutritional elements, but also as phytotherapeutic products. *Cichorium spinosum* ("stamnagkathi") is a highly valued and extensively consumed endemic plant of the Mediterranean region [1]. Preliminary studies exploring *C. spinosum's* effect in *Drosophila melanogaster* diet, have demonstrated the extract's ability to enhance the proteasome activity and to activate the antioxidant Nrf2 pathway [2].
In the present study, an extensive characterization of the decoction's chemical profile was performed using UPLC-HRMS & HRMS/MS. The extract was found to be rich in simple phenolic acids, lactones, hydroxycinnamic acids, as well as in flavonoid glucuronides. In parallel, an untargeted metabolomics protocol was developed using a UPLC-ESI-Orbitrap in order to further investigate the effects of the addition of *C. spinosum*'s extract on *D. melanogaster* diet. The characterization of *D. melanogaster* endogenous metabolites was followed by the analysis of several fly samples whose diet had previously been enriched with *C. spinosum's* decoction at different concentrations. This approach enabled the identification of significant metabolites leading to the exploration of certain biological pathways modulated by the *C. spinosum*'s extract.
Our results indicate that liquid chromatography coupled to mass spectrometry in combination with the model organism *D. melanogaster* [3] in the concept of metabolomics can be considered as a valuable tool for determining the effects of natural products on complex biological systems.
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P-090 Comprehensive chemotaxonomy: mining data from tandem mass spectrometry of lichens

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From as early as the 19th century, it was suggested that the « chemical nature » of plants would have value for their taxonomy [1], although the means of verifying this postulate were scarce at the time. The recent development of untargeted metabolomics offers new tools to address this problem.

Computational solutions have been developed to optimally exploit such untargeted tandem mass spectrometry data. Spectral similarity, deduced from the cosine score, and substructure-linked spectral motifs [2] are notably focusing on metabolite organization and annotation.

In this study, we take advantage of these tools to obtain structural and spectral relationships, which will allow us to classify species (considered here as complex ensembles of molecules). We postulate that the biosynthetic relationships between the chemical features can be inferred from the cosine scores and spectral motifs, which in turn help to classify the producing organisms. We use spectral motifs to compute a modified Junker's BioSynthetically Informed Distance expanding its scope to non-annotated features [3]. Furthermore, we combine it with the weighted Chemical Structural and Compositional Similarity distance [4, 5], introducing a new Structural and Substructural Similarity Informed Distance. Results produced by this method on 32 specimens of the genus *Usnea* (lichenized Ascomycetes) seem to efficiently reflect the phylogeny established by multilocus analysis. We further applied this metric to classify 169 specimens of *Usnea* lacking genetic information.

These tools should efficiently complement the set of approaches currently used to classify living organisms, which mainly so far rely on morphological and genetic characters.

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P-091 Comprehensive study of foliar endophyte communities in a rainforest palm: a model for deciphering host-microbe interactions and exploring metabolite chemo-diversity

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Endophytic microorganisms asymptomatically colonise the internal tissues of plants and constitute the "plant microbiome" [1]. They notably seem to impact the way their plant host interact with its environment. As part of the Swiss-French SECIL project (Study of Endophyte Communities In a Leaf), we sought to investigate the chemodiversity, bioactivity and host-microbe interactions of foliar endophytic fungal communities. The host model *Astrocaryum sciophilum* is an Amazonian understorey palm hosting a diverse fungal community. The aim of the present study is to assess the co-occurrence of metabolites between the host plant and its fungal community to determine the ecological relevance of given strains. We thus performed an in-depth metabolomic investigation of the leaf and its endophytes. 15 strains isolated from the leaves were cultivated and extracted. In parallel, the leaves were extracted and large-scale fractionated for metabolite enrichment by preparative chromatography. To allow for comparison between the plant and the fungal metabolomes, the HRMS/MS data of all the extracts were organised into an informative molecular network (MN) [2] that was annotated against experimental and *in silico* natural product spectral databases. This provided a detailed view of the chemical composition of the metabolomes [3]. Preliminary results show that 25 % of the features are shared by the host plant and the endophytes and common ecologically relevant chemical families such as oxylipins derivaives could be highlighted. The potential for MN to study metabolic element contributions in these interactions will be discussed.

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P-092 Abstract see SL D-03

Abstract see on page 1412

P-093 Cutting-edge analytical technologies for the comprehensive metabolic profiling of *Alkanna tinctoria* roots cultured in greenhouse conditions

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The use of plants containing naphthoquinone derivatives Alkannins & Shikonins (A/S) by humans dates back to ancient times. In recent decades, the use of A/S has seen a resurgence and A/S have risen to a pivotal role as pharmaceutical and cosmeceutical agents, since they possess strong wound healing, antimicrobial, anti-inflammatory, tissue regenerative and antitumor properties. It is thus crucial to enhance the biosynthesis of bioactive A/S in *Alkanna tinctoria* plants, that naturally produce high amounts of these metabolites [1].

In the frame of "MICROMETABOLITE" EU H2020 project, we have optimized a workflow for the metabolic profiling of *A. tinctoria* roots, cultured in the greenhouse from plants obtained by *in vivo* shoot cuttings. A fast and reliable extraction procedure was achieved for comprehensive profiling and identification of A/S and other metabolites biosynthesized in the roots. The aim of this work was to determine the growth stage with peak A/S production, while simultaneously obtaining additional information on the root metabolome. A combination of UHPLC-HRMS and NMR was used for metabolite identification, HPLC was utilized for reliable quantitation of A/S and the extracts were subjected to chiral HPLC analysis [2] for determination of the enantiomeric A/S ratio.

Different A/S derivatives and other metabolites were identified in plant roots using UHPLC-HRMS and NMR. Six A/S derivatives and total A/S were quantified using HPLC-DAD. From six vegetation stages of *A. tinctoria* grown under greenhouse conditions, fruiting period was found to peak A/S production (1% wt/wt of root), while the enantiomeric alkannin/shikonin ratio remained constant (93.7%).

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P-094 Direct injection-electron ionization-mass spectrometry and NMR metabolomics method for analyzing Rhubarb species that inhibit adult T-cell leukemia proliferation

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Metabolic profiling techniques are commonly used to find possible correlations between the metabolic profile of a compound and its biological activity. Direct Injection-Electron Ionization-Mass Spectrometry (DI-EI-MS) [1] and NMR are comprehensive and powerful tools for such analyses, because they allow the simultaneous detection of diverse groups in both primary and secondary metabolites. We were able to select blueberry species whose leaves have anti-proliferative effect in adult T-cell leukemia (ATL) cells, by DI-EI-MS- and NMR-based metabolomic techniques [2]. It is well known that Rhubarb species have also anti-proliferative effect in several cancer cells. However, the active constituents in Rhubarb species are not fully identified. In the present study, we assessed if constituents in Rhubarb species have anti-proliferative effect in ATL cells. Furthermore, we also tried to distinguish the Rhubarb species having better anti-proliferative effect in ATL cells from the other Rhubarb species. Methanol extracts from roots or leaves of some Rhubarb species showed anti-proliferative effect in ATL cells. Therefore, DI-EI-MS- and NMR-based metabolomics can be used to classify bioactive extracts. Principal component analysis of the extracts interpreted from the loading plot analysis showed that each extract had unique ion peaks or chemical shifts. This unique information observed in Rhubarb species were associated with high anti-proliferative effect in ATL cells. These results suggest that Rhubarb species could be classified based on the results of metabolite profiling with DI-EI-MS and NMR.

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P-095 Efficient isolation of new bioactive metabolites from the marine endophytic fungi *Fusarium solani*

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Endophytes are organisms, often fungi and bacteria that live between plant cells. The relationship they establish with the plant varies from symbiotic to almost pathogenic. Endophytes have proven to be a promising source of bioactive natural products with interesting structural diversity. In the present study, the secondary metabolites of the endophytic fungi *Fusarium solani* was investigated. This fungus was isolated from the rhizomes of the marine herbaceous plant *Posedonia oceanica* harvested in the Mediterranean Sea. The ethyl acetate extract of *F. solani* presented antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In order to isolate the compounds responsible for these activities, the culture of *F. solani* was up-scaled and purified by semi-prep. HPLC. A new procedure based on dry charge injection was used in order to maintain the column resolution over high charges. This approach resulted in the successfully one-step isolation of 23 compounds, 16 of them are original natural products possessing interesting antimicrobial activities. known compounds were dereplicated by in silico databases search based on UHPLC-HRMS. A molecular network of structure similarity was created within the extract based on MS-MS spectra. The structure elucidation of the isolated compounds was performed based on NMR and HRMS data analysis.

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P-097 Abstract see SL YRW-03

Abstract see on page 1396

P-098 Gas chromatography-based metabolomics in the identification of potential anti-quorum sensing compounds in commercial essential oils

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The increase in antimicrobial drug resistance has prompted researchers to find alternative ways to combat bacterial infections. Alternative drug targets such as anti-quorum sensing (AQS) have been identified as possible ways to reduce the risk of developing drug resistance. The aim of the research was to investigate the AQS activity of 40 commercial essential oils against *Chromobacterium violaceum* and identify potentially active constituents using an untargeted metabolomics approach. Chemical profiling of the oils was performed by gas chromatography coupled to mass spectrometry (GC-MS). The AQS activity was determined using the microdilution assay and spectrophotometric quantification of violacein production in *Chromobacterium violaceum*. Orthogonal projections to latent structures-discriminant analysis (OPLS-DA) was applied to the data in SIMCA-P+ 14.0, to investigate correlation between AQS activity and the essential oil chemistry. Possible biomarkers were identified using the S-plot. The essential oils with noteworthy AQS activity (percentage inhibition ≥ 90%) at 0.25 mg/ml were *Cymbopogon* sp., *Citrus limon, Eucalyptus dives, Eugenia caryophyllus, Mentha* sp., *Myrtus communis* and *Pinus ponderosa*. Using OPLS-DA models, eugenol, geraniol, geranial, menthol and pulegone were identified as putative biomarkers that may be associated with the activity observed. The results demonstrate the ability of essential oils to interfere with bacterial quorum sensing thus providing a possible alternative drug target to combat infections. Furthermore, with the aid of chemometrics, it was possible to identify potential bioactive constituents in essential oils with AQS ability.

P-099 Identification of antifungal quinolizidines using GC/MS-based metabolomics

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Genisteae plants synthesize quinolizidines as a defense strategy against herbivores and pathogens [1], so their production may depend by a particular biotic pressure. The variation of these alkaloids can be exploited to detect promptly bioactives in extracts through integration of chemical and biological activity datasets using supervised statistics. Thus, several accessions of *Genisteae* plants (from different locations in Bogotá plateu) were investigated through a comprehensive targeted GC/MS-based metabolomics approach in order to explore the quinolizidine variation between sample set and its implication on antifungal activity against *Fusarium oxysporum*. Quinolizidine-enriched extracts were obtained from leaves of *Genisteae* plants (n>66) by means of ultrasound-assisted acid-base extraction. Resulting extracts were separately tested against *F. oxysporum*, through amended-medium method in 12-well plates at 0.1-500 µg/mL, and mycelial growth inhibition was then recorded. Chemical differences in extracts were examined by partial-least squares (PLS) discriminant analysis using MS features data (retrieved from GC/MS profiles using MZmine 2.37). Single-*Y* PLS was also used to integrate the chemical and biological datasets. Extracts showed antifungal capacity at different levels (2>IC_{50}(µg/mL)>55). PLS-DA indicated several differences between samples and clustered according characteristic chemical constituents and/or activity. Single-*Y* PLS analysis evidenced three aphylline-like compounds to be related to antifungal activity (AUC>0.9), which were confirmed by independent antifungal *in-vitro* assays using the isolated quinolizidines (IC_{50}>3 µg/mL). This chemical and antifungal activity integration led to the rapid detection of bioactive quinolizidines in extracts improving the efficiency during antifungals discovery against *F. oxysporum. Product derived from Project IMP-CIAS-2924 financed by UMNG - Validity 2019.*

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P-100 Integrated UPLC-HRMS based metabolomics investigating hydroxytyrosol effect in human obesity

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Based on World Health Organization recent data, obesity is regarded as a major public health problem [1]. So far, several *in vivo* studies and experimental models investigate the effect of olive oil (OO) biophenols and particular hydroxytyrosol (HT), a strong antioxidant entity of OO, in these metabolic diseases [2]. Promising results have been uncovered and nowadays research focuses on the exploration of HT effects on human metabolome.
In this study two different UPLC-HRMS platforms were incorporated for the quantification of HT in human biological fluids and the identification of metabolites-biomarkers thereof, after the administration of an encapsulated biophenols extract enriched in HT. In particular, urine and blood samples were collected in three time points from 30 overweight/obese women, randomized in three groups according to HT intake (A–15mg HT/day, B-5mg HT/day, C-placebo). After investigation and application of the appropriate extraction protocols, a UPLC-triple-quadrupole method was developed for HT detection and quantification, using multiple reaction monitoring mode and suitable collision energies. Successively, extracts were subjected to untargeted metabolomics via UPLC-Orbitrap-MS in negative and positive ionization mode using full scan and data-depending methods. After pre-treatment, data were subjected to multivariate statistical analysis assisted by chemometric tools. Well-defined groups were revealed and statistical significant metabolites-biomarkers were identified among groups. The detected HT concentration were correlated with the identified biomarkers and the corresponding weight loss of each group. Significant interrelations were observed associating HT administration with certain trends and metabolic markers.

Acknowledgements Author would like to thank IKY financial support.

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P-101 Integrating UPLC-MS/MS-based molecular networking and NMR structural determination for the untargeted phytochemical characterization of the fruit of *Crescentia cujete* (Bignoniaceae)

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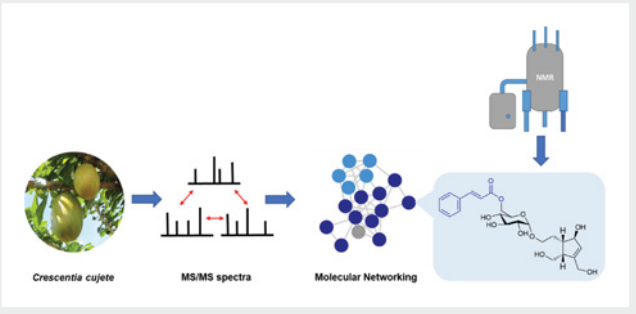
Crescentia cujete (Bignoniaceae), commonly known as calabash tree, is distributed and cultivated throughout most of Tropical America. The fruit pulp is used in folk medicine for the treatment of respiratory diseases such as colds, bronchitis and asthma, and also for gastrointestinal illness [1, 2]. Even though food supplement-derived products from the fruit are commercially available as oral syrup preparations, a comprehensive description of the phytochemical composition of *Crescentia cujete* and appropriate quality control are still lacking.

In this work, we aimed to characterize the chemical profile of the fruit of *Crescentia cujete* using an untargeted metabolomics approach including UPLC-MS/MS-based molecular networking, generated at the Global Natural Products (GNPS) Molecular Networking website [3], and conventional isolation and NMR methods as identification strategy.

The untargeted phytochemical characterization allowed for the identification of 65 metabolites, including 8 *n*-alkyl glycosides, 23 phenolic acid derivatives (such as cinnamoyl and benzoyl derivatives), 15 flavonoids (flavones, and flavanones), 4 phenylethanoid derivatives and 15 iridoid glycosides. Among these, four phenylpropanoid derivatives were described for the first time within this species. In addition, 8-epi-eranthemoside, crescentiol A and crescentiol B are reported as three new iridoid glucosides.

The use of molecular networking as a tool for phytochemical characterization of the fruit of *Crescentia cujete* provides a detailed phytochemical overview of this plant. The reported results provide a useful guide for the development of analytical methods for the quality control of commercial products containing this plant species and further interpretation of their related pharmacological effects.



▶ **Fig. 1**

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P-102 Investigation of Greek honeys using HR-NMR and LC-HRMS metabolomics, for determination of their geographical, botanical origin and authenticity

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Honey is a natural source of nutrition used since antiquity. Nowadays, it is well known that can be also applied, among others, in cosmetics, food industry and environmental engineering [1,2]. However, due to its high economic importance authenticity and adulteration are major issues mainly towards geographical and botanical origin. It should be noted that the sugars have been mainly studied, though limited studies are focused on other polar metabolites, especially phenols, which are related not only with geographical but mainly with botanical origin. Many methodologies based on liquid chromatography, and lately, NMR have been used towards this scope. The aim of this study was to analyze Greek honeys and draw conclusions about their authenticity. Special attention was given to their content in phenolic compounds. Honeys were collected from different islands of the northern east Aegean Sea in Greece (Agios Efstratios, Ikaria, Lesbos, Lemnos, Samos, Fournoi, Chios and Psara), with different herbal origin. Extraction procedure previously reported [3] was carried out with slight modifications. The profiles of the extracts were obtained with both LC-HRMS and NMR. Dereplication based on high resolution mass spectra gave information about the composition of the different honeys, while in NMR profiling in combination with multivariate data analysis enabled the classification of samples. Moreover, significant markers responsible for the observed clusters were identified. Finally, statistical data correlations from both platforms are in progress.

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P-103 Investigation of the phytochemical profiles of *Boronella* spp (Rutaceae) using molecular networking

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Recently, the genus *Boronella* (Rutaceae), endemic to New Caledonia and comprising four species, was transferred into *Boronia* on the basis of molecular phylogenetic analyses [1].

Nevertheless, chemical data on *Boronella* species are scarce. Quinolinone, furoquinoline, dihydrofuroquinoline, pyranoquinoline alkaloids along with koniamborine, an original pyrano[3,2b]indole and an isatine derivative have been described, together with coumarins, lignans and triterpenes [2–4].

In order to deeply explore the metabolome of this poorly studied section in the genus *Boronia*, LC-MS^2 and molecular networking were applied. Aiming to identify unreported derivatives from the four following species, *Boronia hartleyi* (synonym Boronella crassifolia), *Boronia koniambiensis* (syn. *Boronella koniambiensis*), *Boronia pancheri* (syn. *Boronella pancheri*) and *Boronia parvifolia* (syn. *Boronella parvifolia, Boronella verticillata*), extracts from several plant parts were investigated.

After this step, purification of the methanolic stems and leaves extracts of *B. koniambiensis* was performed by a combination of centrifugal partition chromatography, HPLC and MPLC. Among others, the isolation and structure determination of an original coumarin, 3,5-dimethoxypsoralene, was succeeded, together with bergapten, two alkaloids (koniamborine, medicosmine) and three lignans (sesamin, (8*R*,8′*R*)-3-methoxy-3,4-methylenedioxy-9-9′-epoxylignan-4′-ol, *S,S*-(+)-dehydroxycubebin).

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P-104 LC-MS/MS and molecular networking: a complementary approach for chemical composition evaluation of plant extracts – case study with Orchidaceae species

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Due to the high ornamental and medicinal value of many Orchidaceae, it is desirable to quickly evaluate their chemical composition for quality or research purposes. Despite the numerous studies achieved on orchid extracts characterization by liquid chromatography tandem mass spectrometry (LC-MS/MS) strategies [1], there is a lack of available experimental MS fragmentation patterns that tremendously complicate the dereplication process within this plant family.

Molecular networking (MN) has the potential to speed up the dereplication process by highlighting structural analogues within the sample but also across different samples [2].

In this context, we expose here a dereplication strategy based on LC-HRMS/MS and MN representation for orchid extracts characterization. Three orchids extracts have been dereplicated and implemented in molecular networks together. Two of them are from well-known studied orchids (species A from *Gastrodia* and B from *Dendrobium* genus) to assist the dereplication of the third one from another poorly studied orchid species (C).

11 molecules were putatively identified in the extract of species A and 5 molecules in the extract of species B. The method was tested by comparing some identified compounds (gastrodin; parishin, parishin B, C and E, naringenin, N-trans coumaroyltyamine and coumaric acid) with their commercial standards.

The resulting MN representation highlighted the identification of various adducts and analogues compounds among the three species extracts by establishing clusters or nodes according to fragmentation similarities.

As a result, the method is satisfactory, and MN seems to be a promising complementary step to assist the dereplication of plant extracts, especially when the sample of interest is compared with well characterized samples.

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P-106 Metabolic profile elucidation after *in vitro* biotransformation of *Herniaria hirsuta* by an innovative data analysis strategy for dynamic multiclass experiments

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Urinary stone disease is considered as an important healthcare problem. An aqueous extract of the aerial parts of *H. hirsuta* (Caryophyllaceae) is a widely used herbal medicine. However, little is known about the active compounds and the mechanism of action. [1,2] Previous phytochemical research on *Herniaria* species revealed the presence of saponins, flavonoids and coumarins. [2] It is suggested that metabolites of phytochemicals present in *H. hirsuta* (most likely saponins) are responsible for the beneficial effects.

In vitro gastrointestinal biotransformation followed by automated data analysis was optimized using hederacoside C as a model compound for saponins. Samples were analyzed with UHPLC-PDA-HRMS before, during and after biotransformation.

To analyze the longitudinal LC-MS data, XCMS and EDGE were used to extract significant differential profiles from the raw data. [3,4] An interactive Shiny app was developed in R to rate the quality of the resulting 10307 features. These ratings were used to train a random forest model. [5] A performance analysis revealed a high capability of the model to correctly predict experts response (AUC 0.93 with 10 fold cross validation). The workflow revealed the biotransformation of hederacoside C. All major metabolites were ranked in the top 100 out of 10307 features.

When subjecting an extract of *H. hirsuta* to the experimental workflow, herniariasaponin H, the most abundant saponin, showed stepwise elimination of sugar moieties resulting in medicagenic acid. Hepatic biotransformation using S9 fractions of this aglycon resulted in hydroxylated and oxydated metabolites. This insight smooths the path for hepatic biotransformation of the extract.

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P-107 Abstract see SL AR-08

Abstract see on page 1390

P-108 Metabolomics, genetics and epigenetics analysis of European yew for the selection of antineoplastic taxol/taxane producing plant material in Greece

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Taxus L species (yews) are the primary source of the leading anticancer agent taxol and generally of antineoplastic taxanes. As taxane demands exceed supply new sources, such as the marginal south-eastern *T. baccata* populations in Greece, are explored. Results of a metabolic (UPLC-MS/MS, MRM) analysis of taxane content of two populations (Mt Cholomon, Mt. Olympus) showed no significant differences in paclitaxel production, but the docetaxel and paclitaxel precursor DAB was significantly higher Mt Cholomon by 29.83%. Higher yielding paclitaxel trees (HYT) appear to be lower yielding in DAB at the population level: the 10 paclitaxel HYT of Mt. Cholomon produced 25% less that the 10 HYT of Mt. Olympus, while the 10 DAB HYT trees of the former produced 14% more than the 10 HYT of the latter. Populations presented taxane concentrations on the low end of the range reported in the literature (paclitaxel 1.1–5.7 mg 100^{-1}g dw; DAB 12.7-81.1 mg 100^{-1}g dw). Notable levels of genetic (SSR) and epigenetic (MSAP) diversity were found in both populations; the former were lower though than values reported in other relevant studies for *Taxus* (SSR). Preliminary analyses on the relations of genetic and epigenetic diversity to HYT and LYT for both taxanes were inconclusive. This analysis presents a first insight regarding the potential of future commercial exploitation of these genetic resources for taxol production.

P-109 Multi-informative bioactivity-based molecular networking of a large chemodiverse plant collection allows efficient identification of trypanocidal natural products

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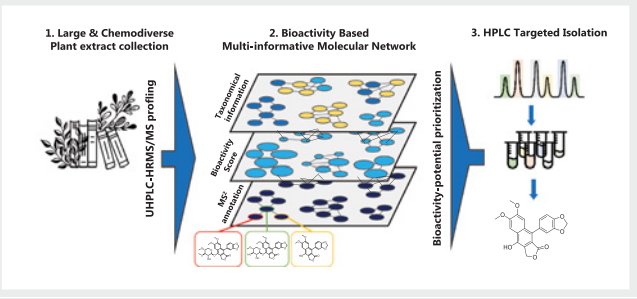
Nature is an inexhaustible source of chemical diverse and bioactive molecules. Given the complexity of natural matrices and the considerable work required to isolate pure compounds, targeting potentially active compounds prior to isolation allows considerable savings of time and resources.

We previously proposed bioactivity based multi-informative Molecular Network (MN) as a strategy to prioritize high-value natural products [1]. In this respect, we recently performed a massive metabolite profiling of a set of biodiverse extracts (1,600 plant extracts: 783 species, 533 genera and 156 families) from the library of plant parts from Pierre Fabre Laboratories made available through a collaboration. Integration of LC-MS/MS based dereplication annotations, combined with the bioactivity results mapping on three trypanosomatids and toxicity assessment, allows highlighting potentially bioactive scaffolds within the extracts library. Based on this comprehensive MN oriented prioritization [2], a targeted isolation of potentially active molecules was performed.

As a proof of concept, we were able to annotate and highlight in the MN *Melochia umbellata* quinoline alkaloids derivatives such as waltherione G, a known trypanocidal compound [3]. Using this approach, we highlighted diphyllin derivatives specific to a *Rotula aquatica* extract. We then proceeded to an efficient one step targeted isolation using semi-preparative HPLC with dry-load injection of the crude extract leading to the purification of ten compounds including five diphyllin derivatives [4].

In conclusion, we demonstrate that coupling dereplication strategies, bioactivity information mapping and efficient semi-preparative HPLC isolation grants a direct access to specific and potentially active NPs from a large and chemodiverse plant collection.



▶ **Fig. 1**

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P-110 NMR metabolic profiling of Greek *Pistacia lentiscus* leaves and fruit extracts for the identification of biomarkers with skin beneficial effects

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The healing properties of Mastic resin are well-known in the Mediterranean basin for more than 2500 years. Nowadays, the resin, a PDO product, has been well-studied and showed antimicrobial, anti-inflammatory, and other activities [1]. However, the fruit and leaves of *Pistacia lentiscus* L. shrub from Chios have not been thoroughly investigated and additionally, they are discarded as considered by-products of the mastic tree cultivation.

In continuation of our research [2], this study emphasises on the exploration of the biological properties of *P. lentiscus* leaves and fruit collected from the region of Mastichochoria. Leaves (36 samples) and fruit (89 samples) were collected from different altitudes and distance from the sea (Fig. 1) during the maturing period. All samples were extracted with various NMR solvents and their NMR metabolic profiling spectra were acquired. In parallel, their antioxidant capacity as well as their enzyme inhibition properties (tyrosinase, elastase, collagenase, hyaluronidase) related to skin whitening, moisture and elasticity were evaluated. Finally, NMR spectral data were processed with SIMCA software (Umetrics) and multivariate statistical analysis (PCA, OPLS-DA) was used to evaluate the quality characteristics of the samples and to identify potential biomarkers. This approach resulted in the detection of more than 40 spectral features (biomarkers) in all of the extracts and facilitated the classification of samples. To the best of our knowledge, this research effort is the first to study the NMR-based chemical profiling of *P. lentiscus* leaves and fruit from different regions of Chios and correlate their enzyme inhibition activity by Multivariate Data Analysis.

▶ **Fig. 1**

Acknowledgments The authors are grateful to the Chios Mastic Gum Growers Association and especially to agronomist Telemachos Vassilakis. The research is supported from "EXANDAS" project, H2020-MSCA-RISE-2015.

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P-111 Plant extracellular vesicle lipids and secondary metabolites

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Extracellular vesicles (EVs) are lipid bilayer delimited vehicles, carrying small noncoding RNAs, besides other bioactive compounds [1]. While plenty of information is available on mammalian EVs, our knowledge on plant EVs remains little. Their effects on human body are currently being investigated, while it is still unexplored how EV lipids vary between distinct species or whether secondary metabolites such as polyphenols or alkaloids are packaged into plant derived EVs. Due to manifold differences in plant families, regarding secondary metabolites and structural characteristics, the lipid composition of EV membranes probably also varies. In order to get insight into the lipid composition of EVs, an extraction method was developed and lipophilic compounds profiled by TLC. In general, EV fractions, isolated by differential centrifugation, were shown to enrich the added membrane dye 3,3′-Dihexyloxacarbocyanine iodide ($DiOC_6$) compared to the supernatant. Further, preliminary data suggest phosphatidylserine to be present in EV preparations from *Hypericum perforatum* L. and *Viscum album* L. It is still puzzling how plant EVs act in gastrointestinal environment. Since secondary metabolites such as polyphenols have the potential to inhibit gastrointestinal enzymes [2] and thereby prevent EV degradation, we investigated if secondary metabolites are packaged into EVs. Although TLC analysis revealed the abundance of several not yet identified structures, nicotine was absent in EV preparations from *Nicotiana tabacum* L. callus culture.

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P-112 Polyphenol composition of disease-resistant grapevine hybrids

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DOI 10.1055/s-0039-3399842

In modern grapevine production, sustainability and a minimal use of fungicides are desired goals. However, most of the commonly used *Vitis vinifera* varieties are susceptible to fungal pathogens. One approach is the use of disease-resistant grapevine hybrids, which result from the cross of *Vitis vinifera* species with disease-resistant American or Asian species.

For breeding purposes, it is of great importance to assess the grape quality of resulting hybrids. Polyphenols are secondary metabolites and play an important role for wine quality as well as human health and nutrition [1]. In the present study, the polyphenol composition of 74 fungus-resistant grapevine hybrids was analyzed by LC-MS-MS. Multiple Reaction Monitoring (MRM) allowed accurate quantification of 41 phe-

nolic metabolites, such as benzoates, phenylpropanoids, stilbenes, dihydrochalcones and flavonoids [2]. Flavan-3-ols were found to be the most abundant class with values ranging from 171 mg/kg FW to 1687 mg/kg FW (fresh weight). Stilbene levels varied from 0.3 mg/kg FW to 75 mg/kg FW, the well-studied trans-resveratrol was present at 7 mg/kg FW in one accession only. Total polyphenol content ranged from 249 mg/kg FW to 2022 mg/kg FW. The obtained data were further evaluated by multivariate statistical methods, i.e. Principal Component Analysis (PCA). In conclusion, this study gives a comprehensive insight in the polyphenolic profile of disease-resistant grapevine hybrids and will assist in the selection of promising hybrids for future breeding strategies.

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P-113 Strategy for exhaustive plant metabolomes characterization from a qualitative and quantitative perspective

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Plant extract metabolome characterization is of growing interest for the academic research community and regulatory affairs, quality control and research & development at the industry level. In the case of industry, reliable analyses are crucial for quality assessment of a final product. Current analytical methods mainly focus on the quantitation of specific and often non-bioactive markers of plant extracts. Therefore, efficient procedures are needed to provide an exhaustive analysis of the metabolome composition of a natural extract, addressing qualitative and quantitative aspects. We argue that combining semi-quantitative data with the fingerprint of minor metabolites could improve quality, authenticity, origin or toxicity assessments. In the context of such original classification, we used untargeted UHPLC-HRMS/MS-based metabolomics, which is a qualitative and highly sensitive approach together with semi-quantitative detection based on ELSD. Four known medicinal plants of industrial interest containing bitter principles (*Quassia amara* L., *Swertia chirayta* (Roxb.) Karst., *Gentiana lutea* L. and *Aloe ferox* Mill.) were selected as model. After a generic untargeted workflow, we mined the data to extract relevant information, using taxonomy and chemometric approaches to query *in silico* annotated Molecular Networks (MN). [1,2] This allowed the rapid and confident annotation of 23% of 16′624 MS2 spectra from diverse specialized metabolites. Mapping semi-quantitative information on the networks also provided a preliminary global evaluation of their relative abundance within the extract. With both qualitative and quantitative data combined, enriched molecular networks represent relevant and detailed compositional information on natural extracts from a generic perspective.

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P-115 Targeted isolation of selaginellin derivatives using molecular networking strategy enhanced by *in silico* annotation

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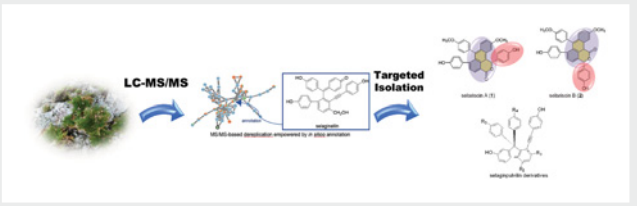
Selaginellins, unique pigments found in the genus *Selaginella*, were reported as potent phosphodiesterase-4 (PDE4) inhibitors in recent studies. To isolate diverse natural selaginellin derivatives, we applied a MS/MS based molecular networking strategy enhanced by *in silico* structural annotation to the *Selaginella tamariscina* extracts. It led to the prioritization of chromatographic peaks predicted as previously unknown selaginellin derivatives. As a result, we could isolate ten unknown compounds containing two unusual selaginellin analogs with 1*H*,3*H*-dibenzo[*de*,*h*]isochromene skeleton named selariscins A (**1**) and B (**2**) along with eight diarylfluorene derivatives, selaginpulvilins M –T (**3**–**10**). The absolute configurations were elucidated by computational electronic circular dichroism (ECD) spectral calculations. Some isolates showed PDE4 inhibitory activity with IC_{50} values in the range of 2.8–33.8 μM, and their binding modes were suggested using a molecular docking study.



▶ **Fig. 1**

P-116 Targeted LC-MS/MS analysis for the quantification of taxanes: assessment of chemodiversity in different European yew (*Taxus baccata*) populations from Greece

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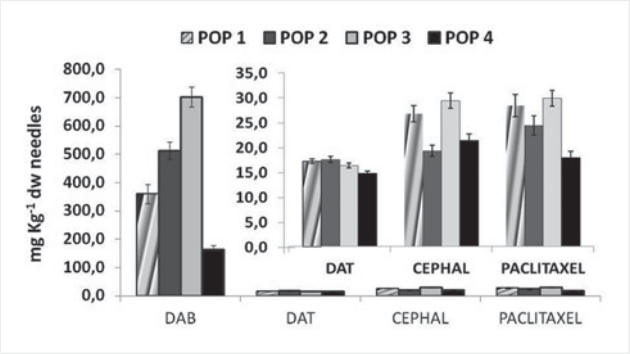
DOI 10.1055/s-0039-3399845

Taxus baccata is a native to Greece medically interesting conifer due to its tetracyclic diterpenes. Amongst them, the anti-cancer agent paclitaxel is the most known. This study evaluated the content of five diterpenes found in the needles of 130 *T. baccata* trees belonging to three populations from different mountainous areas and one *ex-situ* collection, as an alternative source of taxanes. In order to assess the chemodiversity of the populations, fresh needles were collected in August from second and third year stems/branches with northern exposure from each tree and freeze-dried. The phytochemical extraction in triplicate was performed according to Mubeen et al. (2018) with modifications, by mixing 500 mg of dried needles with 4 ml acetone:water (3:2 v/v). After evaporation of

acetone the pooled water phase was extracted twice with 4 mL dichloromethane. and the two phases were evaporated until complete dry. The residues were dissolved in 1 ml methanol and filtered into glass vials. An analytical method was developed using external standards for quantification, according to Kobusiak-Prokopowicz et al. (2016) and validated to quantify paclitaxel, 10-deacetylbaccatin III (DAB), 10-diacetyltaxol (DAT), baccatin III and cephalomanine, using UPLC-MS/MS (MRM). Analysis revealed that mean concentration of DAB, DAT, cephalomanine and paclitaxel ranged from 162.3-703.4, 14.7-17.5, 19.2-29.3 and 17.8-29.7 mg Kg^{-1} dw respectively in the needles of the examined populations (▶**Fig. 1**), while baccatin III was identified only in traces. Such chemodiversity could lead to the selection of superior germplasm native to Greece for further exploitation destined to the production of important taxanes.



▶**Fig. 1** The variation of major taxanes (DAB, DAT, Cephalomanine and Paclitaxel) identified in the needles of trees from four different Greek populations of *Taxus baccata*. Values are expressed as means ± standard error. Biological replicates were n = 23-33 trees *per* population.

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P-117 Taxonomically informed metabolite annotation and data organization in natural products research

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Natural products, more precisely defined as specialized metabolites, are by definition strongly linked to the taxonomical position of the producing organisms. Considering taxonomy when exploring natural products thus appears as an evidence. Such principles were already formulated in 1816 by De Candolle who postulated that 1) *Plant taxonomy would be the most useful guide to man in his search for new industrial and medicinal plants* and 2) *Chemical characteristics of plants will be most valuable to plant taxonomy in the future*. [1] We adhere to De Candolle's postulate and aim to establish their validity using computational approaches.

Regarding the first postulate, we show that the consideration of taxonomic position is beneficial in the metabolite annotation process leading to a systematic improvement (> 50% of correct annotation at rank 1) of current *in silico* metabolite annotation solution (Sirius, MSFinder, CFM+Tremolo). [2] This increased confidence in metabolite annotation efficiently improves the natural products drug discovery process and should complement orthogonal information already shown to strengthen classical spectral scoring systems. [3,4] Regarding the second postulate, we demonstrate the interest of considering the chemical dimension (structural or spectral relationships) when seeking to organize plants or lichens extracts. We present a novel metric, Spectral and Substructural Similarity- Informed Distance (SSS-ID) for such classification and compare it to classically established taxonomies (genetic material or morphological character-based organizations), demonstrating its interest. The principles and implementation as well as practical applications of such approaches will be discussed.

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P-118 6-Metoxyflavonols from the aerial parts of *Tetragonia tetragonioides* (Pall.) Kuntze and their potential as anti-inflammatory agents

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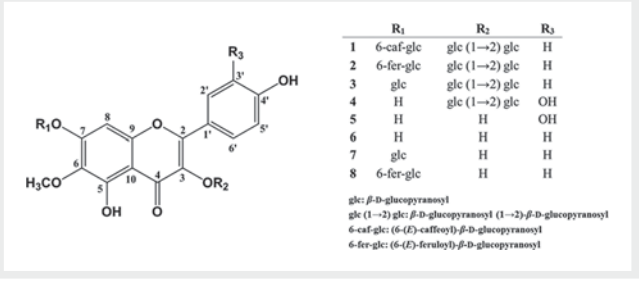
Tetragonia tetragonoides (Aizoaceae) has been used in Korean traditional medicine for treatment various diseases [1]. *T. tetragonoides* contains a variety of secondary metabolites such as flavonoids, alkaloids, and terpenes [2-5]. These compounds have been reported to treat ulcers and stomach cancer [1, 5]. However, pharmacological studies of *T. tetragonoides* on anti-inflammation is barely carried out. Therefore, the present study focused on the isolation and identification of active materials from this plant, as well as examination for the anti-oxidant and anti-inflammatory activities of the isolated compounds. Dried aerial parts of *T. tetragonoides* were extracted with 70% aqueous EtOH, and the concentrated extract was partitioned into EtOAc, *n*-BuOH, and H_2O fractions. As a result of repeated normal, reverse-phase, and Sephadex LH-20 column

chromatographies on each fraction, four new flavonoids (**2-4, 8**) along with four known ones (**1, 5-7**) were isolated. The structures were determined using classical spectroscopic methods such as (NMR, IR, MS). All the compounds except compound **1** were isolated for the first time from *T. tetragonoides* in this study. Some compounds showed significant anti-oxidant and anti-inflammatory activity. Also quantitative analysis of contents 6-methoxyflavonols in the aerial parts of *T. tetragonoides* was conducted through HPLC-UV experiment. These results supported that



▶**Fig. 1** 6-Methoxyflavonols 1-8 isolated from the aerial parts of *Tetragonia tetragonoides*.

the 6-methoxyflavonols isolated from the aerial parts of *T. tetragonoides* are potential as anti-oxidant and anti-inflammatory agents.

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P-119 A triterpene glycoside from the black sea cucumber *Holothuria atra*

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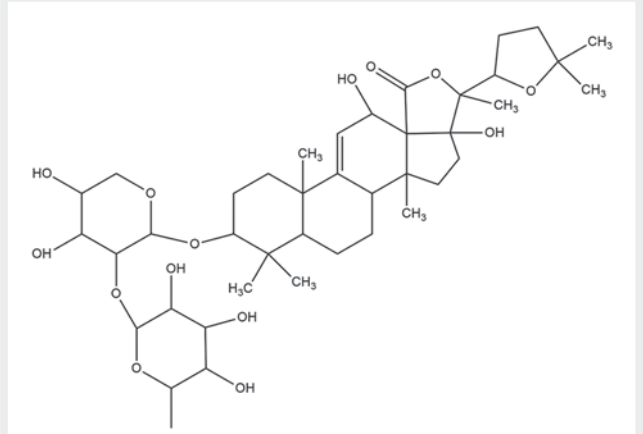
DOI 10.1055/s-0039-3399848

Sea cucumber is a soft-bodied, worm-like, echinoderm. It is consumed in its dried form, which is called “bêche-de-mer”. Many reports described the presence of triterpene glycosides (saponins) in sea cucumbers, and especially the Cuvierian tubules are a rich source of this type of compounds [1,2]. The black sea cucumber, *Holothuria atra* Jaeger, is one of the commercially important species in Indonesia. However, in contrast to most species of sea cucumber, *H. atra* does not possess Cuvierian tubules, but saponins were previously isolated from its body wall [3]. In this project, an extensive study of the composition of the body wall of *H. atra* is carried out, with special interest in its saponin content. Black sea cucumbers were collected in East Java, Indonesia, dried, and extracted with dichloromethane and 80% methanol. Fractionation was carried out by means of liquid partitioning and column chromatography (diaion HP20). Finally, semi-preparative HPLC-DAD-MS allowed for the purification of various saponins, according to their ^{1}H-NMR spectra. Up till now, the structure of one compound could be fully elucidated, based on 1D and 2D NMR and HRMS data. All NMR data were in correspondence to the data reported by Kitagawa, for desulfated holothurin B [4]. Moreover, the HRMS data of this compound were recorded for the first time and confirmed the identification of the compound as desulfated holothurin B (*m/z* 803.4250, [M +Na]$^+$; calculated for $C_{41}H_{64}O_{14}Na$: 803.4194; and *m/z* 485.3267, aglycon).



▶**Fig. 1** Desulfated holothurin B of black sea cucumber *Holothuria atra*

Further research with regard to the structure elucidation of other purified compounds is ongoing.

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P-120 Absolute configuration of sesquiterpene lactones with potent immunosuppressant activity

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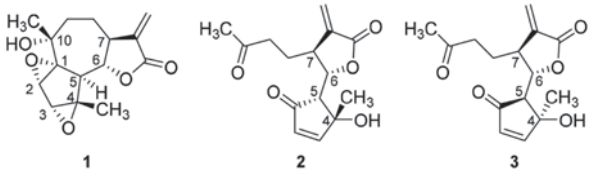
In a screening of *Artemisia argyi* (Asteraceae) and subsequent HPLC-based activity profiling canin (**1**) and the *seco*-tanapartholides **2** and **3** (stereoisomers) were identified as compounds with potent immunosuppressant activity *in vitro*. These isoprenoids were first discovered in 1969 and 1982,

respectively, and have been extensively studied in the past for various biological activities. Although many studies have been published examining their molecular structure and relative configuration by means of NMR and X-ray [2], the absolute configuration remained unresolved. We here established the absolute configuration of compounds **1-3** by a combination of electronic circular dichroism spectroscopy (ECD) and vibrational circular dichroism spectroscopy (VCD).

ECD spectra of **1-3** were measured and compared to spectra calculated *ab initio* for different possible stereoisomers. The most frequently reported relative stereoisomer of canin (**1**) was established as (1*R*,2*S*,3*R*,4*S*,5*S*,6*S*,7*S*,10*R*)-canin. For compounds **2** and **3**, the ECD data reduced the number of possible configurational isomers to four. The absolute configuration was finally established by VCD. Compound **2** was identified as (4*R*,5*R*,6*S*,7*S*)-*seco*-tanapartholide, and **3** as (4*S*,5*S*,6*S*,7*S*)-*seco*-tanapartholide. The combination of ECD and VCD is a powerful approach for resolving the absolute configuration of conformationally flexible molecules.



▶ **Fig. 1**

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P-121 An epigenetic modifier induces the production of new metabolites by *Aspergillus terreus* AST0006

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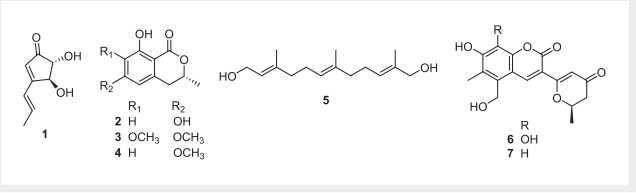
The use of epigenetic modifiers to activate silent gene clusters of secondary metabolism in fungi has demonstrated to be an important strategy for producing novel metabolites [1,2]. In this study, we investigated the use of histone deacetylase (HDAC) inhibitor, suberoylanilide hydroxamic acid (SAHA), on the production of metabolites by *Aspergillus terreus*. In potato dextrose broth (PDB) medium, *A. terreus* produced (+)-terrein (**1**), (-)-6-hydroxymellein (**2**), (-)-6,7-dimethoxymellein (**3**), (-)-6-methoxymellein (**4**), and farnesol (**5**). Incorporation of SAHA into PDB culture medium of this fungus affected the metabolites pattern and produced two new 3-pyrone-coumarin metabolites **6** and **7** together with (+)-terrein (**1**), (-)-6-hydroxymellein (**2**), (-)-6,7-dimethoxymellein (**3**), (-)-6-methoxymellein (**4**), and farnesol (**5**). The identification of metabolites **1-5** was established by comparison of their spectroscopic data with those reported and the structures of new metabolites **6** and **7** were elucidated with the help of their 1D and 2D NMR and HRMS data. The absolute configurations of the new compounds were determined through the comparison of experimental and calculated ECD data.

These results support the use of epigenetic modifiers for obtaining new fungal secondary metabolites.



▶ **Fig. 1** Structures of compounds 1–7.

Acknowledgements FAPESP (Grants 2015/11058-5, 2018/05905-5 and 2014/25222-9).

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P-122 Analytical tools for students to study anthocyanins and carotenoids from autumn leaves

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Previous studies have shown that students' interest in botany tends to decrease with age, but interest in topics of microscopic level such as DNA and proteins increases [1]. To test students' knowledge of plant chemistry we asked Finnish secondary school students (N=41) to list compounds or compound groups in plants. Majority of them could name only water and glucose. To increase student knowledge and interest in plant chemistry, we have created an inquiry task where students study the anthocyanins and carotenoids of autumn leaves utilizing both simple and more sophisticated analytical methods.

The first steps of the task include the extraction of anthocyanins and carotenoids from the leaves, and the separation of these compounds into different phases by liquid-liquid extractions. These steps can be performed in a regular secondary school laboratory using student collected samples. From the separated phases, the amount of pigments are quantified spectrophotometrically. Finally, the anthocyanins are analyzed by a rapid and simple UPLC-MS/MS method to discover if the sample contains delphinidin, cyanidin, pelargonidin, peonidin, petunidin or malvidin type anthocyanidin derivatives.

The task was tested with 18 secondary school students and 16 university students. Based on the feedback, students enjoyed both the extraction, which is visually impressive because of the colorful extracts, as well as the sample collection. Comparing students' results with results determined by a trained chemist showed that the student groups could carry out the analyses reliably.

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P-125 Antidiabetic constituents from the aerial parts of *Tiliacora triandra*

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DOI 10.1055/s-0039-3399852

Tiliacora triandra belongs to the family Menispermaceae and it is native to Southeast Asian countries of Thailand and Laos. *T. triandra* is a climbing plant with deep green leaves and yellowish flowers [1]. The leaves and roots are used traditionally for treating fever, diabetes and malaria [2]. Previous phytochemical and pharmacological study on the plant have shown that bisbenzylisoquinoline alkaloids are the major bioactive constituents and they have anticancer, anti-pyretic, antimycobacterial and antimalarial activities [3,4]. In this study, the chemical constituents from the aerial part of *T. triandra* was carried out for the first time. Two new fatty acids derivatives, 5,7-dihydroxy-6-oxoheptadecanoic acid (**1**) and ethyl-5,7-dihydroxy-6-oxooctadecanoate (**2**), along with two known compounds, ethyl linolenate (**3**) and ethyl linoleate (**4**) were identified. The structures of the compounds were determined based on spectroscopic data analyses including 1D and 2D NMR. All compounds were evaluated for their α-glucosidase and α-amylase inhibitory activities. Compound 1 exhibited strong α-glucosidase and α-amylase inhibitory activity (IC_{50}; 11.58±0.32 and 26.27±1.11 μM, respectively), while compounds **2-4** showed moderate inhibitory activity against α-glucosidase (IC_{50}; 104.77-424.06 μM).

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P-126 Anti-inflammatory compounds from *Xylaria* sp SWUF09-62 fungus

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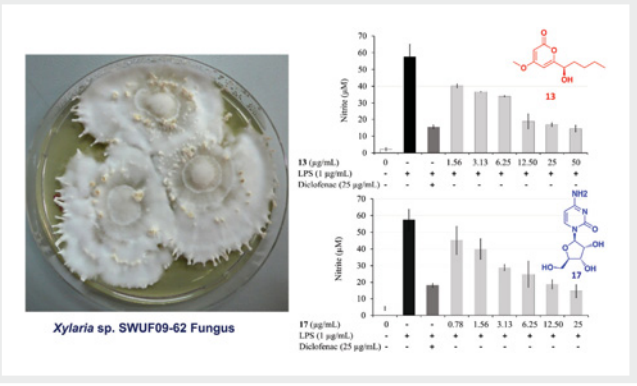
Cancer is one of the leading causes of death in humans worldwide and because of this there is a concerted effort to try to treat and eventually eradicate this disease. The use of natural and synthetic chemoprevention and chemotherapeutic drugs is just one ways that researchers are trying to achieve this [1,2]. Chronic inflammation is one of the factors responsible for inflammation-associated carcinogenesis, therefore anti-inflammation compounds could play a vital role as chemoprevention drugs [3–5]. The aim of this work is to identify the anti-inflammatory compounds from *Xylaria* sp SWUF09-62 fungus, from the Xylariaceae family, which are found in the wild throughout Thailand.

The stroma of the fungus was isolated and cultivated in liquid media, then extracted with organic solvents and finally purified by chromatographic methods. Seventeen (**1-17**) compounds were isolated. There were five chromone (**1-5**) and six mullein (**6-11**) derivatives, together with 5,6-dihydro-4-methoxy-2*H*-pyran-2-one (**12**), (1′*R*)-dehydropestalotin (**13**), 5-hydroxymethyl-2-furfural (**14**), ergosterol (**15**), ergosterol peroxide (**16**) and cytidine (**17**). Anti-inflammatory activity was screened by measuring the level of nitric oxide (NO) production in lipopolysaccharide-stimulated RAW264.7 cells. The results showed that **13** and **17** reduced NO production more than 70%, which was better than the control drug diclofenac.

In conclusion, the Xylariaceae family has proven to be a vital source of bioactive compounds and it is imperative that the search for potential drug candidates from these fungi be continued. New species are often being reported thus increasing the probability of discovering new bioactive compounds for potential pharmaceutical applications.



▶**Fig. 1** Characteristic of fungus on agar plate and some anti-inflammatory compounds

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P-129 Antioxidant activity and phenolic composition of costmary (*Chrysanthemum balsamita* L.) flower

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Costmary, *Chrysanthemum balsamita* L., is a widely cultivated ornament plant. Previous studies have demonstrated that extracts of costmary show several bioactivities including antioxidant, diuretic, astringent, antiseptic, stomachic and anthelmintic [1]. Nevertheless, its phytochemical exploration is still incomplete. Our aim was to characterize the scavenger activity of herb methanolic extracts using *in vitro* ABTS method as well as identify the potential bioactive compounds by LC-ESI-QqQ and LC-ESI-high-resolution Orbitrap MS/MS methods. Based on preliminary screening notable amounts of polyphenols (64.50 ± 4.21 mg/100g) and flavonoids (21.58 ± 1.12 mg/100g) were presented in the extract. Using ABTS method an excellent antioxidant activity was measured with 1.2 ± 0.1 µg/mL IC_{50} value which is comparable with the scavenger activity of quercetin standard (IC_{50} = 3.4 ± 0.2 µg/mL). In the extract altogether 34 phenolics, comprising 14 hydroxycinnamoylquinic acids (mainly different caffeoylquinic acid derivatives) and 20 flavonoids (glycosides of apigenin, luteoilin, diosmetin, acacetin and quercetin) were identified with combination of different LC-MS techniques. Our results clearly show that costmary herb is an inexpensive, readily available natural source of phenolic compounds with a possible pharmacological and cosmetic applications.

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P-130 Antioxidant and antibacterial activities of selected Thai medicinal plant-derived Galactogogue extracts

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Plant-derived galactogogues are compounds produced by plants that help to increase the production of prolactin. This group of medicinal plants has long been used in traditional recipes to stimulate breastmilk production in breastfeeding women [1, 2] but information of their antioxidant and antibacterial activities has limited. Therefore, this study aims to determine the antioxidant and antibacterial activities of selected Thai medicinal plant galactogogue extracts collected from northeast Thailand. Sixteen plant samples were extracted to obtain water and ethyl acetate extracts and the antioxidant activity, total phenolic content (TPC) and antibacterial activity of the extracts were evaluated. From the results, the water extracts of *Caesalpinia sappan* and *Ochna integerrima* (IC_{50} 9.47 ± 0.59 and 10.86 ± 0.10 ppm, respectively) and the ethyl acetate extracts of *C. sappan*, *Salacia chinensis* and *O. integerrima* (IC_{50} 9.85 ± 0.84, 9.17 ± 0.30 and 13.25 ± 0.52 ppm, respectively) exhibited strong DPPH scavenging activity (IC_{50} of standard ascorbic acid 5.39 ± 0.32 ppm). The highest TPCs were found in the water extract of *C. sappan* and ethyl acetate extract of *O. integerrima* (682.67 ± 6.11 and 985.34 ± 0.76 mg GAE/g DW, respectively). In adition, the water extracts of *C. sappan* and *O. integerrima* displayed potent antibacterial activity against gram-positive bateria. The antioxidant activity, TPC and antibacterial activities results were in good agreement. These information help to considerate the use of these medicinal plants as natural antioxidant and antibacterial sources. However, their cytotoxicity, active ingredients and other biological activities need to be concerned for safe use.

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P-131 Antiproliferative property of sea fan extracts against colon (HCT116) and lung (A549) cancer cell lines

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DOI 10.1055/s-0039-3399856

The Philippines is endowed with rich marine resources, which, for the most part, is still untapped. This study explored the potential of the bioactive fractionsfrom sea fanswith the hope of finding potential drug candidates for cancer treatment. Sea fansamples were collected from the island of Mindanao, Philippines.Bioactivity-guided separation of polar and nonpolar extracts were done using colon (HCT116) and lung (A549) mammalian cancer cell lines employing the MTT assay. Chromatographic separation afforded a nonpolar (hexane) fraction of sea fan (coded DT03), which exhibited a percent cell viability of 30.06 ± 0.14 at 30 mcg/mL against HCT116. Its IC_{50} was calculated to be 54.51 ppm. Semi-purification of this extract is ongoing. Other sea fan polar and nonpolar extracts showed noncytotoxic to mildly cytotoxic activities against both colon and lung cancer cell lines. The antibacterial property determination of these extracts is the next phase of this study.

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P-132 Arbuscular mycorrhizal fungi as tool in pharmaceutical and cosmeceutical industry for the enhanced production of secondary metabolites of *Anchusa officinalis*

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Arbuscular mycorrhizal fungi (AMF) are biotrophe microorganisms that establish a symbiotic association with host-plants. These plant-dependent microorganisms have already shown their strong effects concerning plant growth promotion and were reported to stimulate the secondary metabolites (SM) production of their host-plant.

In this study, *Anchusa officinalis* plants, well-known for their therapeutic properties, were analyzed to assess the AMF effects on SM production. Two different experiments were developed on a semi-hydroponic system to compare treated *A. officinalis* plants with different AMF. In the first investigation, plants were inoculated with *Rhizophagus irregularis*, cultivated for 30 days and harvested two times. Concerning the second experiment, four different strains of AMF (*R. clarus*, *R. intraradices*, *R. irregularis* and *Glomus aggretum*) were tested separately on plants and one sampling was performed after one week. Roots and shoots were separated, lyophilized, ground and extracted using ultrasounds with EtOAc and MeOH (35:65 v/v) at 25°C. After centrifugation, the supernatants were removed and evaporated to dryness. Samples were analyzed with UHPLC-HRMS as well as HPTLC and HPLC-DAD-ELSD.

Preliminary results of a targeted metabolomic analysis, showed that the concentration of main compound, rosmarinic acid, present in all the treated plants had no statistically difference from the controls. However, a discernable up-regulation and down-regulation of specific minor SMs in colonized plants was observed, suggesting that the aforementioned AMF affect specific biosynthetic pathways. Further experiments and analyses is needed but the cultivation of medicinal plants with AMF looks a promising way to enhance bioactive metabolites with applications in pharmaceutical and cosmeceutical industry.

Acknowledgments This work has been financed by the EU H2020-ITN-MICROMETABOLITE project (Grant No 721635)

P-134 Bioactive polyketides from a marine green alga-derived fungus *Aspergillus* sp. NTU967

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DOI 10.1055/s-0039-3399858

In the preliminary screening, the bioactivities of 343 fungal strains isolated from marinemacroalgae collected from northeast coast of Taiwan were evaluated intensively. Of these, the ethyl acetate extract of the fermented broth of *Aspergillus* sp. NTU967 was found to exhibit significant antimicrobial activity against *Staphylococcus aureus* ATCC29213.

To disclose the active principles from the ethyl acetate extract of the fermented broth of *Aspergillus* sp. NTU967.

Eleven compounds including seven new compounds, namely aspergilsmins A–G (**1**–**7**), were identified by spectroscopic analysis. Among these, aspergilsmins E (**5**) and F (**6**) exhibited significant growth inhibition against *S. aureus* ATCC29213 with MIC values of 32 µg/mL. Aspergilsmin C (**3**) showed potent cytotoxicity against PC-3 prostate cancer cells and SK-Hep-1 hepatocellular carcinoma cells with IC_{50} values of 7.3 ± 0.2 and 2.7 ± 0.2 µM, respectively. Additionally, **3** also exhibited potent antiangiogenic activity by suppressing the tube formation of human endothelial progenitor cells with an IC_{50} of 4.6 ± 0.3 µM.

leven chemical entities including seven new compounds were isolated from an alga-derived fungus *Aspergillus* sp. NTU967. Of these, compounds **3, 5**, and **6** were the new compounds with bioactivities.

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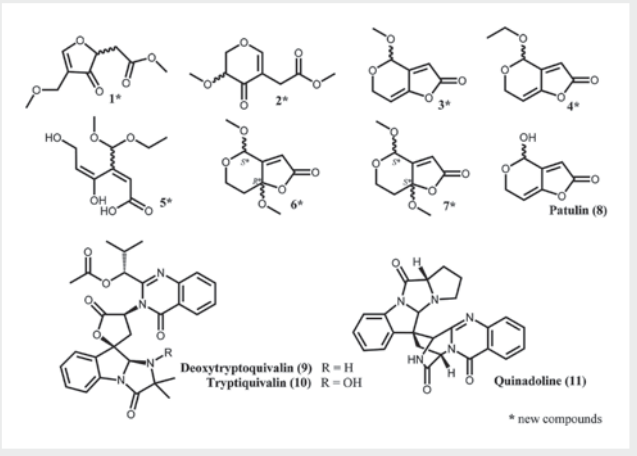

▶ **Fig. 1**

P-135 Bio-guided fractionation of prenylated benzaldehyde derivatives as potent antimicrobial and antibiofilm from *Ammi majus* L. fruits-associated *Aspergillus amstelodami*

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DOI 10.1055/s-0039-3399859

Ammi majus L. (Apiaceae) is an indigenous plant in Nile Delta regions of Egypt. Its fruits contain bioactive compounds (furanocoumarins and flavonoids) of important biological activities. This work aims to identify *A. majus* endophytes An endophytic fungus was isolated from the fruits and identified as *Aspergillus amstelodami* by morphological and microscopic characterization in addition to molecular identification using phylogenetic analysis. The ITS rRNA gene sequence was submitted at the GenBank under the accession number (MK215708). The antimicrobial activity of the 70% ethanol fruits extract and ethyl acetate extract of *A. amstelodami* were investigated where the fungal extract showed higher antimicrobial activity (using colorimetric broth micro-dilution method to determine MIC), against all the tested standard strains. Phytochemical investigation of the fungal ethyl acetate extract using VLC and preparative HPLC yielded five prenylated benzaldehyde derivative compounds, isolated for the first time from this species, and named: dihydroauroglaucin (**1**), tetrahydroauroglaucin (**2**), 2-(-3,6-dihydroxyhepta-1,4-dien-1-yl)-3,6-dihydroxy-5-(dimethylallyl) benzaldehyde (**3**), isotetrahydroauroglaucin (**4**) and flavoglaucin (**5**). Structure elucidation of the isolated compounds was carried out based on their spectral data analysis (^{1}H-, ^{13}C-NMR and MS). Fractions as well as the major isolated compound were evaluated for their antimicrobial and antibiofilm activity. Compound **1** showed highest antimicrobial activity especially against Escherichia coli (MICs=1.95 µg/ml), *Streptococcus mutans* (MICs=1.95 µg/ml) and *Staphylococcus aureus* (MICs=3.9 µg/ml). It showed the highest anti-biofilm activity with MBIC=7.81 µg/ml against both *Staphylococcus aureus* and *Escherichia coli* biofilms, and moderate MBIC= 15.63 µg/ml against

Streptococcus mutans and *Candida albicans* biofilms and MBIC= 31.25 µg/ml against *Pseudomonas aeruginosa* biofilm.

P-136 Bio-guided phytochemical study of *Plectranthus mutabilis* Codd.

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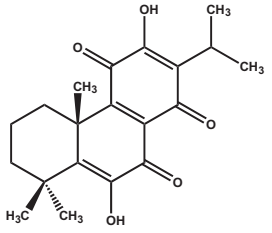
DOI 10.1055/s-0039-3399860

Natural products from medicinal plants represent major resource of novel therapeutic substances for combating serious diseases including cancer and infections. The *Plectranthus* genus (Family: Lamiaceae) represents a large and widespread group of species with a diversity of traditional uses for the treatment of several ailments [1]. *P. mutabilis* Codd. chemical constituents have been cited on its HPLC analysis that revealed the presence of Nepetoidins A and Nepetoidins B [2]. We therefore aimed to study the composition and biological activity of this plant to reinforce the low phytochemical information. In this study, the air-dried *P. mutabilis* whole plant was extracted in acetone using the ultra-sound assisted extraction method. Furthermore, a bio-guided fractionation was performed followed by an *Artemia salina* general toxicity assay, DPPH-antioxidant activity and antimicrobial activity [3]. The extract was subjected to different column chromatographies using silica or polyamide with increasing polarity to afford the metabolite coleon U (**1**). The fully structure characterization was done mainly by 1D- and 2D-NMR and comparison with literature data. Compound **1** was tested against two Gram positive (Minimum Inhibitory Concentration - MIC values: *Staphylococcus aureus* = 1.56 µg/mL, *Enterococcus faecalis* = 25 µg/mL) and two Gram negative bacteria (*Klebsiella pneumoniae* = >100 µg/mL, *Escherichia coli* = >100 µg/mL). Additionally, the isolated diterpenoid showed moderate cytotoxicity in Human cancer and normal cell lines (Colo 205, multidrug resistant overexpressing ABCB1 Colo 320 and MRC-5) showing slight selectivity to resistant cancer cells. Further phytochemical studies are ongoing.



▶**Fig. 1** Coleon U (1)

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P-137 *Bjerkandera adusta* as a source of benzoic acid derivatives targeting 26S proteasome and cathepsins B&L

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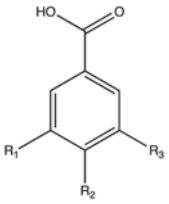
One of the main pathways involved in aging process is the proteostasis network, which is an assembly of protein degradation machines, molecular chaperones and stress response pathways that control the functionality of the proteome during protein synthesis, folding, trafficking and degradation. Ageing process is followed by a gradual dysfunction of the proteostasis network and this leads to proteome instability due to accumulation of damaged and/or misfolded proteins. The two main machineries of protein degradation in eukaryotic cells are the ubiquitin-proteasome and the autophagy-lysosome pathways [1, 2].

The aim of the present study was to investigate the strain CF-092983, identified as *Bjerkandera adusta*, as a potential source of natural products able to activate proteasome and/or lysosomal cathepsins and thus to contribute to the maintenance of protein homeostasis affected by several exogenous and/or endogenous factors.

Chemical investigation of the EtOAc L-L extract led to the isolation of four hydroxybenzoic acid derivatives (▶**Fig. 1**). All the isolated compounds significantly enhanced the activity of cathepsins B and L. Additionally, compounds **1, 3** and **4**, promoted the main degrading activity of the 26S proteasome at a final concentration of 5 µM. Moreover, using the crystal structure of the 26S proteasome, and of cathepsins B and L we run *in-silico* simulations to explore the possible binding mode of the active compounds.

Overall, the findings of this study further support the antioxidant and/or the potential anti-ageing effects of fungal extracts, and the fact that fungal natural products can be used as effective agents for promoting human health.



	R_1	R_2	R_3
1	-H	-OH	-H
2	-H	$-OCH_3$	-H
3	-H	$-OCH_3$	-Cl
4	-Cl	$-OCH_3$	-Cl

▶**Fig. 1** Chemical structures of isolated compounds

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P-138 Camoreoside A-I, novel triterpene saponins, from the seeds of *Camellia japonica*

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Camellia japonica L. (Theaceae) is an evergreen shrub, which is cultivated as a popular ornamental tree in Korea, China, and Japan and its seeds have been used as a source of cooking oil, in cosmetic uses and as a traditional medicine in Asia. Intensive phytochemical works have revealed that oleanane-type saponins are the characteristic compounds of the seeds of *C. japonica*. The purpose of the present study is to isolate and determine oleanane-type saponins from *C. japonica* using high-performance countercurrent chromatography (HPCCC) coupled with reversed-phase high-performance liquid chromatography (RP-HPLC) and spectroscopic evidences, respectively. HPLC-ESI-Q-TOF-MS observed a variety of saponins with di-, tri-, tetra-saccharides as oligo-glycoside moieties from the enriched saponin extract of *Camellia japonica* seeds. HPCCC and RP-HPLC methods were applied to give nine undescribed saponins, camoreosides A-I. The structures were determined utilizing ESI-Q-TOF-MS, 1D-, 2D-NMR, and optical rotation. This result indicates that combination HPLC-ESI-Q-TOF-MS analysis and HPCCC coupled with RP-HPLC are excellent tools for discovering novel saponins from natural sources.

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P-139 Chalcone dimers from the twigs of *Pistacia chinensis*

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Pistacia chinensis, family Anacardiaceae, is a perennial arbor distributed mainly in southern China and cultivated in Japan as street trees. Its galls have been used as traditional Chinese medicine for the treatment of sore, cough, and hemostasis. The chemical constituents of this plant had been fastened mainly on phenolic compounds [1, 2]. Previously, two neoflavone (4-arylcoumarines) dimers has been isolated from this plant [3]. These dimers are of interest due to the inconsistent isomers that result from the chalcone framework. Over 100 representatives of natural 4-arylcoumarins is known. The unique structure, broad popularity in traditional plant medicines containing these compounds, and the valuable pharmacological properties make neoflavones highly interesting. Herein, phytochemical investigation of the twigs of *P. chinensis* is indicated to explore new chemical constituents.
Air-dried and pulverized aerial parts of *P. chinensis* (1.8 kg) were extracted with MeOH (4 × 10 L) at room temperature. After removal of the combined solvents, a crude extract (109.4 g) was obtained. The crude extract was suspended in H_2O and partitioned with EtOAc and *n*-BuOH (each 3 L) in succession. The resulting four fractions were evaporated to dryness in vacuo, to yield EtOAc (46.5 g), *n*-BuOH, and H_2O extracts. The EtOAc extract was subjected to a column chromatography over silica gel, Sephadex LH-20, Sep-pak C_{18} cartridge, and semi-preparative HPLC. The structures of the isolated compounds were established on the basis of extensive analysis of NMR spectroscopic data, MS spectra analyses, and experimental and calculated electronic circular dichroism spectra.

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P-140 Characterization of chemical constituents from *Fissistigma polyanthoides* stems

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The Dao ethnic community in Vietnam traditionally uses *Fissistigma polyanthoides* stems for treating inflammation and arthritis, as well as recuperating women after childbirth. The present study was conducted to provide detailed insights into the chemical composition of this medicinal plant, which has barely been studied in phytochemical terms in the past. Our efforts resulted in the isolation of 34 compounds, including 16 new and 18 known natural products. For structural elucidation, one- and two-dimensional NMR and MS techniques were used, the absolute configuration of compounds **4**, **5**, and **10** could be established by ECD calculation. Among all isolated compounds, fifteen terpenoids (**1-15**), fourteen flavonoids (**16-29**), one phenylpropanoid (**30**), and four alkaloids (**31-34**) were identified. Our results revealed that a substitution of the sesquiterpenoid scaffold with (2′-*O*-*trans*-cinnamoyl)-β-D-glucopyranoside (**1-9**) are characteristic features of *F. polyanthoides* constituents.

P-141 Chemical compositions from *Biscogniauxia* sp. PK15-040 fungus

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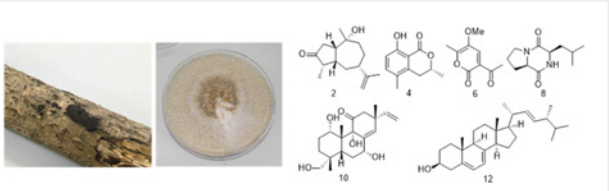
The genus *Biscogniauxia,* which was once a member of wood-decaying fungi Xylariaceae family, has been resurrected and has become a member of new Graphostromataceae family. This fungus is naturally found on the bark, trunks, and branches of shrubs and trees [1-3]. Unlike its previous co-family, *Xylaria* genus, only a few reports on natural products were published from *B.* spp. [3-4]. The objective of this research is to investigate the chemical compositions from *Biscogniauxia* sp. PK15-040 fungus collected from the Phu Khieo Wildlife Sanctuary, Thailand. The fungus was identified by its morphological characteristics and molecular data. It was cultivated in PDB medium for 6 weeks and purified by chromatographic methods. Its structures were established by the interpretation of spectroscopic data including 1D and 2D NMR, and IR.

Fifteen compounds were isolated and categorised into eight classes of compounds, including three guaiane sesquiterpenes, pogostol (**1**), xylaranone (**2**), and xylaranol B (**3**); two mellein derivatives, 3,5-dimethyl-8-hydroxy-3,4-dihydroisocoumarin (**4**) and 3,5-dimethyl-8-methoxy-3,4-dihydroisocoumarin (**5**); two pyrone derivatives (**6-7**); two cyclodipeptides (**8-9**); two isopimarane diterpenes (**10-11**); two steroids, ergosterol (**12**) and ergone (**13**); *p*-tyrosol (**14**) and a bergamotene derivative (**15**). The results indicate that *Biscogniauxia* sp. PK15-040 is an example of a marvellous source of diverse secondary metabolites and possibly diverse bioactive compounds, therefore we believe that *B.* spp. needs to be investigated further.



▶**Fig. 1** Nature of *Biscogniauxia* sp. PK15-040 and structures of some isolated metabolites.

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P-142 Chemical constituents and cytotoxic activity from *Xylaria* spp. fungi

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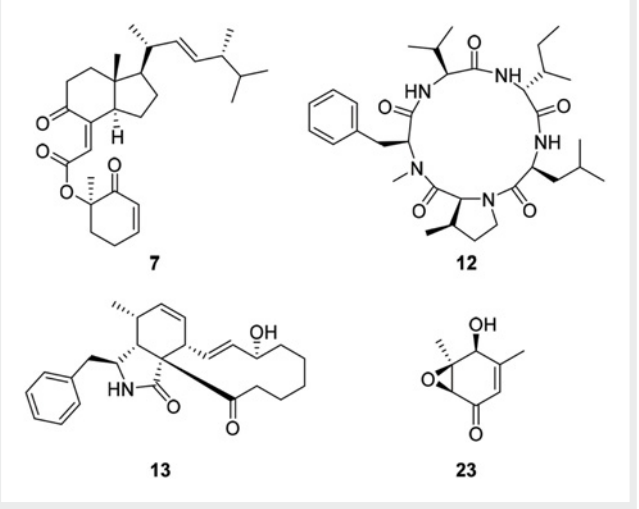
Cancer is by far a leading cause of human deaths worldwide and as such there is an ongoing search for drugs that can halt it, cure it or prevent it from happening. [1]. More than two-thirds of the marked chemotherapeutic agents are based on natural products [2]. However, only a few of the identified anti-cancer natural product candidates entered the drug development process, hence the search for potential candidates is still essential. *Xylaria*, one of the wood-decaying fungi genus belonging to Xylariaceae family, has been shown to be a potential source of diverse secondary metabolites, especially cytotoxic compounds [3]. Thus, this research aimed to help in this search for new effective anti-cancer drug candidates from this genus found in Thailand.

Three selected *Xylaria* spp., namely *X. allantoidea* SWUF76; *X.* spp. SWUF08-37 and SWUF15-05 were studied, from which eleven (**1-11**), thirteen (**12-24**) and twelve (**25-36**) compounds were isolated, respectively. Their structures were determined based on NMR, MS, ECD and X-ray data. The isolated compounds were evaluated for cytotoxic activities against cancer cell lines and normal Vero cell lines by MTT assay. Compound **7** showed substantial cytotoxicity against HeLa (IC_{50} = 2.24 µg/mL), HT29 (IC_{50} = 2.51 µg/mL), HCT116 (IC_{50} = 3.50 µg/mL) and MCF-7 (IC_{50} = 3.77 µg/mL) cells. Compound **23** showed reasonable cytotoxicity and compounds **12** and **13** showed slight cytotoxicity against all tested cell lines.

These results show that the selected *Xylaria* spp. produced diverse secondary metabolites, some of which exhibited cytotoxic activities against various cancer cell lines.



▶**Fig. 1** Cytotoxic compounds from selected *Xylaria spp.*

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P-143 Chemical constituents and their anti-inflammatory activity from the fungus, *Diaporthe phaseolorum* var. *caulivora*

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DOI 10.1055/s-0039-3399867

Secondary metabolites of microbes are widely considered to be one of the best reservoirs for drug discovery from natural sources [1]. *D. phaseolorum* var. *caulivora*, which was isolated from *Neolitsea daibuensis*, was processed through solid-state fermentation, and its solid fermentate showed anti-inflammatory activity based on the preliminary screening.

The solid fermentate of *D. phaseolorum var. caulivora* was partitioned into the *n*-BuOH-soluble layer and water-soluble-layer. In our previous study, we have reported six new geranylcyclohexenetriols, phomentrioloxins A-F (**1-6**), along with seven known compounds, phomentrioloxin (**12**), peribysin A (**13**), adenosine (**14**), mellein (**15**), de-*O*-methyldiaporthin (**16**), palmitic acid (**17**), and ergosterol peroxide (**18**) from the *n*-BuOH-soluble fraction of solid fermentate of *D. phaseolorum var. caulivora*. Continuing investigation of this fungus led to the isolation of four additional new compounds, including one new pyrone, caulivopyrone (**7**), one sesquiterpene, caulivobysin (**8**), two new isobenzofuranones, cauliphthalide A (**9**), and cauliphthalide B (**10**), and one compound isolated from nature for the first time, 3-*O*-desmethyl phomentrioloxin (**11**) [2]. Phomentrioloxin (**12**) exhibited NO inhibitory activity with IC_{50} value of 12.5 ± 2.2 μM and without cytotoxicity.

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P-144 Chemical constituents, molecular docking, and acetylcholinestrase inhibitory activity of *Macaranga gigantea*

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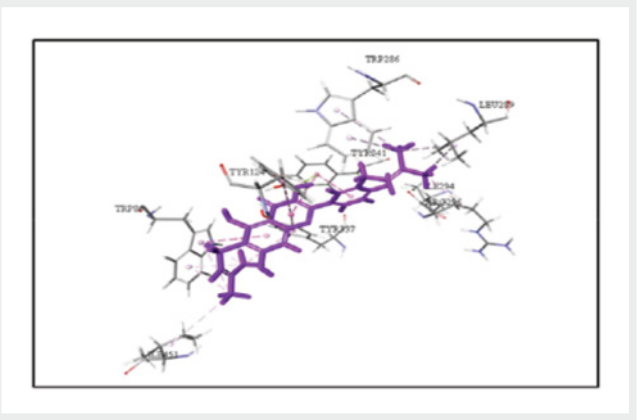
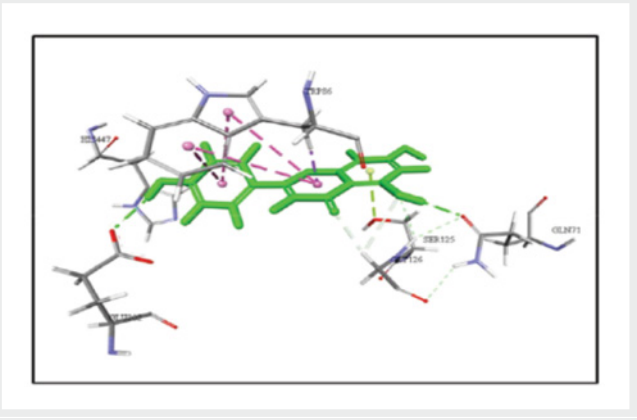
Genus *Macaranga* comes from the family of Euphorbiaceae and known for their mutual associations with ants. Fresh or dried leaves of some *Macaranga* species was used by traditional healers to treat swellings, cuts, sores, boils and bruises. The isolation of chemical constituents from this genus have produced phenolic compounds, such as flavonoids and stilbenoids [1, 2]. Phytochemical study was done on *Macaranga gigantea,* known as Mahang gajah. The purpose of this study was to isolate the chemical constituents from the leaves of *Macaranga gigantea,* to evaluate the anti-acetylcholinesterase property, and to discuss the binding conformation of the isolated compound with the enzyme, using molecular docking. Several chromatography techniques such as vacuum liquid, radial, column, and preparative thin layer chromatographies were used to isolate of the pure compounds. The structure of the isolated compound was confirmed by means of spectroscopy methods such as infrared (IR), ultraviolet-visible (UV-Vis), nuclear magnetic resonance (NMR), mass (MS) spectroscopy and by comparison with the literature data. Two flavonoids were successfully isolated from the leaves of *Macaranga gigantea,* which are glyasperin A and apigenin [3, 4]. The molecular docking study showed that both glyasperin A (▶**Fig. 1**) and apigenin (▶**Fig. 2**) are situated in a peripheral anionic site surrounded Trp86, Tyr124, Trp286, Leu289, Arg296, Ile294, Tyr337, Tyr341, and Ile451 which suggested that both compounds can potentially block the entrance of the active site gorge, thus prevent the ACh bind to the AChE [5]. This result is further supported by the percent inhibition at 100 μM of the two compounds.



▶**Fig. 1** Binding interaction of glyasperin A in active site gorge of AChE



▶**Fig. 2** Binding interaction of aplgenis in active site gorge of AChE

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P-145 Chemical profiling of mycosporine-like amino acids in *Bostrychia* spp. and novel sulphated and brominated compounds from *Bostrychia calliptera* (Ceramiales, Rhodophyta)

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The genus *Bostrychia* (Ceramiales, Rhodomelaceae) consists of approximately 40 species which are taxonomically accepted at present [1]. However, cryptic species are known to occur in several species complexes of *Bostrychia*, such as *B. tenella, B. simpliciuscula, B.calliptera* and *B. intricata* [2, 3]. All of them produce a variety of chemically different mycosporine-like amino acids (MAAs), compounds that are known for their outstanding UV absorption capacity and their photoprotective role in nature [4]. In order to get a deeper understanding of the chemical diversity of MAAs in the genus, a previously developed LC-MS method was successfully applied for the analysis of various *Bostrychia spp*. This approach enabled the investigation of their MAA pattern and revealed distinct differences among the species. *Bostrychia scorpioides*, for example showed a unique MAA pattern, thus leading to the isolation and identification of six new MAAs. Regarding *B. calliptera*, earlier studies suggested the existence of either two or three genetic lineages within this species based on molecular DNA sequencing data [3, 5]. Phytochemical profiling of those samples that were used for the DNA sequencing also revealed distinct differences in their chemical composition. Overall, the presence or absence of brominated and sulphated compounds or variations in their MAA pattern allowed a clear discrimination between the first and the other two lineages. The most important marker substances were isolated from *B. calliptera* and their structures elucidated. The respective compounds are considered as suitable biomarkers for chemotaxonomic studies within this polyphyletic group.

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P-146 Chemical profiling, biostatic and biocidal dynamics of *Origanum vulgare* L. essential oil

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DOI 10.1055/s-0039-3399870

Origanum vulgare L. (Lamiaceae) is a widespread flavoring culinary and medicinal herb. The present study aimed at investigating the antimicrobial activity of *Origanum vulgare* (OV) essential oil (EO) through illustrating its biostatic, biocidal and the dynamics of the biocidal activity against 11 different microorganisms. GC/MS of OV EO allowed the identification of 32 compounds representing 99.94% of the oil. The two major identified compounds were terpinen-4-ol (38.35 %) and *trans*-sabinene hydrate (10.06 %). Different methods were employed to illustrate the biostatic activity of OV EO. Results of the biostatic studies on OV EO using agar and broth dilution methods showed that *Staphylococcus aureus (S. aureus)* was the most sensitive organism; with a Minimum inhibitor concentration (MIC) 1.18 mg/ml. Agar diffusion method showed that the highest activity was observed against *Bordetella bronchiseptica (Br. bronchiseptica), Saccharomyces cerevisiae (S. cerevisiae), Bacillus subtilis (B. subtilus)* and *Staphylococcus epidermidis (S. epidermidis)* with inhibition zones 38 ± 1.5, 29.5 ± 0.8, 26.9 ± 0.9 and 26.9 ± 1.1 mm, respectively. Studying the dynamics of 1% v/v OV essential oil emulsion over a period of 6 h revealed that *Escherichia coli (E. coli), B. subtilis, S. epidermidis* and *S. cerevisiae* had the fastest response. Also increasing concentrations of OV oil emulsion increased the rate of cell killing and the duration of growth lag phase increased correspondingly. These data indicated that OV EO produces a concentration and time-dependent antimicrobial activity.

P-147 Chilean “chaura” berries (*Gaultheria phillyreifolia* and *G. poeppigii*): isolation of secondary metabolites and antioxidant activity

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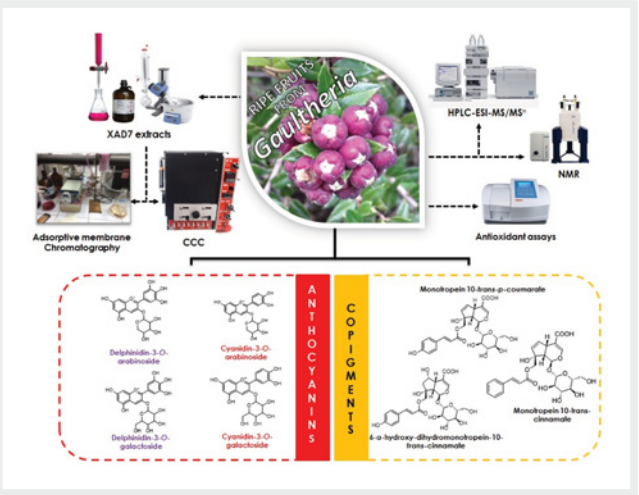
Berries from the *Gaultheria* genus (Ericaceae) are used worldwide as food and in traditional medicine [1]. Most studies have been carried out with Asian [2], North American and European species [3, 4]. Five edible *Gaultheria* species occurs in Chile [5] and little is known on their secondary metabolite chemistry. The aims of the present work were to isolate the main secondary metabolites from *Gaultheria phillyreifolia* and *G. poeppigii* berries, and to evaluate their antioxidant activity. A combination of membrane chromatography (MC) and counter-current chromatography (CCC) was used to isolate the main compounds. The products were characterized by spectroscopic and spectrometric means. The antioxidant activity was measured by different spectrophotometric assays. The fruit extracts were submitted to MC to separate anthocyanins from copigments. Four anthocyanins were isolated by CCC and identified as galactoside and arabinoside of cyanidin and delphinidin. From the copigment fraction, CCC allowed the isolation of quercetin-3-arabinoside, 3-rutinoside and 3-rhamnoside, 3-caffeoylquinic acid and the iridoids monotropein-10-*trans*-coumarate, monotropein-10-*trans*-cinnamate and 6α-hydroxy-dihydromonotropein-10-*trans*-cinnamate. The latter two compounds are new natural products. Other 34 compounds were tentatively identified by HPLC-DAD-ESI-MS^n. The *G.*

phillyreifolia samples showed better antioxidant activity in the FRAP, TEAC and CUPRAC assays. On the other hand, the *G. poeppigii* samples showed better results in the DPPH and ORAC assays. The observed differences between both species were validated by a Partial Least Square Discriminant Analysis (PLS-DA). This is the first report on the isolation and characterization of secondary metabolites from Chilean *Gaultheria phillyreifolia* and *G. poeppigii* berries.



▶ **Fig. 1**

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P-148 Combined HPLC-PDA-MS and NMR study of *Matricaria pubescens* extracts from Algeria

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Matricaria pubescens (Desf.) Schultz (Asteraceae), known as hairy chamomile, is endemic in North Africa, especially in the Algerian Sahara and is used for several ailments such as rheumatic and muscular pains, coughs, allergies, ocular affections, dysmenorrhoea, scorpion stings, dehydration and toothaches [1]. Previous phytochemical studies on this species had revealed the presence of flavonoids and coumarins [2]. As a part of a project aiming at studying *Matricaria* sp. the analysis of *M. pubescens* was undertaken, but this time through an integrated chemical profiling combining HPLC-PDA-MS, NMR and phytochemical analyses, where appropriate. A medium polarity extract was prepared according to the Bohlmann protocol (cyclohexane:diethylether:methanol 1:1:1), slightly modified [3]. This extract was further subjected to fractionation and all separation steps thereof were monitored by both HPLC-PDA-MS and NMR. Analyzes did not show the presence of sesquiterpene lactones, but instead evidenced several flavonoids and phenolic compounds. More than 20 compounds have been unambiguously identified up to now, among them luteolin-4′-O-glucopyranoside and quercetagenin-3-O-glucopyranoside, reported for the first time in *Matricaria* sp. Furthermore, two polyamines previously reported in other Asteraceae sp. [4,5] have been isolated and characterized. Fingerprint analysis and comparison of this species to the officially recognized in Europe *M. recutita* L. shows many similarities in the chemical content and justifies the ethnopharmacological uses in Algerian traditional medicine.

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P-149 Comparative phytochemical study of various extracts from *Limonium gmelinii*

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Limonium is a genus of plants that includes more than 300 different species worldwide, out of which in Kazakhstan, the most industrially significant and productive is *Limonium (L.) gmelinii* (Willd.). Major part of previous research was conducted on roots, from which four drugs were obtained, included at the National drugs register issued by the Ministry of Health Care. Underestimated aerial part might have some practical advantages, in particular large mass and less damage to the plant population along with novel biological activities. The object of the current research is plant material, harvested in Almaty region in 2018, dried, milled and assessed for its good quality in accordance with the requirements of the State Pharmacopeia. Preliminary phytochemical study revealed presence of the following groups of biologically active compounds: tannins (4.17 %), flavonoids (6.84 %), organic acids (4.07 %), and carbohydrates (3.89 %). In lipophilic fraction, tricosane was dominating (47.44 %). High content of phytol acetate (12.88 %) related to derivatives of acyclic diterpene alcohols was established. In addition, the 3,7,11,15-tetramethyl-2-hexadecan-1-ol, detected in the amount of 5.17%, was identified. After extraction with 50% ethanol in 1:5 ratio, with ultrasonic intensification of the extraction process at a frequency of 37 kHz and a temperature of 40°C, the resulting extract was dried under vacuum to completely remove the solvent. As a result, the degree of extraction was 53.55% of the weight of processed raw material, with increased amount of tannins (24.05 %), flavonoids (10.17 %), organic acids (5.84 %), and carbohydrates (4.13 %). Comparative analysis of biological activities is underway.

P-150 Comparative study of biological activities and chemical constitution of *Agaricus subrufescens* and *Pleurotus ostreatus* mushrooms

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There are approximately 2,000 species of edible mushrooms found in the world, of which only 25 are cultivated on a large scale for commercial purposes. Among these, the main species cultivated in Brazil are: *Pleurotus ostratus* (shimeji), and, recently, *Agaricus subrufescens* (mushroom of the sun) [1, 2]. Thus, the aim of this study is to evaluate the antioxidant activity, the total phenolic content, and to identify the bioactive compounds of *A. subrufescens* and *P. ostreatus* by LC-MS/MS. The antioxidant activity of hydroalcoholic extract was evaluated by 1,1-diphenyl-2-picryhydrazyl radical (DPPH$^{\bullet}$) method and oxygen radical absorbance capacity (ORAC) assay, while the phenolic content was quantified by Folin-Ciocalteu method. The *A. subrufescens* extract presented higher antioxidant activity (54.71 ± 3.45 g of the Gallic acid equivalent (GAE) 100 g^{-1}) than the *P. ostreatus* extract (28.99 ± 0.63 g GAE 100 g^{-1}). The same results were obtained by ORAC method: *A. subrufescens* presented higher antioxidant activity (5.95 ± 0.092 g GAE 100 g^{-1}) than *P. ostreatus* (0.76 ± 0.029 g GAE 100 g^{-1}). The *A. subrufescens* also presented higher values of phenolic content 92.94 ± 0.30 mg GAE 100 g^{-1} compared to *P. ostratus* (16.48 ± 0.65 mg GAE 100 g^{-1}). Three compounds were identified by LC-MS/MS in both species based on the fragmentation pattern reported in the literature [3]: *p*-coumaric acid, caffeoyl hexose, and saccharopine, among which the first two presents antioxidant metabolites reported in the literature. Therefore, the higher phenolic content observed for *A. subrufescens* can explain its potent antioxidant effect.

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P-151 Composition of the essential oil of *Tagetes tenuifolia* Cav. *and Tagetes filifolia* Lag. from Guatemala

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Tagetes tenuifolia Cav. grows in México, Central America, Colombia and Peru at 300–2700 m high. *Tagetes filifolia* Lag. is found in México, Central America, Argentina, Bolivia, Colombia, Ecuador, Peru and Venezuela. The purpose of the study was to determine the composition of the essential oil of both plants growing wild in Guatemala to evaluate their potential for oil production. Thus, during 2018 aerial parts of *T. tenuifolia* and *T. filifolia* were collected in Sololá at 1500 m high at west of Guatemala and in Jutiapa at 800 m high at east of Guatemala respectively, yielding 0.6% and 0.1% of essential oil respectively by hydrodistillation. The main components in the oils analyzed by GC/MS and GC/FID were: 3-caren-2-one (44.5 %), (*Z*)-tagetone (11.5 %), (*E*)-ocimenone (10.5 %) and dihydrotagetone (5.5 %) for *T. tenuifolia* differing of results obtained for *T. tenuifolia* cultivated in Italy which presented (*E*)-ocimenone as the major component [1]. (*E*)-anethole (73.4 %) and methyl chavicol (7.2 %) were the main components found for *T. filifolia* in this study similarly to the results reported in Peru [2] which presented (*E*)-anethole as the major component for the oil, but differing from the oil of the plant cultivated in Italy which presented methyl chavicol as the major component [1]. The domestication and cultivation of the plants for oil production could be an alternative to improve the income of the population of the regions characterized by high poverty indexes but further studies regarding biological and pharmacological activities are recommended.

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P-152 *Corylus avellana* as source of antioxidant diarylheptanoids

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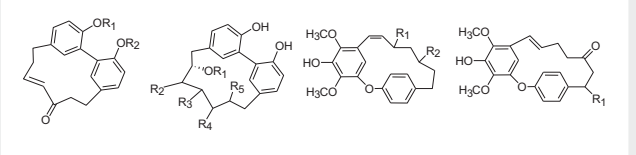
Hazelnut (*Corylus avellana* L.), belonging to the family Betulaceae, is one of the most popular tree nuts [1]. Among Italian cultivars, 'Tonda di Giffoni' and 'Tonda Gentile delle Langhe' gained the European Protected Geographical Indication (PGI) label for their processing quality, which guarantees the origin, the typical features, the high physico-chemical quality and the organoleptic properties [2].
In the frame of a research programme aimed at exploiting *C. avellana* biomasses as source of bioactive compounds, cyclic diarylheptanoids and diaryletherheptanoids named giffonins A-V have been isolated from leaves, leafy covers, male flowers and seeds of *C. avellana*, cultivar 'Tonda di Giffoni' [1-5]. Diarylheptanoids are a class of natural products based on the 1,7-diphenylheptane skeleton. The most representative compound belonging to this class is the well known curcumin, possessing various biological activities, including antioxidant, cancer-preventing, anti-inflammatory [4]. The isolated compounds have been evaluated in different models showing that, as for curcumin, some isolated giffonins were able to prevent oxidative damages of human plasma lipids, induced by H_2O_2 and H_2O_2/Fe^{2+}.
Our work has been extended to the leaves and seeds of the cultivar 'Tonda Gentile delle Langhe' explored by analytical approaches based on LC-HRMS/MS and NMR. In the phenolic profiles the occurrence of cyclic diarylheptanoids corresponding to giffonins or to closely related compounds along with linear diarylheptanoids has been highlighted, confirming *C. avellana* leaves as a rich source of antioxidant diarylheptanoids.



▶ **Fig. 1**

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P-155 Discovery of novel skin-whitening agents produced by endophytic fungi associated with desert plants. The case of *Comoclathris* spp

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Endophytic fungi have been identified as a significant source of novel natural bioactive products with potential application in several fields as agriculture, medicine and food industry [1, 2, 3]. In order to explore the potential of endophytic fungi to produce secondary metabolites with applications in the cosmeceutical industry, 142 diverse endophytes from a wide variety of Spanish arid plants were selected to be screened for their skin-whitening activity, applying the OSMAC approach. Cytotoxicity evaluation was performed to discriminate cytotoxic fungal extracts [4]. Two fungal strains CF-090361 and CF-090766, isolated from *Sedum sediforme* and *Nerium oleander,* were selected as the most promising. Phylogenetic analyses support that both strains could represent a new species of *Comoclathris.* The aim of this study was to assess the effect of different fermentation conditions on the tyrosinase inhibitory activity and to isolate and identify the active secondary metabolites. Tyrosinase bio-guided assay of both strains led to the isolation of the main active components. Two known compounds Brevianamide M (IC_{50}= 6.81 µM), Graphostrin B (IC_{50}=86.32 µM), and three new compounds with molecular formula $C_{12}H_{12}O_3$ (IC_{50}=3.5 µM), $C_{11}H_{16}O_3$ (IC_{50}=853.72 µM) and $C_{12}H_{10}O_4$ (IC_{50}=0.16 µM) were identified as the main responsible for the whitening activity.

Volcano-plots based on LCMS were applied to determine significant metabolomic differences between both strains and the different fermentation conditions tested, highlighting these bioactive secondary metabolites. Overall, these results can serve as proof of concept that endophytic fungi can be a source of bioactive secondary metabolites that can potentially be used in the cosmeceutical industry.

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P-156 Effect of different culture media on secondary metabolite production of *Xylaria* sp. SWUF08-81

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Fungi are known to produce a diverse range of bioactive metabolites. This diversity occurs because of a combination of species complexity and growth environments. A number of research projects have investigated the effects of fungi growth conditions on the compounds they produced including pH, temperature, and type of culture media [1,2,3]. This allowed the researchers to establish the various compounds profiles and their potential use as drug candidates. There are numerous reports on bioactive compounds from *Xylaria* spp. but only a few have concentrated on the possible impacts of culture media on the compounds [4].

The aim of this research is to study the effect of different culture media, namely GM and PDB, on the metabolites produced and their biological activity, from the wood-decaying fungus *Xylaria* sp. SWUF08-81. There were sixteen compounds isolated from GM medium, five of which were cytochalasin derivatives (**1-5**) together with three mellein, six simple monocyclic and three potential new compounds such as dibenzofuran, mellein and pyrone derivatives. From the PDB medium, nine compounds were identified including two new and five known dihydroisocoumarins derivatives (**6-10**) and two auroglucins. The structure elucidation of the other compounds is ongoing. The preliminary bioassay screening at 100 µg/mL showed that **6, 7** and the new dibenzofuran were toxic against HCT116, HT29, MCF-7, Hela and Vero cells at a percentage of cell viability < 30 %.

It can be concluded that the *Xylaria* sp. SWUF08-81 fungus produced entirely different classes of compounds when the growth culture media were altered.

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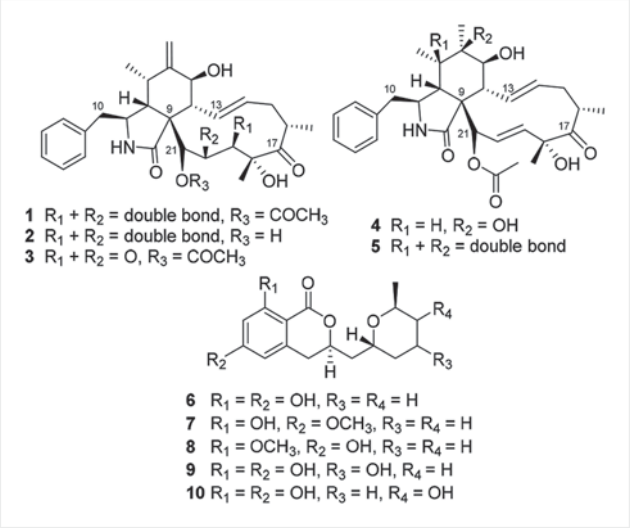

▶**Fig. 1** Some isolated compounds from *Xylaria* sp. SWUF08-81 fungus.

P-158 Essential oil composition of roots, aerial parts and fruits of *Ferulago pauciradiata* Boiss. & Heldr

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Ferulago W. Koch is a genus of the family Apiaceae and 35 taxa grow naturally in our country [1, 2]. Members of this genus are used in traditional medicine for their various biological activities, and mostly known for their aphrodisiac activities [3]. In this study we analyzed the essential oil of different parts of *F. pauciradiata,* an endemic species for Turkey.

Essential oils of the grounded roots, aerial parts and fruits were obtained by hydrodistillation using a Clevenger type apparatus for 3h and they were analyzed both by GC-FID and GC-MS, simultaneously.

The main components of the plant organs are as follows: Roots: 2,5, dimethoxy-*p*-cymene (69.7%), *p*-cymene (12.7%), γ-terpinene; Aerial parts: 2,5, dimethoxy-*p*-cymene (33.0%), α-pinene (9.0%), nonacosane (8.9%); Fruits: bornyl acetate (30.4%), germacrene D (8.1%), α-pinene (7.0%)

In the literature, there is only one study present regarding the essential oil composition of the fruits [4] and the content and amount of major components are somewhat different which may be due to the different collection localities. As far as we are concerned, the compositions of the roots and aerial parts have been analyzed for the first time.

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P-159 Protective effects of ethanol extract of a Mexican propolis on indomethacin-induced gastric mucosal damage in mice.

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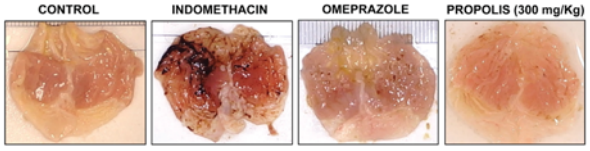
DOI 10.1055/s-0039-3399880

Peptic ulcer is a lesion in the gastric mucosa. Current treatments exhibit limited efficacy and several side effects, which makes it necessary to search for new alternatives[1]. One of these alternatives can be propolis. This natural product contains mainly phenols and flavonoids, which are directly related to its antioxidant, antimicrobial and anti-inflammatory activities[2]. The aim of this project was to evaluate the gastroprotective effect of the ethanolic extract of Mexicali propolis (EeMeP) in a model of indomethacin-induced gastric ulceration in mice.

Gastric lesions were induced by intragastric administration of indomethacin at a dose of 20 mg/Kg[3]. Treatment with EeMeP (100, 200 and 300 mg/Kg) was administered 2 hours before indomethacin.

The results show that the EeMeP contains chrysin, kaempferol, luteolin, pinocembrin, quercetin, baicalein, apigenin and myricetin, as well as a high free-radical scavenging capacity (89.92 µg/mL) which is related to the concentration of phenols (28.66 %) and flavonoids (6.02%). EeMeP showed aniinflammatory and antimicrobial activity. Group treated with EeMeP presented a reduction in the percentage of lesion area, attenuation of histological damage and maintenance of the mucus barrier, which can be translated as a protection of the gastric tissue compared with the group without treatment. In the group treated with EeMeP, the activity of MPO, and the levels of TNF-α, IL-1β and IL-6, decreased significantly. In the same group, an increase in the activity of GPx and SOD was observed.

We show that EeMeP has a gastroprotective effect and that this effect can be attributed to its flavonoid content.



▶**Fig. 1**

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P-160 Exploitation of *Streptomyces lopnurensis* isolated from the marine environment of Golf of Thailand for the discovery of novel skin whitening agents

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In continuation of our research on investigating the marine environment, more than 300 microorganisms were isolated from invertebrates of the Indian Ocean, the Red Sea and the Mediterranean. The microorganisms were cultivated and extracted by optimized protocols in and a library of more than 1200 extracts was established. The initial screening revealed that 0.5% of the extracts showed inhibitory activity against tyrosinase and elastase. Subsequently, selected extracts were further evaluated for their ability to inhibit tyrosinase in normal diploid human dermal fibroblasts.

Among the most active extracts, the one from *Streptomyces lopnurensis*; an actinomycete isolated from a sea cucumber of genus *Synaptula* collected from Sattahip Beach (Gulf of Thailand), inhibited tyrosinase in both cell-free and cell-based assays (93.2% at 300 µg/mL and 30.2% at the concentration of 10 µg/mL, respectively).

The microorganism was re-cultivated in 10L scale, under solid and liquid conditions in Marine Broth, using *in-situ* solid phase extraction technology (XAD-16) and it was then extracted with AcOEt and MeOH. Biological activity was confirmed by cell-free tyrosinase enzymatic assay (inhibition activity ranged between 33.61-83.56% at 300µg/mL) and the most active extract was further fractionated. All fractions were purified by preparative techniques (HPLC, TLC), while NMR and LC-MS methodologies were implemented for the identification and structure elucidation of the isolated compounds. All compounds have been further investigated for their biological activity against tyrosinase in cell-free and cell-based assays.

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P-161 Extraction of active compounds of *Sedum roseum* by natural deep eutectic solvent

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DOI 10.1055/s-0039-3399882

Sedum roseum (L.) Scop. (syn. *Rhodiola rosea* L.) is well established phytoadaptogen used in traditional and officinal medicine all over the world [1,2]. Ethanol is most common solvent for the extraction of active compounds from plants. Natural deep eutectic solvents (NDES) are emerging as green and sustainable solvents for efficient extraction of bioactive compounds and as alternative to organic solvents. NDES ultrasonic assisted extraction was developed to extract roots and rhizomes of *S.roseum*. The concentrations of salidroside, rosin, rosavin, tyrosol, and cinnamic alcohol were analysed by HPLC-DAD. NDES have been prepared using mixtures of glucose, fructose, and lactic acid. In result of optimization of extraction conditions the total concentration of 5 analysed compounds in NDES extract was equivalent to those in 40% EtOH extract. The solubility of glycosides in NDES was higher than those of tyrosol, and cinnamic alcohol. This fact could be explained by polarity of solvent used. However, by variation of composition of NDES we were able to receive extracts with increased concentration of tyrosol, and cinnamic alcohol, while the concentration of glycosides was a little decreased.

To the best of our knowledge this is a first report in which NDES was used for effective extraction of *S.roseum*.

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P-162 Flavonoids and oxepinones from the leaves and twigs of *Desmos cochinchinensis* and their α-glucosidase inhibitory activities

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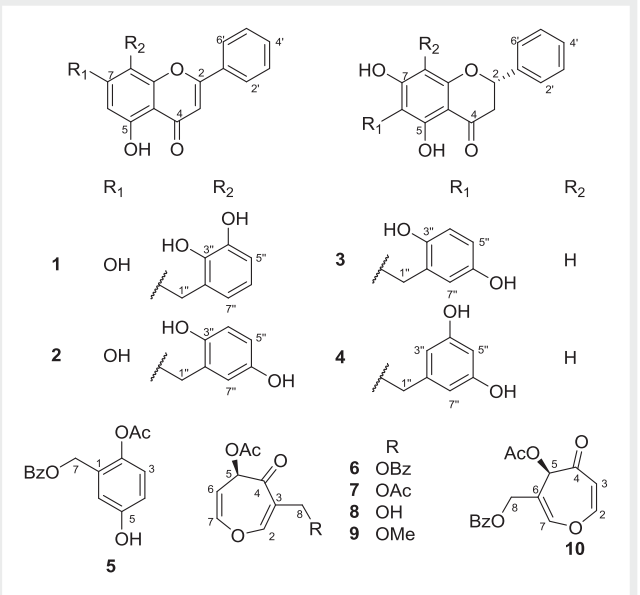
DOI 10.1055/s-0039-3399883

Desmos cochinchinensis, belonging to the Annonaceae family, is a shrub widely distributed in Asia. Several secondary metabolites including, terpenoids, alkaloids, chalcones, and flavonoids have been isolated from this plant. Some of these compounds showed useful biological properties including antitumor, anti-inflammatory, antiviral and anti-HIV activities. Herein, twenty-four compounds comprising four new flavonoids (**1**–**4**), a new benzyl benzoate derivative (**5**), five new oxepinones (**6**–**10**), and fourteen known compounds were isolated and purified from the twigs and leaves of *D. cochinchinensis* by various column chromatographic techniques. Their structures were established by spectroscopic methods. The structure of compound **1** was confirmed from X-



►**Fig. 1**

ray diffraction data with Mo Kα radiation. The absolute configurations of **3**, **4**, and **6–10** were identified from comparisons of their ECD spectra with those reported compounds. Compounds **1**, **2**, **6**, **8**, and **10** showed α-glucosidase inhibitory activities with IC_{50} values of 0.2, 0.2, 3.6, 4.9, and 3.1 μM, respectively, which were better than the positive control, acarbose, (IC_{50} 170.7 μM).

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P-163 Flavonol arabinofuranosides from the methanolic extract of *Hypericum jovis* Greuter.

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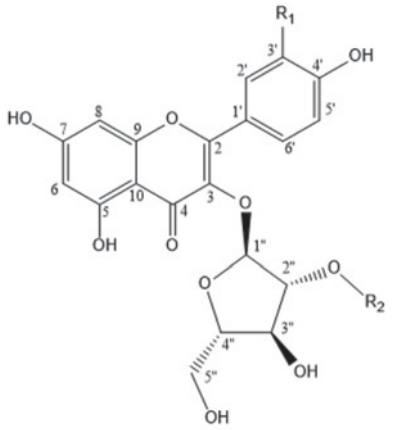
DOI 10.1055/s-0039-3399884

The genus *Hypericum* L. (Clusiaceae) is subject of several studies during the last years because of its promising biological activities, since it biosynthesizes a plethora of active compounds, such as napthodianthrones, phloroglucinols, flavonoids, xanthones and phenolic acids [1]. It comprises more than 450 taxa worldwide [2]; 40 taxa in Greece, 12 of them endemic. Apart from the well-studied species *H. perforatum*, approximately 60% of *Hypericum* spp. have not been evaluated yet. This is the first study of the methanolic extract from *H. jovis*, a narrow Greek endemic species. Three flavonoid arabinofuranoses, namely avicularin, 2''-acetyl-avicularin and 2''-acetyl-juglanin have been isolated and their structures were elucidated by high-field 1D & 2D NMR. Avicularin is a common metabolite of *Hypericum* spp., while 2''-acetyl-avicularin is a rare natural product, which has previously been isolated only twice, namely from *Tibouchina semidecandra* and *Berchemia floribunda* [3,4]. To the best of our knowledge, 2''-acetyl-juglanin is being described for the first time as a natural product.



▶ **Fig. 1**

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P-164 Four new benzoyltyramines from the peels of *Atalantia monophylla* and their cytotoxicity

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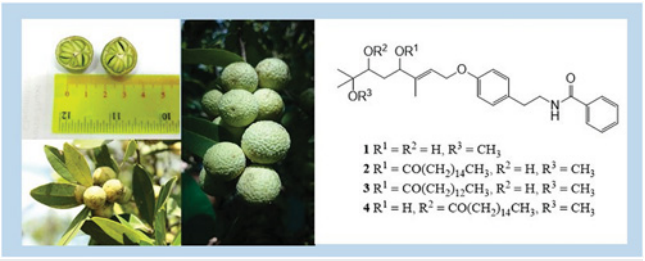
DOI 10.1055/s-0039-3399885

Four new benzoyltyramines, atalantums H-K (**1-4**)[1], and seven known compounds (**5-11**)[2] were isolated from the peels of *Atalantia monophylla*. All compounds were tested for cytotoxicity against HeLa, HCT116 and MCF-7 cell lines, as well as normal cells (Vero cells). Compound **5** showed cytotoxicity against HeLa, HCT116 and MCF-7 cell lines with IC_{50} values ranging from 16-25 μg/mL but was inactive against Vero cells. Compound **6** also showed interesting results as compound **5** with IC_{50} values ranging from 15-18 μg/mL and an IC_{50} value of 80.2 μg/mL against Vero cells. This means compounds **5** and **6** can be used as lead compounds for anticancer agents.



▶ **Fig. 1**

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P-165 HPLC-UV/MS phytochemical characterization of the ethanolic tincture of *Cardiospermum halicacabum*

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DOI 10.1055/s-0039-3399886

Cardiospermum halicacabum L. (Sapindaceae), known also as balloon vine, is an important medicinal plant used in homeopathy and in Ayurveda, but also in folk medicine, for the treatment of a variety of diseases, like

rheumatism, nervous disorders, chronic bronchitis, and lumbago [1, 2]. Phytochemical studies have shown, that different parts of *C. halicacabum* contain phenolic acids, flavonols and their glycosides, triterpenoids, a variety of fatty acids and volatile esters, carbohydrates, proteins, saponins, and tannins [3].

Our key aim was to develop a new UPLC-DAD-ESI-MS/MS analytical method as a part of the phytochemical characterization of the ethanolic tincture of the fresh aerial parts of the flowering plant. Although the chemical profiles of extracts from different plant materials are well documented, there is still a lack of knowledge on the chemical constituents.

Chromatograms were compared to standard reference compounds and with literature data of known constituents. The *C. halicacabum* ethanolic tincture was found to contain the flavones apigenin, luteolin and chrysoeriol, as well as their glucuronides, as already described in the literature [3]. However, the new UPLC method allows also the characterization of nonpolar constituents, such as esters of fatty acids.Consequently, the new method will enable a better quality control based on metabolic profiling.

Acknowledgement We are grateful for the funding of the project by Dr. Willmar Schwabe GmbH & Co. KG.

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P-166 Identification of new labdane diterpenoids from the aerial parts of *Otostegia persica* utilizing NMR and circular dichroism calculations

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DOI 10.1055/s-0039-3399887

The genus *Otostegia* (Lamiaceae) encompasses 33 species growing worldwide [1]. The Iran flora, comprises three species of this genus, which are growing in tropical and subtropical regions. In traditional medicine, *Otostegia persica* is used for the treatment of malaria, headache, diabetes, stomachache, and rheumatoid arthritis, but also to promote wound healing [2,3]. Phytochemical investigations of a petroleum ether subfraction of *O. persica* extract resulted in isolation of three new labdane diterpenes with spiro skeleton (**1-3**) and two derivatives comprising a furan ring (**4** and **5**). The relative configuration of the isolated compounds was established by analysis of NOESY correlations along with ^{1}H and ^{13}C NMR and DP4+ probability calculations, while the absolute configuration was determined by quantum chemical calculation methods and comparison of calculated and experimentally obtained ECD spectra.

Therefore, the isolated compounds were identified as (2''*S*,3*R*,4a''*S*,5*R*,5'*R*,6''*R*,8a''*S*)-6''-hydroxy-5-methoxy-2'',5'',5'',8a''-tetramethyldecahydro-2H,2''H-dispiro[furan-3,2'-furan-5',1''-naphthalen]-3''(4''H)-one (**1**), (2''*S*,3*R*,4''*S*,4a''*S*,5*R*,5'*R*,6''*R*,8a''*S*)-4'',6''-dihydroxy-5-methoxy-2'',5'',5'',8a''-tramethyldecahydro -2H,2''H-dispiro[furan-3,2'-furan-5',1''-naphthalen]-3''(4''H)-one (**2**), (2''*S*,3*S*,4a''*S*,5*R*,5'*R*,6''*R*,8a''*S*)-6''-hydroxy-5-methoxy-2'',5'',5'',8a''-tetramethyldecahydro-2H,2''H-dispiro[furan-3,2' furan-5',1''-naphthalene]-2,3''(4''H)-dione (**3**), (4a*S*,7*R*,8a*R*)-4-(2-(furan-3-yl)ethyl)-7-hydroxy-3,4a,8,8-tetramethyl-4a,5,6,7,8,8a-hexahydronaphthalen-2(1H)-one (**4**), (1*S*,4a*S*,7*R*,8a*R*)-4-(2-(furan-3-yl)ethyl)-1,7-dihydroxy-3,4a,8,8-tetramethyl-4a,5,6,7,8,8a-hexahydronaphthalen-2(1H)-one (**5**).

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P-167 Immunosuppressant flavonoids from *Scutellaria baicalensis*

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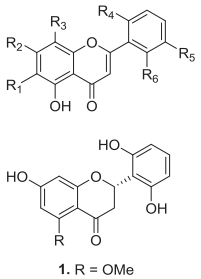
DOI 10.1055/s-0039-3399888

A library of 435 extracts from plants used in Traditional Chinese Medicine was screened for their inhibitory potential on the proliferation of human T-lymphocytes *in vitro*. A dichloromethane extract from roots of *Scutellaria baicalensis* Georgi (Lamiaceae) inhibited T-lymphocyte proliferation with an IC_{50} of 12.9 µg/mL. To localize compounds responsible for the activity, 600 µg of the extract was submitted to HPLC-based activity profiling. Of the 33 one-minute micro-fractions collected, suppression of T-cell lymphocyte proliferation was detected in micro-fractions at r_T 10, 14 and 17 minutes, respectively.

Preparative isolation led to seventeen flavonoids. Twelve compounds (**2**, and **5-15**) were localized in the active time windows, and structurally related flavones **4, 16**, and **17,** and flavanones **1** and **3** were isolated from fractions adjacent to the active time windows. All flavonoids possess an unusual substitution pattern on the B-ring, with absence of substituents at C-3 and C-4.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
2	H	OH	OMe	OH	OH	OMe
4	H	OMe	OMe	OH	H	OH
5	OMe	OMe	OMe	OH	H	OH
6	H	OH	OMe	H	H	OH
7	H	OH	H	H	H	OH
8	H	OH	OMe	OH	H	OMe
9	OMe	OH	H	H	H	OH
10	H	OMe	OMe	OH	H	OMe
11	H	OH	OMe	H	H	H
12	OMe	OH	OMe	H	H	H
13	H	OH	H	H	H	H
14	OMe	OMe	OMe	OH	H	OMe
15	H	OMe	OMe	H	H	OH
16	OMe	OH	H	H	H	H
17	OMe	OMe	OMe	H	H	OH

▶ **Fig. 1**

P-169 *In vivo* anthelmintic activity evaluation of licarin A, a neolignan isolated from leaves of *Nectandra oppositifolia* (Lauraceae)

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DOI 10.1055/s-0039-3399889

Schistosomiasis is a neglected disease affecting people worldwide, and its control is largely dependent on praziquantel. Thus, natural products are considered a valuable source for new prototypes. The hexane extract from leaves of *N. oppositifolia* (Lauraceae) displayed *in vitro* activity against *Schistosoma mansoni*. Based on this result, the bioactivity-guided fractionation of this extract afforded the isolation of lincarin A. The *in vivo* antischistosomal properties of licarin A were investigated for the first time in a murine model of schistosomiasis. For *in vivo* experiments, three-week-old Swiss female mice were subcutaneously injected with a suspension containing 80 *S. mansoni* cercariae (BH strain). Licarin A was administered by oral gavage to groups of five mice. Results showed that oral treatment with a single oral dose of licarin A achieved total worm burden reduction of 46.3% ($P < 0.05$). With respect to egg burdens, licarin A led to a reduction of 53.4% ($P < 0.05$) in the number of immature eggs, whereas analysis of fecal samples revealed a reduction of 76.8% ($P < 0.05$) of eggs. Regarding liver and spleen pathologies, licarin A reduced the infection-promoted increase in liver mass by 19.4% and spleen mass by 22.4% in adult *S. mansoni*-infected mice, in comparison to vehicle treated controls. In conclusion, oral treatment with licarin A at a single dose to mice harboring chronic infection decreased the number of parasites and eggs as well as ameliorated pathology in the liver and spleen, demonstrating the potential of this natural compound in the treatment of schistosomiasis.

Acknowledgments FAPESP (project 2018/18975-1 and 2016/20633-6), CNPq and CAPES for financial support

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P-170 Ingol and *ent*-Atisane diterpenes from the aerial parts of *Euphorbia deightonii*

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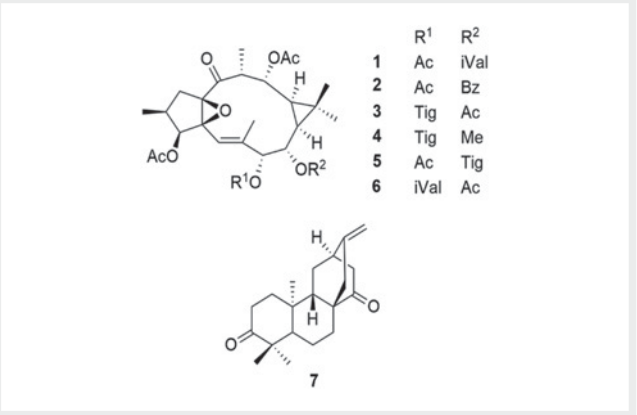
DOI 10.1055/s-0039-3399890

Plants of the Euphorbiaceae family display high diversity of structurally unique diterpenoids, which have attracted great interest from biogenetic, synthetic, biological and toxicological point of view. *Euphorbia deightonii* Croizat is a thorny succulent plant, which grows to 6 m in height. It is endemic to western Africa. In Nigeria, this species is used in folk medicine as a wart remover, and for treatment of leprosy and woman sterility [1]. The irritancy of the latex in a mouse ear inflammatory assay was demonstrated, and subsequently the presence of ingenol esters was reported [2, 3]. In the course of our phytochemical study the aerial parts of *E. deightonii* investigated in order to identify further diterpenes. Fractionation of methanol extract by a combination of solvent-solvent partition, open column chromatography and HPLC afforded six ingol derivatives (**1**-**6**) and one *ent*-atisane type diterpene (**7**). Their structures were established on the basis of 1D and 2D NMR analysis. All compounds were isolated from *E. deightonii* for the first time.



▶ **Fig. 1**

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P-171 Investigation of anti-protozoal activities and metabolite profiling of Helichrysum species based on traditional use

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DOI 10.1055/s-0039-3399891

Tropical diseases such as leishmaniosis constitute a major health concern in developing countries. *Helichrysum* spp. mainly distributed in African countries have been used in traditional and folk medicine for the treatment of infectious diseases such as protozoal problems. We aimed to investigate and compare the anti-protozoal activities and bioactive phytochemical components of *H. leucocephalum*, *H. oocephalum* and *H. oligocephalum*. A sensitive method coupling high-performance liquid chromatography (HPLC) with photodiode-array detector (PDA) and electrospray ionization mass spectrometry (ESIMS) was optimized for the separation and metabolite profiling. The LC-ESIMS metabolite profiles of the fractions from the plants were compared by applying two step workflow using an ACD/MS workbook suite add-in, and data clustering on an open-source web platform freeclust [1]. The metabolites can be categorized into two major types namely flavonoids and phenolic acids, and phloroglucinol and pyrone derivatives. This biological evaluation revealed potent activities for the obtained fractions particularly DCM extracts dominated with pyrone and phloroglucinol derivatives. DCM extract of *H. oligocephalum* showed to be the richest in pyrone and phloroglucinol derivatives with anti-protozoal activities. The data emphasizes on the potential of *Helichrysum* plants which are mostly distributed in South Africa for the treatment of infectious diseases dominated in developing countries particularly Africa itself.

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P-172 Investigation of the marine microorganism *Cladosporium halotolerans* for the isolation and identification of bioactive metabolites with potential anti-aging activity

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The marine environment is an inexhaustible source of secondary metabolites with significant biological activities. In continuation of our efforts to discover bioactive metabolites from under-investigated marine environments[1], invertebrates and their associated microorganisms were collected from areas of the mesophotic zone of the Red Sea that were found to be rich in biodiversity. More than 100 microorganisms were cultivated under solid and liquid conditions, and evaluated for their potential anti-aging activity using elastase and tyrosinase enzymatic assays. In the context of this work, the strain XMLm 133-S2, isolated from *Sarcophyton* sp., a soft coral collected from the Red Sea, was selected for further investigation since it exhibited a promising effect in elastase and tyrosinase enzymes.

The strain, identified as *Cladosporium halotolerans*, was re-cultivated under liquid conditions (10L scale) in marine broth using absorption resin technology and extracted with AcOEt and MeOH. Dereplication techniques were employed in order to investigate the metabolite content in each extract and the isolation of secondary metabolites was performed using different chromatography techniques (CPC, Semi-Prep HPLC, Sephadex LH-20, Prep-TLC). Spectroscopic and spectrometric data (1D & 2D NMR and UPLC-HRMS) were recorded for all isolated compounds, in order to refunambiguously elucidate their structure. In total, eighteen compounds were elucidated. Bioevaluation of isolated compounds shown that among them, six diketopiperazines from AcOEt extract and a nucleoside from MeOH extract displayed remarkable inhibitory activity against elastase or tyrosinase.

Acknowledgement TASCMAR project (www.tascmar.eu) has been funded by the European Union in the frame of H2020 (Grant Agreement No 634674).

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P-173 Investigations of the pharmacological properties of carvotacetones isolated from *Sphaeranthus africanus*

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Sphaeranthus africanus has been used in traditional medicine in Vietnam to treat sore throat, alleviate swelling and as a sedative [1].

Aim To evaluate the anti-inflammatory activity of the plant and explore the pharmacological properties of the isolated compounds.

Results Seven carvotacetones were isolated from the dichloromethane extract. All compounds exhibited significant anti-proliferative activity against several cancer cell lines: CCRF-CEM, MDA-MB-231, HCT-116 and U-251 [2]

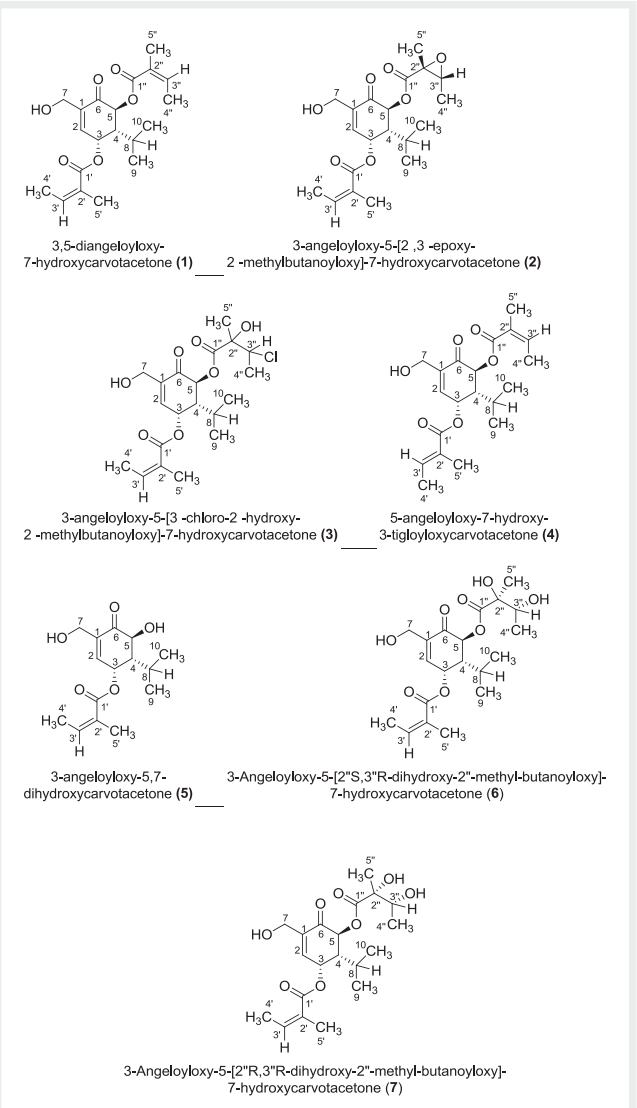
Compounds **1** and **2** possess potent and selective COX-2 inhibitory activity with IC_{50} values of 3.6 and 0.5 µM, respectively.

Compounds **1-7** exhibited inhibitory effects on COX-2 gene expression in THP-1 macrophages. Compound **4** is the most active compound inhibiting the synthesis of COX-2 mRNA by 55% at 2.06 µM.

Carvotacetones inhibited NO production in BV2 and RAW cell lines with IC_{50} values ranging from 0.2 to 2.9 µM. Compound **4** showed potent inhibitory activity with IC_{50} values of 0.2 µM in both cell lines.

Carvotacetones **1** and **6** exerted strong antibacterial activities (MIC ≤ 31.25 mg/L) against *M. aurum* and *M. bovis* BCG. Compound **1** considerably increased the susceptibility of *M. smegmatis* towards ethidium bromide and rifampicin by an 8- and 2-fold reduction of their MICs.

Compounds **1-6** showed anti-plasmodial activity, with IC_{50} values ranging from 1.41 to 3.9 µM.



►**Fig. 1** Structures of compounds 1-7

The activities of major constituents of *S. africanus* support the traditional medical application of this plant for the treatment of inflammation-related disorders. **1-7** may be interesting due to their antiproliferative and anti-plasmodial activities which should be considered for further testing and mechanistic studies.

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P-175 Isolation and characterization of bromophenolic compounds in the red alga *Vertebrata lanosa*

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Vertebrata lanosa (L.) T.A.Christensen is a red alga that grows epiphytically on other algae, especially on the brown alga *Ascophyllum nodosum* (L.) Le Jol. [1]. It is known to contain several bromophenols, compounds that are unique in the plant kingdom. Only marine organisms are able to biosynthesize them, as they have direct access to the bromide contained in seawater, but also by virtue of a rare enzyme called vanadium bromoperoxidase [2]. Some of these brominated molecules are thought to play a crucial role in the chemical protection of marine plants, but their functional role is not fully known [2].

In order to obtain deeper understanding of bromophenolic compounds in red algae, seven bromophenols were isolated from the methanolic extract of *Vertebrata lanosa*, collected in Brittany (France), by using various chromatographic techniques. The structures of the isolated compounds were elucidated by nuclear magnetic resonance and mass spectrometry. Among the isolated substances are compounds like lanosol, methylrhodomelol and 2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-methoxymethyldiphenylmethane and other structurally similar compounds. A simultaneously developed high performance liquid chromatography assay was used to determine the content of each bromophenol in the alga and furthermore applied to screen for respective compounds in other red algal species, too.

In addition, these substances pose very interesting candidates for a pharmacological investigation in different assay systems as a broad range of relevant activities of bromophenolic compounds has been reported in literature, ranging from antioxidant, antimicrobial, anti-thrombotic, anti-diabetic to anti-cancer effects [3].

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P-176 Isolation and identification of Wnt inhibition-specific metabolites from an Amazonian palm tree endophyte *Lasiodiplodia venezuelensis*

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The Wnt signaling pathway controls multiple events during embryonic development of multicellular animals of almost all taxa and, when aberrantly activated in the adult, is carcinogenic. Breast cancers are dependent on Wnt pathway overactivation mostly through dysregulation of pathway component proteins expression, which necessitates search of the therapeutically relevant compounds targeting them [1]. In the present work, the objective was to explore the chemical diversity and the presence of selective Wnt inhibitors within a unique collection of 54 fungi isolated as endophytes from the long-lived Amazonian understorey palm *Astrocaryum sciophilum* and also from its near environment. The fungi were cultured, extracted with ethyl acetate and screened for their effects on cell proliferation and in the Wnt pathway [2]. 13 extracts exhibited a specific Wnt inhibition activity, 6 of them being true endophytes of *A. sciophilum*. The endophytic strain *Lasiodiplodia venezuelensis*, was prioritised for scaled up fractionation for its selective activity and taxonomical originality. Application of geometric transfer from analytical HPLC conditions to semi preparative scale and use of dry load injection enable the isolation of 14 pure compounds in a single step [3]. The structural elucidation of these compounds were performed by conventional spectroscopic methods such as HRMS and 2D NMR. Among the molecules identified, 5 are original natural products described for the first time, and 9 are new to this species. Some of the isolated compounds are potent selective inhibitors of the Wnt pathway.

Acknowledgement This work has benefited from a joint ANR-SNF grant (SECIL, ref ANR-15-CE21-0016, SNF N° 310030E-164289).

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P-177 Isolation and structure elucidation of several tri- and sesquiterpenes from *Commiphora myrrha* (Nees) Engl.

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Commiphora myrrha (Nees) Engl. (Burseraceae) is a spinous tree that is endemic to Somalia, Kenia and Äthiopia. The plant is known to produce a gum resin, called myrrh, which is traditionally used for the treatment of a variety of diseases e.g. pain, arthritis and inflammations [1,2,3]. For a further evaluation of the antiphlogistic potential of myrrh, an ethanolic extract of the drug was subjected to a bioassay guided fractionation, using an ICAM-1 *in vitro* model. During the isolation process liquid-liquid-partition-, silica gel flash-, centrifugal partition- and preparative high-pressure-liquid-chromatography were used to gain a variety of pure compounds. The chemical investigation

yielded several triterpenes of the cycloartane-type with diverse hydroxylation patterns in the A-ring, as well as sesquiterpenes and sesquiterpenlactones of the guajane-, eudesmane-, germacrane- and elemane-type. The structure elucidation was done by 1D and 2D spectroscopy and confirmed by MS experiments. Some of the substances were found for the first time in the entire genus of *Commiphora* and represent the next step to complement the chemical characterization of this plant product. These compounds might also show an important contribution to the anti-inflammatory effect of myrrh.

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P-178 Isolation of tetramer stilbenoids from *Shorea leprosula* Miq., acetylcholinesterase inhibitory activity and molecular docking study

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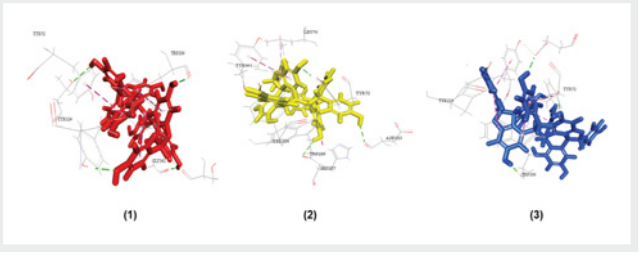
DOI 10.1055/s-0039-3399897

Shorea leprosula Miq. or locally known as "meranti tembaga", belongs to the family of Dipterocarpaceae [1]. The wood of *S. leprosula* is used for utility furniture and ceilings whilst the resin is beneficial as an adhesive and medicine [2]. Besides being of importance in timber production, the genus of *Shorea* is also rich in oligostilbenoids which possess various biological activities [3,4]. Due to that, *S. leprosula* has been selected to be phytochemically studied. The aim of this study are to isolate the chemical constituents from the stem bark of *S. leprosula*, to evaluate the acetylcholinesterase (AChE) inhibitory activity and to investigate the molecular docking study of the compounds. The isolation process was done by using several chromatographic methods to afford three pure compounds. Based on the analyses of UV, IR, NMR and comparison with the literature data, the pure compounds were elucidated as hopeaphenol (**1**), isohopeaphenol (**2**) and hemsleyanol D (**3**). The acetylcholinesterase inhibitory assay displayed good activity in compound **1** with the IC_{50} value of 10.00 □M while compound **3** showed moderate activity with the IC_{50} value of 19.45 □M. The capability of compounds **1-3** to form interactions with Tyr 72, Tyr 124 and Trp 286 is highly important due to the location of amino acid at the peripheral anionic site of AChE which suggest these compounds may block the entrance of active site gorge in preventing acetylcholine from binding to AChE [5] (▶**Fig. 1**). Thus, the molecular docking study is in good agreement with the AChE inhibitory assay.



▶**Fig. 1** Binding interactions of compounds **1-3** in acetylcholinesterase enzyme

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P-179 Isolation of three flavonoids and a phenolic compound from *Macaranga hypoleucaei*

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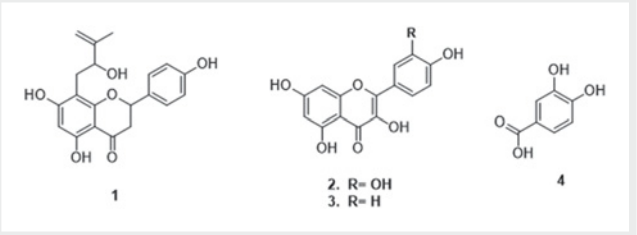
Macaranga (mahang) belongs to the Euphorbiaceae family with over 300 species and is native to tropical Africa, Madagascar, South-East Asia, Australia and the Pacific region [1]. The leaves of many *Macaranga* species are used to treat swelling, fresh cuts, sores, bruises and boils [2]. This genus is known as a source of flavonoids and stilbenoids which possess various biological activities [3]. Powdered leaves of *M. hypoleuca* (Reichb.F. & Zoll.) Müll. Arg. (2.5 kg) were macerated in methanol for 24 h at room temperature three times. The crude extract (700 g) was subjected to liquid-liquid partition using *n*-hexane and ethyl acetate. The ethyl acetate fraction (370 g) was fractionated using vacuum liquid chromatography (VLC) to give eight major fractions (HL1-HL8). Fraction HL5 (2.34 g) was subjected to fractionation to yield seven fractions (HL51-HL57). Fraction HL54 was further purified using column chromatography (CC) and preparative thin layer chromatography (p-TLC) to get one pure compound. Fraction HL6 (10.6 g) was fractionated using VLC to give eight fractions (HL61-HL68). Fraction HL65 and HL66 were subjected for fractionation and purification using column CC, high performance liquid chromatography (HPLC) and p-TLC to afford three pure compounds. The pure compounds were analyzed based on their NMR, UV-Vis and IR data as well as comparison with literature data. A flavanone tomentosanol D (**1**) (5.8 mg), two flavonols namely quercetin (**2**) (26.4 mg) and kaempferol (**3**) (6 mg), as well as 3,4-dihydroxybenzoic acid (**4**) (2 mg) were purified successfully from the leaves of *M. hypoleuca*.



▶**Fig. 1**

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P-180 Isolation, purification and identification of 20 – hydroxymaytenin as a bioactive metabolite from *Maytenus heterophylla* liquid cell culture

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20 – hydroxymaytenin belongs to the group of quinone - methide petacyclictriterpenes (QMTs), which showed a highly cytotoxicity in wide ranges of cancel cell lines. Therefore, they are pharmaceutical attractive metabolites [1]. However, not all of QMT derivatives are available commercially including 20 – hydroxymaytenin. So, the only way to obtain and used QMTs as a standard reference is to isolate from the plant material. Based on the previous studies, there are two main approaches are available. First method involves in the multiple steps of classical column chromatography[1] and second method is using the preparative HPLC with a suitable column[2]. These methods require either time to process or proper instruments. Here, we propose a short time procedure with regular and basic techniques to isolate and purify 20 – hydroxymaytenin.

To modify and establish a fast and simple method to provide accepted in both quality and quantity of 20 – hydroxymaytenin from *M. heterophylla* cell culture.

Overall 35 % yield of high purity (up to 97% by HPLC) of 20 – hydroxymaytenin, confirmed structure by a comparison of a spectroscopic data to the previous report[3], was obtained from this technique after 4 simple steps: extraction with dichloromethane, impurities elimination by solid phase extraction (C-18), isolation by preparative thin layer chromatography and purification by Sephadex LH – 20.

Our modified method, which required only a short time of operation and basic equipment, could provide accepted in both quality and quantity of 20 – hydroxymaytenin from *M. heterophylla* cell culture.

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P-181 Isolation, structure elucidation, and absolute configuration of Germacrane isomers from *Carpesium divaricatum*

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Germacranolides are one of the main classes of sesquiterpene lactones, for which road bioactivities have been reported, including cytotoxicity, anti-inflammatory and anti-malaria. Most of them were isolated from the genus *Carpesium*. The parent nucleus of the germacranolides contains a 5-membered γ-lactone ring fused to a circular 10-membered carbocycle, with different post-modifications to produce complex and diverse structural features. In addition, these germacranolides contain as many as nine stereogenic centers, creating the problem of stereochemical configuration. However, their absolute configurations have rarely been studied.

To investigate the chemical constituents from the whole herb of *Carpesium divaricatum* (Asteraceae), a folk medicine used by the Tu nationality in China; to find more germacranolides with diverse structure to determine their absolute configuration.

101 compounds were isolated from the whole herb of *Carpesium divaricatum*. Among them, 56 were germacranolides including 11 sets of isomers with 5 subtypes. NMR, HRESIMS, ECD calculation and X-diffraction were applied to identify their structures and absolute configurations.

49 new compounds were obtained and 30 of them were germacranolides. Some of them have cytotoxic and anti-inflammatory properties. These findings present knowledge on the structurally diverse and biologically important germacranolides. We also clarified the confusion that exist in the absolute configuration of germacranolides.

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P-182 LC/MS of the soft coral *Sarcophyton ehrenbergi* and its isolated associated endophytic fungi: *Aspergillus oryzae* and *Aspergillus flavus*

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The soft coral Sarcophyton ehrenbergi (Alcyoniidae) contains biologically active secondary metabolites (prostaglandins, diterpenes, sesquiterpenes, ceramides and cerebrosides) and showed cytotoxic and antimicrobial activities. Sarcophyton ehrenbergi was collected from the Red Sea, Hurghada, East of Egypt. This work aims to identify its endophytes. Two endophytic fungi were isolated from it for the first time and identified as Aspergillus oryzae and Aspergillus flavus. Fungal identification was carried out by Phylogenetic analysis and further confirmed by internal transcribed spacer (ITS) sequencing and molecular data analysis. LC/MS analysis was carried out comparatively for the coral and its 2 isolated fungi. Results revealed twenty, nineteen and sixteen compounds identified from Sarcophyton ehrenbergi, Aspergillus oryzae and Aspergillus flavus, respectively. Seven compounds identified as Asperorydine B, 4-methyl-5,6-dihydro-2H-pyran-2-one, Speradine A, Maltoryzine, Aflatoxine B1, Asperorydine G and Asperorydine M were common in both fungal species. No common compounds were detected between the soft coral and its 2 isolated fungi. Further investigations are required to reveal the potential biological activities of the isolated endophytic fungi and /or their identified compounds.

P-183 LC-MS and NMR guided isolation of monoterpene dimers from cultivated *Thymus vulgaris* Varico 3 hybrid and their antityrosinase activity

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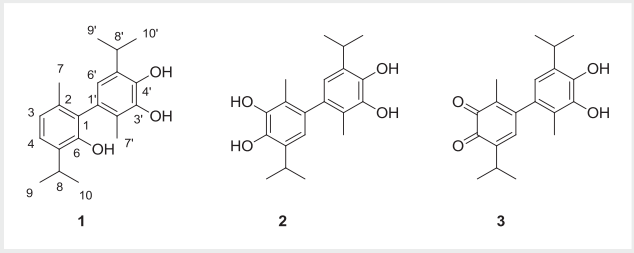
Thymus vulgaris (Lamiaceae), is an important culinary herb and medicinal plant used for the treatment of productive cough and upper respiratory congestion [1,2]. *Thymus vulgaris* L. Varico 3 is a novel hybrid with high yield in essential oil and at least 60% in thymol. As a part of our research program for quality control studies and pharmacological activities on Greek cultivated medicinal plants the chemical investigations on the non volatile constituents of *Thymus vulgaris* Varico 3 were undertaken. Preliminary analyzes of the leaves revealed the presence of biphenyl constituents, which are known to possess deodorant, antioxidant and antiplatelet activities [3,4]. These compounds, which are thymol variants, possessing ortho-hydroxyl groups are structurally similar to tyrosinase substrates. Aims of this work were (i) to investigate the biphenyl content of previously unexplored hybrid "Varico 3" and (ii) to test the monoterpene dimers for their antityrosinase activity. Targeted isolation based on a combination of NMR and HPLC-PDA-MS of the dichloromethane extract afforded one new p-cymene dimer (**1**) together with two known p-cymene derivatives (**2, 3**), as well as thymol, oleanolic acid, ursolic acid, cirsimaritin and xanthomicrol. The structural elucidation of all compounds was performed by NMR spectroscopic analyses and HRESIMS experiments. The biphenyls were assayed for their inhibitory activity on tyrosinase. Compounds **2** and **3** showed negligible activity, while compound **1** effectively inhibited the enzyme with 35% (± 0.3) inhibitory activity, higher than the inhibition of the reference compound kojic acid (18.6 ± 0.02).



▶ **Fig. 1**

Acknowledgements Research was financed by grants of the Aristotle University of Thessaloniki (code 93267).

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P-184 LC-MS guided the efficient isolation of afzelin and quercitrin from herbal plants

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Afzelin (AZ) and quercitrin (QC) are two available flavonoids found in some herbal medicine and show several promising bioactivities as antioxidant, antibacterial, anti-inflammation, and anti-tumor, etc [1-4]. We report here using LC-MS as guided direction to identify and isolate AZ and QC from ethanol extracts of six Vietnamese medicinal plants, *Azadirachta indica* (leaves)-AIL, *Houttuynia cordata* (aerial part)-HCA, *Lophatherum gracile* (aerial part)-LGA, *Loranthus parasiticus* (stem, leaves)-LPS, *Oenanthe javanica* (aerial part)-OJA, and *Persea americana* (leaves)-PAL.

Checking the standard samples of AZ and QC in LC-MS profiles, the ion peaks ($[M-H]^-$) at *m/z* 431 and *m/z* 447 were determined to be AZ and QC, respectively. Other LC-MS profiles indicated that AIL, HCA, and PAL contained AZ and QC; whereas OJA and LPS only possessed afzelin, and LGA missed both AZ and QC. PAL (7 kg, dry) was further chosen to isolate AZ and QC due to the highest content. Extracted with ethanol 95%, then evaporated and partitioned with *n*-hexane and EtOAc sequently, the EtOAc layer (150 g) was yielded and further subjected to an open column with silica gel (*n*-hexane-EtOAc) to obtain four fractions. By LC-MS analyses, AZ and QC were found in fraction 2. Not only employing preparative HPLC but also using a C18 open column, the total amount of AZ (3.1 g) and QC (1.3 g) were yielded from fraction 2 (10 g). These results revealed that LC-MS analysis is a very useful method for providing the potent efficient isolation process to yield afzelin (AZ) and quercitrin (QC) from herbal plants.

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P-185 LC-MS/MS analysis and biological activities of fruits of *Genipa americana*, *Eugenia pyriformis* and *Araucaria angustifolia* from Brazil

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Tropical fruits represent a valuable source of bioactive compounds, and their consumption is increasing on national and international markets due to the growing recognition of their nutritional and therapeutic value [1]. Brazil is one of the largest fruit producers in the world, however, in most cases, the potential of these fruits remains unknown or poorly

studied. Thus, the purpose of this study was to investigate the chromatographic profile of *Genipa americana* (jenipapo), *Eugenia pyriformis* (uvaia), and *Araucaria angustifolia* (pinhao) by LC-MS/MS, and to evaluate the antioxidant capacity of these Brazilian fruits extracts by ABTS method [2]. The peels, pulp and seed were analyzed separately after partition with hexane, ethyl acetate and methanol. The methanolic extract of *A. angustifolia* peels displayed the highest antioxidant activity, resulting in EC_{50} 54.07 µg mL^{-1} after 60 min of incubation, followed by ethyl acetate extract of *Eugenia pyriformis* (EC_{50}= 80.18 µg mL^{-1}). The other extracts were considered inactive (EC_{50} > 100.0 µg mL^{-1}). The chromatographic profile and mass spectra of the *A. angustifolia* extracts obtained by LC-MS/MS showed the presence of 10 phenolic compounds that were identified based on the fragmentation pattern reported in the literature [3]: (epi)gallocatechin, prodelfinidine B3, proanthocyanidin dimer, (epi)catechin, quercetin-3-*o*-glucoside, eriodicitiol-*o*-hexoside, GB-2, myricetin, quercetin and amentoflavone, which may be responsible for the antioxidant effect. Studies like these may lead to the development of functional foods with valuable health benefits, as well as the development of technological applications in the pharmaceutical and cosmetic areas.

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P-186 Metabolite profiles of green, green/red, and red pigmented leaves of *Lactuca sativa* L. cultivars harvested at mature and bolting stages

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Lettuce (*Lactuca sativa* L.) is one of the most popular vegetables in the world. Studying the metabolite content of lettuce at different stages of harvest could be useful to understand their health-benefits and stages of harvest for maximum phytochemical valorization. This study was aimed to identify, characterize, and quantify prominent phenolic compounds and estimate antioxidant activity of lettuce cultivars at mature and bolting stages. The identity and contents of the studied metabolites and antioxidant activity varied significantly. The total content of phenolic acids ranged from 18.3 to 54.6 mg/100g DW and 15.5 to 54.6 mg/100g DW; flavonoids between 9.2 & 25.9 mg/100g DW and 14.9 & 83.0 mg/100g DW in mature and bolting stage, respectively. The contents of cyanidin, lactucin, lactucopicrin, and ABTS values were in the range of 0.3 to 9.7 and 0.5 to 10.2 mg/g DW, 1.8 to 41.9 and 9.7 to 213.0 µg/g DW, 9.9 to 344.8 and 169.2 to 3888.2 µg/g DW, and 12.1 to 29.0 and 15.7 to 30.3 mg TE/g DW, respectively. The score plots of PCA revealed that the green and red pigmented cultivars were distinguished each other and located at the negative and positive sides of PC1, respectively, while the green/red pigmented cultivars were distributed throughout the four quadrants of the PCA plots with no prominent grouping. Lettuce accumulate high amount of sesquiterpene lactones, quercetin malonylglucoside, methylkaempferol glucuronide, kaempferol malonylglucoside, and 3-*O*-caffeoylicquinic acid at bolting compared to the mature stage. In general, red pigmented materials contained higher amount of metabolites.

P-187 Millexatins A-M, antibacterial flavonoids from *Millettia extensa*

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DOI 10.1055/s-0039-3399906

The genus *Millettia* belongs to the family Leguminoseae, which are distributed in the tropical and subtropical regions of Asia, Africa and Australia. Some species of *Millettia* have been utilized as traditional medicines to treat various diseases. This genus is well known to produce flavonoids, chalcones, rotenoids and isoflavonoids and some of these compounds showed interesting biological activities such as cytotoxic, antimalarial, and antiinflamatory activities. *Millettia extensa* (Benth.) Baker is a large climbing shrub that is distributed in the north of Thailand. According to no phytochemical investigation or biological evaluation of this plant has been reported and its crude acetone extract showed good antibacterial activities. The first phytochemical investigation of *Millettia extensa* (leaf, root, and stem extracts) resulted in the isolation and identification of 13 new isoflavones, millexatins A-M, together with 19 known compounds. The structures of these new compounds were determined on the basis of their spectroscopic data. Most of the isolated compounds were evaluated for their antibacterial activities. Some compounds showed good antibacterial activity against the Gram-positive bacteria, Bacillus subtilis TISTR 008, Staphylococcus aureus TISTR 1466 and S. epidermidis ATCC 12228, with MIC values ranging from 2-8 µg/mL.

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P-188 Modern solid support free liquid-liquid techniques turn EVOO to a pool of new natural products and provide gram scale isolation of high importance biophenols

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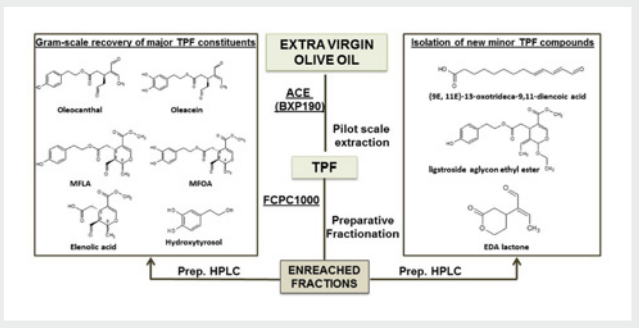
DOI 10.1055/s-0039-3399907

Olive oil is straightly correlated with the Mediterranean diet and the providing health benefits. Most of the beneficial characteristics of EVOO are attributed to olive oil biophenols which compose the Total Phenolic Fraction (TPF). In previous studies of our group, solid support free liquid-liquid extraction and chromatography techniques, were performed on laboratory scale for the extraction of TPF and the further fractionation of EVOO biophenols as well. Following this process, enriched fractions of high importance molecules like oleocanthal, oleacein, MFLA, MFOA, elenolic acid and hydroxytyrosol have been produced. In addition, new natural compounds presented as minor TPF constituents (elenolic acid ethylester, oleocanthalic acid,

oleaceinic acids and EDA acid), were isolated1,2. In the present continuing study, a pilot scale extraction process was developed using Annular Centrifugal Extraction, resulting in the recovery of large amount of TPF. This pilot step gave us the opportunity not only to isolate high quantities of the above mention compounds, but also to investigate the extract chemical composition in deep. The preparative CPC fractionation following by preparative HPLC analysis of the enriched CPC fractions resulted in the gram-scale recovery of the major TPF secoiridoids in a purity higher than 98%. Moreover, three new minor TPF constituents ((9E, 11E)-13-oxotrideca-9,11-diencoic acid, ligstroside aglycon ethyl ester and EDA lactone) have been isolated. The structure elucidation was performed using NMR and UPLC-HRMS analysis. This holistic extraction and isolation methodology is well promising for the identification of EVOO molecules and make them available for *in vivo* tests and clinical studies.



▶**Fig. 1** Schematic diagram of isolation process

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P-189 Modification of oligomeric and polymeric proanthocyanidins via oxidation in alkaline conditions

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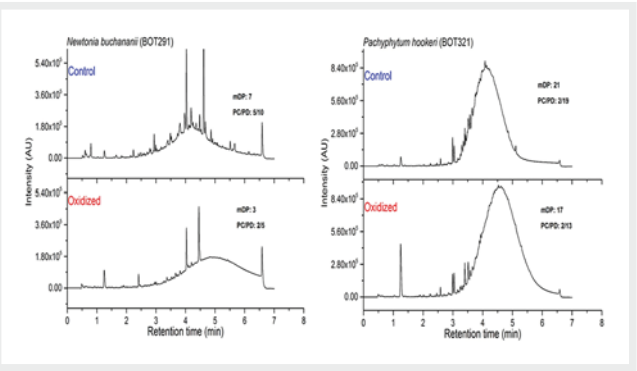
DOI 10.1055/s-0039-3399908

Proanthocyanidins (PAs) are the secondary metabolites that are responsible for many positive effects related to ruminant nutrition and health. We were looking for naturally modified PAs that cannot be found in plants. To develop the production methods of such rare PAs we used a unique method where these are modified by oxidation in alkaline conditions. It included the collection of 300 plant samples from the Botanical Garden of the University of Turku, Finland. From the preliminary screening, 130 plant samples have procyanidin and prodelphinidin rich oligomers and polymers, which has polyphenol oxidase enzyme activity. The PAs in these plant samples were extracted and analyzed as such by UPLC connected to Waters XEVO TQ triple quadrupole mass spectrometer. Then the plant extracts were oxidized by an oxidation test using a pH 10 buffer. These samples were further studied by QExactive Orbitrap mass spectrometers, in order to characterize the modified PA structures. In addition, the proanthocyanidin oligomers and polymers in oxidized and non-oxidized samples were quantified by Multiple Reaction Monitoring (MRM) methods by UPLC-MS/MS. These results suggested that prodelphinidin (PD) rich oligomers have higher chances to modify the PA structure in the alkaline condition whereas procyanidin (PC) rich oligomers and polymers have less chances to modify the structure. However, the PC helps to stable the tannin structure. On the other hand, if the PD is absent in the sample then the structure is more stable, or no oxidation occurs.



▶**Fig. 1** UPLC-DAD profiles of modified proanthocyanidins.

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P-190 Molecular docking as a tool to design new royleanone derivatives for colon cancer therapy based on PKC-δ modulation

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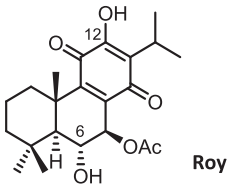
DOI 10.1055/s-0039-3399909

Cancer is one of the most common causes of death worldwide. Many efforts have been made to develop more effective chemotherapeutic drugs. Protein Kinases (PKCs) have wide ranging effects in crucial processes of tumorigenesis and metastatic dissemination. PKC-δ behaves as tumor suppressor in colon cancer, one of the most prevalent cancers and a leading cause of cancer mortality worldwide [1].

Plectranthus genus (Lamiaceae) is a good source of cytotoxic compounds. Abietane diterpenes isolated from this genus have revealed promising anticancer activity [2] particularly as PKC modulators [3]. 7α-Acetoxy-6β-hydroxyroyleanone (Roy, ▶**Fig. 1**) obtained from *P. grandidentatus* exhibits biological properties including antitumoral activity[4]. Thus, Roy is a potent lead molecule for future drug development with the ultimate purpose of modulate the PKCs. A small library of Roy derivatives prepared by us, was tested on a yeast-based screening assay, and showed promising ability to activate PKC isoforms. Furthermore, the patented 6β-benzoyloxy-12-*O*-benzoylroyleanone (Roy-Bz) was obtained by semi-synthesis of Roy. This derivative has shown selective modulation in PKC-δ and consequently potently inhibits the proliferation of colon cancer cells [3].

In this work, several theoretical derivatives of Roy were designed through modification of the C-12 and C-6 hydroxyl groups. In addition, molecular

docking simulations were carried out against the 3D structure of PKC-δ. These results allowed the identification of the most promising compounds for PKC-δ modulation. Based on the docking achievements new hemi-synthetic abietanes are currently in study for structure-activity relationships and new drug development based on the royleanone scaffold.



▶**Fig. 1** 7α-Acetoxy-6β-hydroxyroyleanone (Roy) isolated from *P. grandidentatus*.

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P-191 Monomeric and oligomeric pigments in red wines: contribution to color and differences in compositions between wine varieties and vintages

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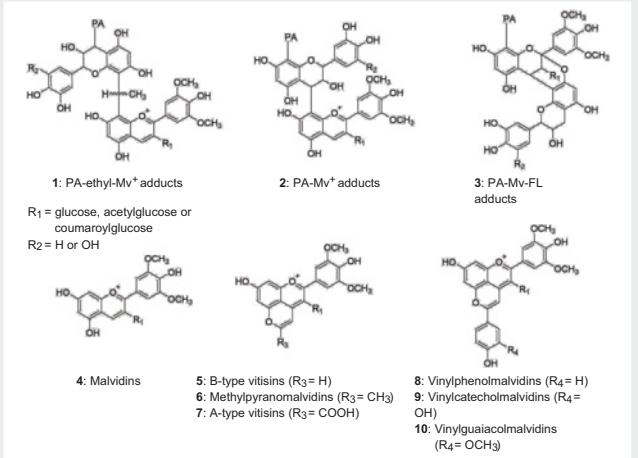
Recently, a group-specific UPLC–MS/MS method was published, which can separately detect three different types of proanthocyanidin-malvidin adducts (**1**–**3** in Fig. 1) directly from any wine sample [1]. Furthermore, the method can detect small, medium-sized and large oligomeric adducts separately, and it can detect some of the most typical monomeric anthocyanin adducts as well (**4**–**10** in Fig. 1). Since, we have analyzed with the method a large sample set consisting of 317 unique red wines to study how the quantitative and qualitative variation in the red wine pigments explains the variation in the color intensity, and how the adduct profiles of different types of wine varieties and vintages compare to one another.

With a statistical model, we could explain 85% of the variation in the color intensity with only three compound groups (**1, 2** and **7**) out of the original 10 (Fig. 1). Information about the relative sizes of the oligomeric adducts **1** and **2** were needed to achieve the high explanatory power of the model. In the young wines, the differences in the adduct composition between the wine varieties were found to be mostly quantitative. The evolutionary trends were partly similar between oligomeric adducts **1**–**3**: the average sizes of the adducts increased towards older wines in all three groups, but the quantitative evolution of the concentrations differed between the groups. In conclusion, the group-specific methodology enabled the precise conclusions about the basic functions and properties of the oligomeric adducts in red wines.



▶**Fig. 1** The malvidin-based compound groups, which the UPLC–MS/MS method utilized in this study is able to detect. The abbreviation PA stands for proanthocyanidin.

References [1] Laitila JE, Suvanto J, Salminen JP. Liquid chromatography–tandem mass spectrometry reveals detailed chromatographic fingerprints of anthocyanins and anthocyanin adducts in red wine. Food Chem 2019; 294: 138–151

P-193 Natural deep eutectic solvents: an eco-friendly alternative for the extraction of naphthoquinones from *Alkanna tinctoria* roots

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DOI 10.1055/s-0039-3399911

Plant derived natural products have an important role in pharmaceutical, cosmeceutical and food supplements industries. Their increasing demand is leading to the over-exploitation of plant resources and to the over-consumption of organic solvents, widely recognized to be of great environmental concern. For this reason, the design of green extraction methods of natural products is currently a key research topic. [1-3]

In order to address this issue an eco-friendly natural deep eutectic solvents (NADESs) were used instead of organic solvents for the extraction of naphthoquinones from the roots of *Alkanna tinctoria* Tausch, a Greek endemic plant from Boraginaceae family. More than twenty NADESs containing choline chloride-, and betaine-, as hydrogen bond acceptor combined with different hydrogen bond donors (sugars, organics acids) were investigated for their potential to extract this particular class of compounds. As a result of the statistical evaluation, the most relevant deep eutectic mixture with the highest extraction efficiency was found to be composed of lactic acid and sucrose (LaS). To maximize the extraction yield, a further optimization step of extraction parameters was followed, including the optimum LaS ratio (w/w), the solid-to-solvent ratio, temperature, water content as well as the application of solid phase extraction techniques for the recovery of the naphtoquinone fraction from the NADES extraction solution. Our results revealed the optimized LaS mixture as a valid green alternative for the extraction of naphthoquinones from *Alkanna tinctoria*. In fact, by using a ratio of lactic acid-sucrose 5:1 (w/w), a solid-to-solvent ratio of 60:1 (w/v) at 40°C, with 30% of water content, targeted compounds were successfully recovered.

Acknowledgments This work has been financed by the EU H2020-ITN-MICROMETABOLITE project (Grant No 721635)

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P-194 New amide, chalcone, and flavonoid derivatives from the leaf and fruit extracts of *Melodorum siamensis*

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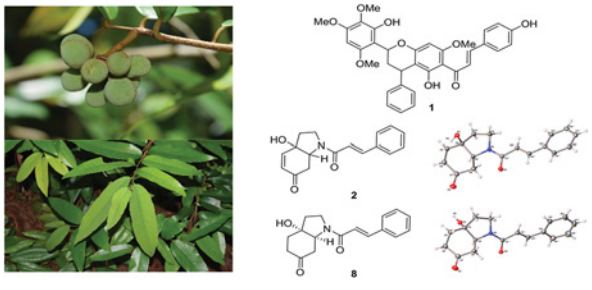
DOI 10.1055/s-0039-3399912

Melodorum siamensis, locally known as Nom-Maeo, is a scandent shrub native to Thailand belonging to the Annonaceae family. Previous phytochemical investigations described chalcones, flavonoids, and amides from the leaves of *M. siamensis*. Some chalcone and amide derivatives showed interesting biological activities, including, cytotoxicity and anti-inflammatory activities. In our study, four new chalcones, a new flavonoid, a new amide, and 19 known compounds were acquired from the fruits and the leaves of *M. siamensis* using chromatographic techniques. Scelemic mixtures of chalcone dimer (**1**) and the amide derivative (**2**) were obtained and the enantiomers were separated by semipreparative chiral HPLC. The structures were established by 1D and 2D NMR, and MS data analyses. The absolute configuration of **1** was determined by experimental and calculated ECD data, while the configurations of **2** and **8** were determined by X-ray diffraction analysis. A biosynthetic pathway for **1, 2**, and **8** was also proposed. One compound showed significant activity against NF-κB with an IC_{50} value of 9 μM in MIN-6 cells, the other compounds were inactive.



▶**Fig. 1**

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P-195 New flavonoids from the flowers of *Coreopsis lanceolata* and their pharmacological activities

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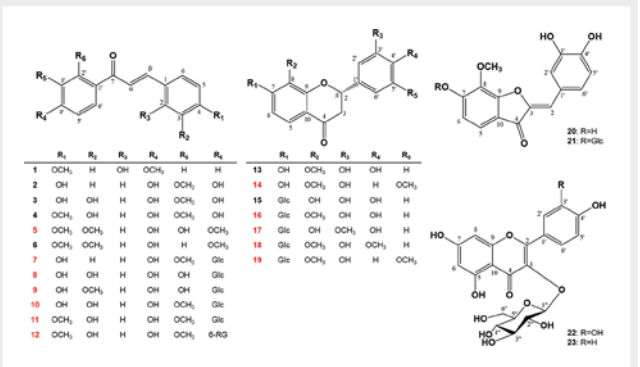
The *Coreopsis* (Asteraceae) genus is one of the most common phanerogams that blossom in summer. *C. lanceolata* is a herbaceous perennial plant originated in America, South Africa and Eastern Asia, reported to have antioxidant [1], antiallergic, antibacterial, antileukemic [2], and nematicidal [3] effects. Despite several reported pharmacological activities of *C. lanceolata*, there is only one paper to isolate flavonoids in *C. lanceolata* flowers [1]. Therefore, the present study focused on the isolation and identification of active materials from the flowers of this plant, as well as evalutaion of various pharmacological activities.
Seven new chalcones, lanceolein A–G (**5** and **7-12**), five new flavanones, flavanolanceotin A–E (**14** and **16-19**), five known chalcones, two known flavanones, two known aurones, and two known flavonols were isolated from the methanolic extract of *C. lanceolata* flowers. All isolated flavonoids showed radical scavenging activities in DPPH and ABTS radical experiments and inhibited NO production in LPS-stimulated RAW264.7 macrophages. Some chalcones prevented the growth of human colon cancer cells via the induction of cellular cytotoxicity and apoptosis. Some flavanones protected against oxidative stress in PC-12 neuronal, Caco-2 colon epithelial, and RAW 264.7 macrophage cells. Moreover, two aurones and two flavonols recovered the damage of the pancreatic islets caused by alloxan treatment in zebrafish (*Danio rerio*) larvae. These results suggested that *C. lanceolata* flowers can be used as multi-metabolic diseases agents.



▶**Fig. 1** Chemical structures of flavonoids **1**–**23** from the flowers of *Coreopsis lanceolata*. Glc: O-*β*-D-glucopyranosyl, 6-RG: O-*α*-L-rhamnopyranosyl-(1→6)-O-*β*-D-glucopyranosyl.

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P-196 New isocoumarins and flavonoids from *Citrus grandis* Osbeck fruits and their antidiabetic activities

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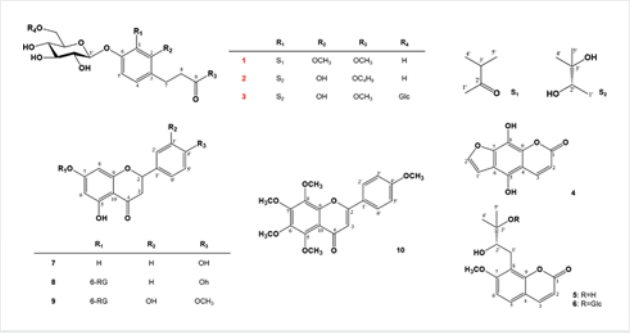
DOI 10.1055/s-0039-3399914

Citrus grandis Osbeck. (Rutaceae) is an evergreen shrub only distributed in Jeju island, Korea. Traditionally, *C. grandis* Osbeck, which is called as “Dangyuja”, “Dangyuji”, or “Daeyuji”, has been used as a folk remedy for fever. The fruits of *C. grandis* were used at various ceremonies in Jeju island, Korea [1]. In recent studies, it was reported that *C. grandis* had two times higher organic acid content than *C. junos* and four times more vitamin C content than lemons [2, 3]. However, there are few studies on the other chemical constituents and biological activities of *C. grandis* fruits. Therefore, the present study focused on the isolation and identification of active compounds from the fruits of this plant, as well as evalutaion of protective effects of the isolated compounds on pancreatic islet damaged by alloxan in a zebrafish (*Danio rerio*) larvae model. *C. grandis* fruits were extracted with aqueous methanol, and the concentrared extract was partitioned into EtOAc, *n*-BuOH, and H_2O fractions. The repeated normal and reverse-phase column chromatographies on EtOAc and *n*-BuOH fractions led to the isolation of three new isocoumarins, citragrandin A–C (**1**–**3**), three known coumarins (**4**–**6**), and four known flavonoids (**7**–**10**). The compounds were identified based in classical spectroscopic methods such as NMR and HRMS. Some of the isolated compounds showed radical scavenging activities in DPPH and ABTS radical experiments and some exhibitied protective effects on pancreatic islet damaged by alloxan in zebrafish (*Danio rerio*) larvae.



▶**Fig. 1** Isolated compounds from the fruits of *Citrus grandis* Osbeck. Glc: O-*β*-D-glucopyranosyl, 6-RG: O-*α*-L-rhamnopyranosyl-(1→6)-O-*β*-D-glucopyranosyl.

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P-197 New sesquiterpenoids from an endophytic fungus *Paraconiothyrium brasiliense* ECN258

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DOI 10.1055/s-0039-3399915

Paraconiothyrium brasiliense was a plant endophyte distributing worldwide and has been ever isolated from a wide range of host plants such as *Coffea arabica* in Brazil, *Ginko biloba* in Canada, and *Prunus* spp. in South Africa. *Paraconiothyrium brasiliense* ECN258 that we used in this study was isolated from a stem of *Cinnamomum camphora* in Japan. To discover new natural resources for beneficial compounds, we have obtainined endophyte strains more than 400 from various plants in Japan and continue to investigate the metabolites produced by them [1, 2]. In this study, eleven new sesquiterpenoids (**1**–**11**) were isolated from cultures of *Paraconiothyrium brasiliense* ECN258 as well as five known sesquiterpenoids (**12**–**16**). The structures of new compounds were elucidated by analyzing IR, MS, NMR, and ECD spectroscopic data. Compounds **1**–**7** are eremophilane sesquiterpenoids, whereas compounds **8**–**10** have new or rare carbon frameworks that are probably biosynthesized by rearrangement of eremophilanes. Furthermore, paraconiothyrins K (**11**) is a C_{18} compound containing an eremophilane within the backbone. Compounds **1**–**5** and **8**–**16** were evaluated for their effects on some nuclear receptors including RXRα, PPARγ, PPARδ, RARα, and LXRα by means of luciferase reporter gene assay for each receptor. Among test compounds, compound **9** exhibited an inhibitory effect on LXRα at the concentration of 50 μM.

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P-198 Norisoprenoids from *Asystasia gangetica* (L) T. Anderson var. *micrantha* (Acanthaceae)

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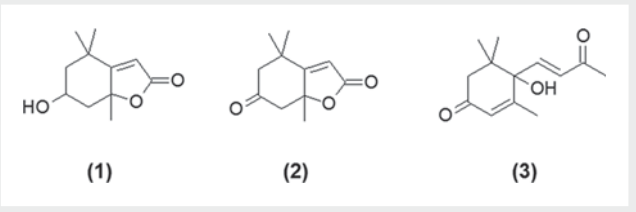
DOI 10.1055/s-0039-3399916

Asystasia gangetica (L) T. Anderson var. micrantha *(Acanthaceae),* commonly known as ‘Chinese violet’ or ‘rumput Israel’, is a straggling herb usually found among short grasses and along the roadsides. Various parts of this plant are also used as remedy for hypertension [1], asthma [2] and to treat treat skin allergies [3]. Present study was designed to isolate and elucidate bioactive compounds from this plant. Methanolic extract of the plant leaves was fractionated by using PLC. Selected fractions were subjected to preparative HPLC and recycling HPLC for further purification. The structures of isolated compounds were characterized by using spectroscopic method including NMR, IR, UV and mass spectral data as well as comparison with literature. Three phytochemical constituents namely loliolide **(1),** dedydrololiolide **(2)** and and megastigmane **(3)** have been purified (▶**Fig 1**). All of the compounds were identified for the first time from genus *Asystasia*.



▶ **Fig. 1** Isolated compounds from *Asystasia gangetica*.

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P-199 Oleoside type secoiridoids from the flowers of *Syringa dilatata* and their potential as anti-inflammatory agents

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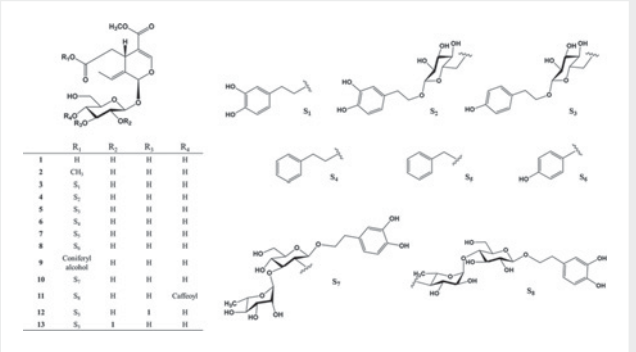
DOI 10.1055/s-0039-3399917

Syringa plants, which have good fragrance and beautiful appearance, are cultivated worldwide. Also, this plants have been reported to contain various secondary metabolites such as iridoids, lignans, and phenylethanoids. In particular, *yringa* plants contain a large amount of iridoids that have pharmacological activities. Due to the effectuation of the Nagoya Protocol, securing of biological resources has become very important. For this reason we have been interested in *S. dilatata*, only a Korea native species of its genus. No study has been reported for components and activities of *S. dilatata* flower. Therefore, the present study focused on the isolation and identification of active compounds from this plant, as well as examination of the anti-inflammatory effect of the isolated compounds.

Dried flowers of *S. dilatata* were extracted with aqueous MeOH, and the concentrated extract was partitioned into EtOAc, *n*-BuOH, and H_2O fractions. Repeated SiO_2, ODS, and Sephadex LH-20 column chromatographies on *n*-BuOH fraction, led to isolation of eight new iridoids (**6-13**) and nine known ones (**1-5, 14-17**). From the results of spectroscopic data the chemical structures were identified without ambiuity. All compounds were isolated for the first time from *S. dilatata* flowers in this study.

Some compounds showed significantly NO inhibition in LPS-stimulated RAW 264.7 macrophages. In addition, quantitative analysis of iridoids in the *S. dilatata* flowers was conducted through HPLC experiments. These results supported that the iridoids isolated from the *S. dilatata* flowers and its extract are potential as anti-inflammatory agents.



▶ **Fig. 1**

P-200 Abstract see SL YRW-04

Abstract see on page 1396

P-201 PEGASUS: an analytical chemometrics platform for the discovery of bioactive natural compounds.

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DOI 10.1055/s-0039-3399919

Natural products have been a source of medicinal agents for thousands of years and the remarkable number of modern drugs that have been derived, are predominantly based on traditional medicine. However, isolation of natural products has always been tedious, as herbal extracts are complicated systems, containing hundreds of chemical entities. It is time and solvent consuming and very often the procedure ends with the re-isolation of known metabolites. Complementary, there has been a pressing need to involve approaches that accelerate the measurement of metabolite levels directly from plant extracts through the implementation of HTS technologies and chemometrics.

The goal of PEGASUS is the establishment of a validated analytical chemometrics platform through the elaboration of contemporary chromatographic and spectroscopic techniques (FCPC, HPTLC, LC-HRMS/MS, NMR) along with sophisticated statistical algorithms for the rapid and effective identification of bioactive compounds, prior to their isolation.

The last five years, the development of an *Het*eroCovariance *A*pproach (*HetCa* algorithms), has been applied at our department for the discovery of bioactive metabolites for several biological targets such as free radicals, enzymes and cancer cell lines [1-4]. *HetCa* is a MATLAB toolbox based on Statistical Total Correlation Spectroscopy (STOCSY) and Statistical Heterospectroscopy (SHY) methodologies. Specifically, *HetCa* has been applied for the discovery of antioxidant, anti-tyrosinase, anti-acetylcholinesterase, anti-hyaluronidase and cytotoxic against several cancer cell lines natural compounds from plant species belonging to the Greek flora.

PEGASUS incorporates for the first time chromatographic, spectroscopic techniques and various bioactivity results along with advanced chemometrics for the rapid identification of bioactive compounds.

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inhibitors from ***Paeonia parnassica*** extracts through a HeteroCovariance Approach. J Ethnopharmacol 2018; doi.org/10.1016/j.jep.2018.10.008

P-202 Pharmacokinetic investigation of hydrogenated natural curcumin metabolites

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DOI 10.1055/s-0039-3399920

Curcuminoids are the main bioactive components of the well-known Asian spice and traditional medicine, turmeric [1]. These compounds have poor bioavailability, *in vivo* they undergo a rapid metabolism, and bioreduced derivatives are among their main *in vivo* metabolites. These metabolites were also reported to exert various bioactivities *in vitro* and *in vivo* [2]. They can be synthetized by batch hydrogenation, however this method has low selectivity [3].

Our objective was to develop a procedure to selectively obtain curcuminoids with different levels of saturation by using a continuous flow hydrogenation reactor. Furthermore, we aimed to evaluate pharmacokinetic properties of the prepared metabolites, such as kinetic solubility, chemical and metabolic stability, and their gastrointestinal and blood-brain barrier permeability with parallel artificial membrane permeability assay (PAMPA).

To this end, we achieved high selectivity in preparing hexahydrocurcumin. The pharmacokinetic tests showed that the reduced metabolites have dramatically increased water solubility and chemical stability as compared to that of curcumin. The metabolic rate by human liver microsomes followed the tetrahydrocurcumin > curcumin ~ hexahydrocurcumin ≫ octahydrocurcumin order. Curcumin showed negligible BBB and GI penetration, similarly to hexahydrocurcumin, tetrahydrocurcumin was however largely superior in this regard. Our results show better bioavailability and pharmacokinetic properties for some hydrogenated curcuminoid derivatives as compared to those of curcumin.

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P-203 Pharmacokinetics of epicatechin and γ-valerolactone in rat plasma and tissues after oral treatment with the *Crataegus* special extract WS® 1442

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DOI 10.1055/s-0039-3399921

WS® 1442 is an ethanolic (45 % w/w) dry extract from hawthorn leaves with flowers (DER 4 - 6.6: 1), registered as a traditional herbal medicinal product to support cardiovascular function. This standardised extract contains 17 to 20 % oligomeric B-type procyanidins (OPCs), which are thought to be primarily responsible for its beneficial cardio- and vasoprotective effects.

However, it is known that higher oligomers do not reach the blood circulation as such, but are metabolised to smaller entities by intestinal bacteria. Therefore, we have investigated whether procyanidin monomers, like epicatechin or further degraded substances, like γ-valerolactone do reach target tissue and examined their pharmacokinetics.

For the quantification of both metabolites, rats were treated twice daily with 300 mg/kg WS® 1442 for three consecutive days. Plasma and several tissues (heart, liver, kidney and aorta) were collected at 8 different time points (t = 0, 1, 2, 4, 5, 6, 8 and 24 h) after the last treatment. All samples were homogenised in a suitable buffer, phase II conjugates were hydrolysed enzymatically and the aglycons were extracted with ethyl acetate. After removal of the organic solvent, the residue was taken up and, submitted to HPLC-MS/MS analysis.

Epicatechin and γ-valerolactone were detectable in plasma and all examined tissues and showed distinct concentration-time profiles.

Particularly interesting is the quite high concentration of epicatechin in aortic vessels (227.1 ± 123.2 ng/g), even higher than in plasma (159.8 ± 93.7 ng/mL), which may in consequence give evidence for the vasoprotective effects of WS® 1442.

P-204 Phenolic constituents isolated from the flower of *Carex kobomugi*

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DOI 10.1055/s-0039-3399922

Cyperaceae is the family of flowering plants known as sage. This family comprises about 5000 species defined in about 70 genera. These species are widely distributed throughout the world and are found growing in low to high land. The genus *Carex* consists of approximately 1500 species distributed throughout the world. Previously, many chemical constituents have been isolated from the family Cyperaceae including sesquiterpenes [1] (genus *Cyperus*), stilbenes [2] (genus *Carex*), diterpenes [3] (genus *Killinga*), and acetophenones [4] (genus *Schoenus*). *Carex kobomugi, a* perennial plant, widely grows in the coastal zones in Japan. Some stilbene oligomers have been reported from this plant root. These oligomers are of interest due to the inconsistent isomers that result from the resveratrol framework. While *C. kobomugi* have been shown to possess several interesting metabolites, no reports have yet been published that describe the chemical constituents found in flowers. Herein, we report the isolation and the structural elucidation of several compounds. Fresh flowers of *C. kobomugi* (3.1 kg) were extracted with methanol (3 × 5L) at room temperature. The combined extracts were concentrated under reduced pressure to provide 182 g of a dark extract, part of which (172 g) was suspended in H_2O and successively extracted with EtOAc and *n*-BuOH. The EtOAc soluble fraction was chromatographed/purified on silica gel, Sephadex LH-20, Sep-pak C_{18} cartridge, and preparative HPLC. All compounds were isolated from the EtOAc fraction by repeated column chromatography as noted above. We report the isolation of five new phenolics along with seven known compounds.

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P-205 Phenolic content and antioxidant potential evaluation of unexploited byproducts from *Vitis vinifera* L.

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Vitis vinifera L. (Vitaceae family) is a popular woody perennial plant commonly used in the winemaking. Unfortunately, viticulture generates huge amounts of residues (i.e. leaves, twigs, seeds, skins), which can cause environmental issues when discarded in open areas. It is therefore extremely important to evaluate the potential reuse of these matrices, to reduce the amount of waste at landfill sites [1,2]. Among the main grapevine by-products, no data on the green pruning residues (GPRs) produced by the annual spring pruning have been reported. Thus, the aim of this work was the analysis and valorization of GPRs from 16 red and white *V. vinifera* cultivars from Piedmont (Italy), by comparing the results with those obtained from the leaves of the same cultivars. An experimental design optimization was performed and the obtained hydroalcoholic extracts where then quali- and quantitatively analyzed by HPLC-PDA-MS/MS, with similar polyphenolic profiles for both GPRs and leaves. Quercetin 3-*O*-glucuronide, caftaric acid and quercetin 3-*O*-glucoside were the main components of the investigated matrices, although in variable proportions. Considering the prevalent presence of polyphenols in the analyzed extracts their antioxidant activity was evaluated with colorimetric *in-vitro* assays, off-line combined with HPLC-PDA analysis. In this way the contribution of each compound to the antioxidant activity, in terms of radical scavenging abilities, was determined.

These findings could suggest GPRs as a potential source of natural compounds, and therefore promote their use in the food field, as food supplements, which could increase their economic value together with a positive effect on the environment.

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P-207 Phytochemical and biological investigations on potentially antimicrobial and anticancer Guinean plant species

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Cancer and microbial infections are global public health problems, and therapeutic management remains a challenge, especially in developing countries. In Guinea, medicinal plants are still widely used for the management of these diseases. The purpose of this study was to deepen the phytochemical knowledge and investigate the therapeutical potential of selected plants, through the purification of secondary metabolites, and the evaluation of their antimicrobial and anticancer activity.

Twelve medicinal plants selected following an ethnobotanical survey carried out in Guinea, were subjected to a preliminary biological screening. Promising activities against *Candida albicans, Staphylococcus aureus* and *Plasmodium falciparum* were obtained for extracts from *T. albida, Landolphia heudelotii, Swartzia madagascariensis, Tetracera* sp. and *Hannoa* sp. ($IC_{50} \leq 10$ µg/ml). *Combretum paniculatum* and *Pavetta crassipes* were the most cytotoxic ($CC_{50} \leq 10$µg/ml). Based on these results, *Terminalia albida* was selected as one of the most promising plants and was subjected to bioassay-guided fractionation. All extracts and fractions were evaluated for their antimicrobial, antiprotozoal and cytotoxic activities. Liquid chromatography (Flash chromatography and/or semi-preparative HPLC-DAD-MS) and NMR spectroscopy were used for isolation of compounds and their identification.

The bioguided fractionation of *T. albida* root led to the isolation of 13 compounds, among which sericoside, arjunglucopyranoside, seric acid, 3-hydroxy-4, 4-dimethyldihydro- (2-13C)-furan-2-one, 28-*O*-β-glucopyranosyl-arjunic Acid, 3,4,3′-tri-*O*-methoxy-ellagic-acid and 2α,3β,21β,23-tetrahydroxyolean-12-en-28 oic acid.

These results may validate at least in part the traditional use of the selected plant species. Further research, aiming for the purification and bioactivity assessment of additional compounds from *T. albida* and other promising plant extracts is ongoing.

P-210 Phytochemical investigation of *Cyperus malaccensis* subsp. *monophyllus*

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The genus Cyperus belongs to the family Cyperaceae with approximately 600 species distributed in China, Indonesia, Japan, and Vietnam. Some of *Cyperus* plants are used in folk medicine. Rhizomes of *Cyperus rotundus*, for example, have been used in traditional Chinese medicine and Japanease 'Kampo' medicine as estrogenic and anti-inflammatory agents for the treatment of menstrual disorders, stomachache, and bowel disorders [1]. Coumarins [2], quinones [3], benzofurans [4], aurones [5], sesquiterpenes [6], and flavonoids [7] have been reported from *Cyperus* species. Herein, we describe our exploration of the chemical diversity within *Cyperus malaccensis* subsp. *monophyllus*, and report the isolation and the structural elucidation of several compounds.

Air-dried and powdered aerial part (3.5 kg) of *C. malaccensis* subsp. *monophyllus* were extracted with Acetone (5 x 22 L) at room temperature to yield the crude extract (49 g). A part of extract was separated by repeated column chromatography (CC), including silica gel CC (Si-CC), Sephadex LH-20 CC, octadecylsilanized silica gel (ODS) CC, and preparative HPLC to yield 11 known compounds. Their structures were elucidated through extensive spectroscopic methods, including 1D and 2D NMR experiments and MS spectra analyses.

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P-211 Phytochemical investigation of the fruits of *Paliurus spina-christi* Mill.

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Paliurus spina-christi Mill. (Rhamnaceae) is a much-branched, deciduous, thorny shrub with green fruits that become brown during maturation. The shrub is found in dry slopes in Mediterranean, Southwest and Central Asia and North America. Its common name is 'Christ's thorn' because it is said that its spiny branches were used to make the crown of thorns which had been placed on Christ's head before his crucifixion. *P. ramosissimus* Lour., *P. orientalis* Franch. and *P. hemsleyanus* Rehder are well known species of the Eastern Asia [1]. Traditionally, *P. spina-christi* is used for its diuretic and anti-hypercholesterolemic properties, as well as a palliative against gastrointestinal pain [2-5]. Thus, it was decided to investigate the shrub's fruits phytochemically.

Powdered mature fruits were exhaustively extracted with cyclohexane, dichloromethane, methanol and water. In order to remove fats and sugars, the dried methanolic extract was subjected to liquid-liquid extraction with c-Hex., EtOAc and water. The EtOAc fraction was initially chromatographed on Si-gel using Vacuum Liquid Chromatography (NP-VLC), followed by prepTLC and NP-HPLC for the final separations and isolation of the pure secondary metabolites. The water phase was treated using adsorption resin technology and the obtained fractions were further chromatographed using Reversed Phase-Solid Phase Extraction (RP-SPE) and RP-HPLC. Structure determination was based on NMR spectroscopy and LC-MS.

So far, there have been isolated and identified 12 secondary metabolites, 1 flavonoid-diglycoside, 2 phenolics, 2 triterpenoids, 1 lignan, 3 phytosterols and 3 cyclopeptide alkaloids (CPAs). One of the CPAs, metabolite 8, has been recognized as a new natural product.

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P-213 Abstract see SL YRW-01

Abstract see on page 1395

P-214 Protein precipitation activity of refined proanthocyanidin fractions

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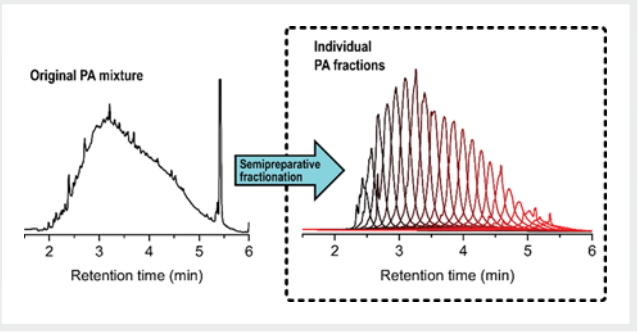
Proanthocyanidins (PAs) are plant specialized metabolites, which consist of flavan-3-ol type subunits. PAs possess a variety of bioactivities such as anthelmintic activity against ruminant gastrointestinal nematodes [1, 2]. PAs are found in multiple legume species and thereby they could form a solution to the global problem of ruminant gastrointestinal nematodes [3]. In biological activity studies, PAs have been usually utilized either as plant material or crude extracts and fractions. Usually PA composition of a single plant species can be extremely complicated consisting of even hundreds of compounds with different flavan-3-ol combinations. Thus, the structural complexity of PAs makes the structure–bioactivity linkage examinations challenging.

The aim of this study was to utilize chromatographically refined PA fractions produced from 11 Finnish plant species [4] to study their protein precipitation capacity (PPC) – the most probable mode of action of PAs against nematodes – via novel well plate reader method. The fractionation is illustrated in ▶**Fig 1**. The refined fractions were analysed for their subunit content, polymer size and galloylation by UPLC-MS/MS [5]. The novel well-plate reader method was a turbidimetry based measurement of the insoluble complex formed during the PA–protein interaction. This approach revealed how the PPC of a complex PA mixture is divided in different retention time areas and how the PA subunit content, polymer size and galloylation affect the PPC.



▶**Fig. 1** UPLC-DAD (λ = 280 nm) chromatogram comparison of pre-purified PA fraction (original PA mixture) and refined PA fractions (individual PA fractions).

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P-215 Qualitative and quantitative determination of flavonoids in different organs of *Atriplex nitens* Schkuhr and evaluation of anti-hyaluronidase activity

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Atriplex nitens Schkuhr (*Amaranthaceae*), also known as *A. sagittata* is a common weed resistant to harsh environmental conditions, such as salinity or drought. The plant is also valued as fodder due to high biomass production. Its phytochemical composition is poorly known, except for the presence of proteins, fats and ecdysteroids in seeds [1, 2]. Related species are reported to have high anti-oxidant properties and are rich sources of phenolics, including flavonoids. These were detected eg. in *A. hortensis, A.* lentiformis, or *A. littoralis* [3].

Flavonoids are known for various biological activities that determine their medicinal use. One of these is anti-hyaluronidase activity [4]. Therefore, the aim of our study was qualitative and quantitative determination of flavonoids in crude extracts from different organs of *A. nitens*, together with estimation of their effect on this enzyme.

Flavonoids have been analysed by LC-ESI-MS/MS. They were detected only in the aboveground parts of the plant. Nine compounds were quantified. Among them, kaempferol-3-glucoside-7-rhamnoside was predominant in stems and leaves (159.00 ± 6.93 µg/mg and 219.67 ± 13.65 µg/mg, respectively) whereas isoquercetin was most abundant in flowers (259.33 ± 6.11 µg/mg). The highest anti-hyaluronidase activity *in vitro,* determined by turbidimetric method [5], was observed for the flower extract (IC_{50} = 84.67 µg/mL). This corresponds to the high amount of total flavonoids in this plant part (624.09 µg/mg), including the predominant isoquercetin.

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P-216 Quantitative determination of phenolics compounds in *Origanum vulgare* extract by high-performance liquid chromatography coupled with tandem mass spectrometric detection

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Phenolic compounds in 80% MeOH extract of oregano (*Origanum vulgare* L.) were analysed by reversed phase HPLC, followed by tandem mass spectrometric detection.

For the first time thirty nine phenolics, corresponding to flavonoids, flavonoid glycosides, benzoic acids, cinnamic acids, coumarins and lignans were quantitified in oregano extract.

Quinic acid, naringenin, luteolin-7-*O*-glucoside, caffeic acid, apigenin, luteolin, chlorogenic acid, vitexin, protocateuchic acid, kaempferol, rutin, syringic acid, quercetin-3-*O*-glucoside, quercitrin, gentisic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, chrysoeriol, vanillic acid, apigenin-7-*O*-glucoside, hyperoside, kaempferol-3-*O*-glucoside, aesculetin, ferulic acid, apiin, myricetin, epigallocatechin gallate, epicatechin, baicalin, catechin, baicalein, synapic acid, matareisinol, 3,4 dimethoxycinnamic acid, scopoletin, secoisolariciresinol, amentoflavon, umbelliferon and *o*-coumaric acid were were present at levels above the reliable quantification limit.

Quinic acid, naringenin, luteoline-7-*O*-glucoside, caffeic acid, apigenin and luteolin were the most abundant phenols detected in oregano extract.

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P-217 Rapid and effective recovery of oleanolic and maslinic acid from olive products using centrifugal partition extraction

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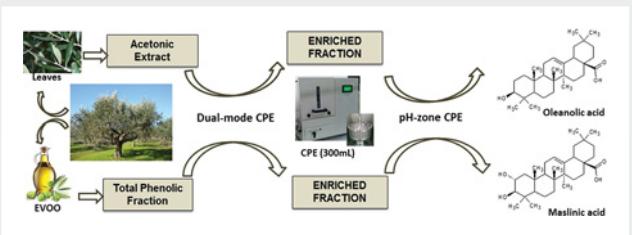
DOI 10.1055/s-0039-3399931

Oleanolic acid (OA) and maslinic acids (MA) represent the main triterpens of Olea europaea and are characterized as high-value natural compounds with important biological activities. Due to the increased scientific interest of these compounds, large amounts of pure metabolites are urgently needed. In the present study, a separation method based on Centrifugal Partition Extractor (CPE) was developed, resulting in rapid and effective isolation of oleanolic and maslinic acid in pure form. The preliminary study of our group revealed that the unpolar extract of olive leaves (OLUE) contains an important amount of OA while total phenolic fraction (TPF) obtained from the liquid-liquid extraction of EVOO is a good source of MA. Both OLUE and TPF extracts have been initially fractionated by dual-mode CPE using the biphasic system n-hexane-ethylacetate-ethanol-water 3:2:3:2 (v/v/v/v). The analysis was run in ascending mode and lasted 40 min for both extracts resulting in the fast recovery of the enriched fractions. The second step of the process included the treatment of the enriched fractions using pH-zone refining CPE method. The biphasic system consisted of n-hexane-ethylacetate-ethanol-water 8:2:5:5 (v/v/v/v) while TFA and TEA were added in the stationary and mobile phase respectively. Following this two-step procedure, 640 mg of OA and 550mg of MA were recovered with greater than 95% purity, as determined by



▶ **Fig. 1**

HPTLC and ^{1}H-NMR analysis. This methodology can be efficiently used for the large-scale purification of oleanolic and maslinic acid from olive products as well as for the isolation of acidic terpenoids from natural sources.

P-218 Rearranged clerodanes from the stems of *Tinospora baenzigeri*

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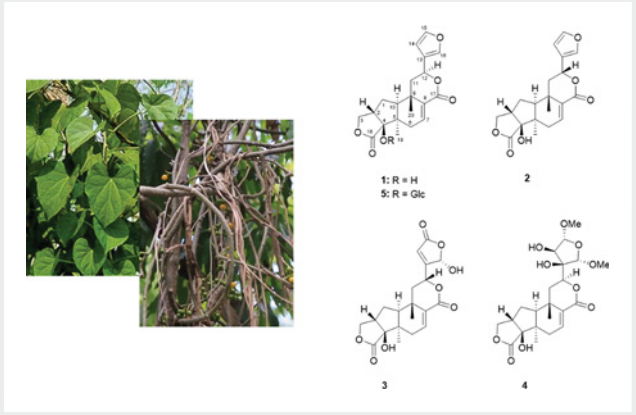
The genus *Tinospora* (Family Menispermacae) comprises approximately 30 species, widely distributed throughout Asia, Africa, Australia, and the Pacific. *T. crispa* and *T. baenzigeri* are well known in Thailand and used as herbal remedy, particularly *T. crispa*. By contrast, *T. baenzigeri* has not been extensively studied. Previous investigations of the plant resulted in the isolation and characterization of a number of rearranged clerodane furanoditerpenoids and their glucosides. Their claimed antimalarial activity against *Plasmodium falciparum* could not be confirmed, and other biological activities have not been examined yet. In order to discover natural compounds with diverse structure and biological activity from Thai medicinal plants and to extend the study on *T. baenzigeri*, the plant specimens were examined for their phytochemical constituents. Four new rearranged clerodane-type diterpenoids (**1**–**4**), a new glucoside (**5**), and six known compounds (**6**–**11**) were obtained from the EtOAc crude extract of *T. baenzigeri* stem. The structures of the new compounds were elucidated by interpreting their spectroscopic data, particularly 1D and 2D NMR. Single-crystal X-ray diffraction analysis was subsequently performed to confirm the structures and relative configurations of compounds **1**–**4**. These compounds are rare examples of rearranged clerodanes, particularly compound **4** possessing a fully oxidized tetrahydrofuranyl ring. The isolated compounds were assayed for their protective effect against *N*-acetyl-*p*-aminophenol (APAP)-induced HepG2 cell damage. Compounds **8**, **9**, and **11** showed hepatoprotective activity at 10 M with 17.0, 19.2 and 39.0% inhibition, respectively, whereas rearranged clerodanes (**1**-**3** and **5**) were weakly active.



▶ **Fig. 1**

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P-219 Roxydienone, a novel cyclohexenone from *Trachyspermum roxburghianum*

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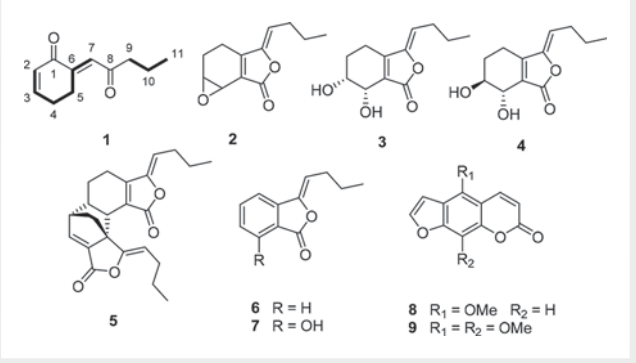
Roxydienone (**1**), a novel cyclohexanone and 8 known compounds (**2**–**9**) were isolated from the seeds of *T. roxburghianum*. The structures of these compounds were established on the basis of extensive analysis of its spectroscopic data. Compounds **1**, **2** and **7**–**9** showed cytotoxicity against NCI-H187 cell line while compounds **1** and **9** showed cytotoxicity against KB cell line.



▶ **Fig. 1**

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P-220 Secondary metabolites and their anti-inflammatory activity from the root of *Machilus zuihoensis*

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Most of the time, inflammation is a short and protective response. However, prolonged inflammation might lead to tissue destruction, organ dysfunction, or even death. Continuous inflammation may cause chronic human diseases, such as cancer, diabetes, arthritis, Alzheimer's disease, and so on. There are many side effects on current medication of inflammation, which limit the use of anti-inflammatory drugs. Therefore developing new anti-inflammatory drugs is an urgent issue. Approximately sixty indigenous Formosan Lauraceous plants have been screened for anti-inflammatory activity. Among them, the methanolic extract of the root of *Machilus zuihoensis* Hayata showed potent anti-inflammatory effect. This study aims to investigate the isolates of the root of *M. zuihoensis* and to evaluate their anti-inflammatory activity.

The methanolic extract of the root of *M. zuihoensis* was partitioned into the ethyl acetate layer and water layer. Bioassay-guided fractionation of the active ethyl acetate layer of the root of *M. zuihoensis* led to the isolation of three new compounds, including one new butanolide, machizuihonol (**1**), two new lignans, machilolinol (**2**) and marphenol L (**3**), together with nineteen known compounds, including four benzenoids, two butanolides, one coumarin, one flavonoid, six lignans, three sesquiterpenoids and two steroids. The structures of these compounds were elucidated by spectral analysis. Anti-inflammatory activity of some isolates have been evaluated, and the isolation of the active subfractions of this plant is still in progress.

P-222 Terpenoids from the whole plant of *Vaccinium emarginatum* Hayata and their anti-inflammatory activity

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The metabolite characterization of the *Vaccinium* species have been well documented as rich sources of naturally occurring phytochemicals, which are represented by iridoids, steroids, terpenoids, coumarins, lignans, flavonoids, and anthocyanins, with promising bioactivities including antioxidative, anti-inflammatory, antinociceptive, antimicrobial, antidiabetic, and anticancer activity [1]. *Vaccinium emarginatum* Hayata (Ericaceae) is an endemic epiphytic shrub found at an altitude from 1,500 to 3,000 m in Taiwan. It has been used in folk medicine for treating cystitis, dysentery, enteritis, rheumatoid arthritis, and urinary tract infections in Taiwan. Its root nodules have been used as a Yang Tonic. In preliminary assessment, the *in vitro* anti-inflammatory activity against NO production of the *V. emarginatum* whole plant methanolic extract was mainly associated with its EtOAc-soluble fraction. The phytochemical investigation of this fraction afforded 2 new iridoids (**1**, **2**), 22 pentacyclic triterpenoids including 2 new coumaroyl triterpenes (**3**, **4**), and 4 steroids. Compounds **3** and 3*β*-*O*-*trans*-feruloyl-19*α*-hydroxyurs-12-en-28-oic acid (**5**) showed potent anti-inflammatory activity against NO production in LPS-induced RAW 264.7 cells (▶**Tab. 1**).

▶**Tab. 1** Anti-inflammatory activity of compounds 1 and 3 against NO production in LPS-induced RAW 264.7 cells.

Compound	IC50 (μM)
3	12.36 ± 0.86
5	24.21 ± 1.28
Dexamethasone	1.52 ± 0.05

a Values are expressed as mean ± SD of three independent experiments.
b Dexamethasone was used as a positive control.

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P-223 The alkamide constituents of *Zanthoxylum rhetsa* (Roxb.) DC. fruits

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Alkamides, a class of lipophilic protoalkaloids or pseudoalkaloids, have been drawing attention of scientists due to their diverse pharmacological activities [1, 2]. *Zanthoxylum* species, frequently used in Asian cuisine e.g. Sichuan pepper for their tingling sensation [3], have been well documented to be a rich source of alkamides. *Zanthoxylum rhetsa* (Roxb.) DC., Rutaceae, originating in subtropical regions, a typical spice and "the soul" of Northern Vietnamese culinary art, is traditionally employed for treatment of inflammatory-related ailments especially toothache [4]. The target of this study was to investigate the alkamide compositions of this spice. From the dichloromethane fraction of the *Z. rhetsa* fruit extract, fourteen alkamides could be isolated. Two compounds, rhetsamide A and rhetsamide B, were identified as C12 fatty acid isobutylhydroxyamides. The remaining twelve compounds which comprise rhetsamide C-J, zanthoamides G-I and the known sanshool derivatives hydroxy-ζ-sanshool, hydroxy-γ-sanshool, hydroxy-γ-isosanshool, the major compounds of the investigated extract, were determined as C14 fatty acid isobutylhydroxyamides. To our knowledge, none of these compounds except for hydroxy-γ-isosanshool was previously described for this species and this is the first report of the ten new compounds, rhetsamide A-J. These results revealed that the fruits of this plant are a valuable source of new natural products as well as promising biological principles, which are worthy to be further investigated and exploited.

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P-224 The antioxidant activity of *Caesalpinia sappan* heartwood extracted with different ethanol concentrations

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The effect of different ethanol concentrations, used for the extraction of *C. sappan* wood, on the antioxidant capacity was investigated. The heartwood of *C. sappan* was extracted with 95%, 75%, and 50% ethanol and subsequently partitioned using deionized water, dichloromethane, and ethyl acetate. The fractions were subjected to different antioxidant assays based on free radical scavenging activity (DPPH, $ABTS^{\bullet+}$ and *in-vitro* lipid peroxidation). Additionally, the content of total phenols and flavonoids was determined. The present study provides evidence that the total phenolic content and antioxidant capacity of *C. sappan* heartwood extracts were significantly dependent on

the ethanol concentration used for extraction. However, the subsequent ethyl acetate fraction showed the overall strongest anti-oxidative activity in the assays. The crude extract prepared by using 75% ethanol contains a considerable amount of total phenols (741.8 mg GAE/g *C. sappan* heartwood), with low IC_{50} values (2.4 µg/mL in DPPH assay, 2.98 µg/mL in ABTS assay, and 14.2 µg/mL in lipid peroxidation assay). Interestingly, the ethyl acetate fraction obtained from the 75% ethanol crude extract showed the highest scavenging activity (2.24 µg/mL for DPPH radical assay, 2.38 µg/mL for ABTS assay). Accordingly, total phenolic content and antioxidant capacity were closely correlated (R^2 <0.83 and *p*-value <.0.01).

P-225 The chemical constituents from the stems of *Dalbergia stipulacea*

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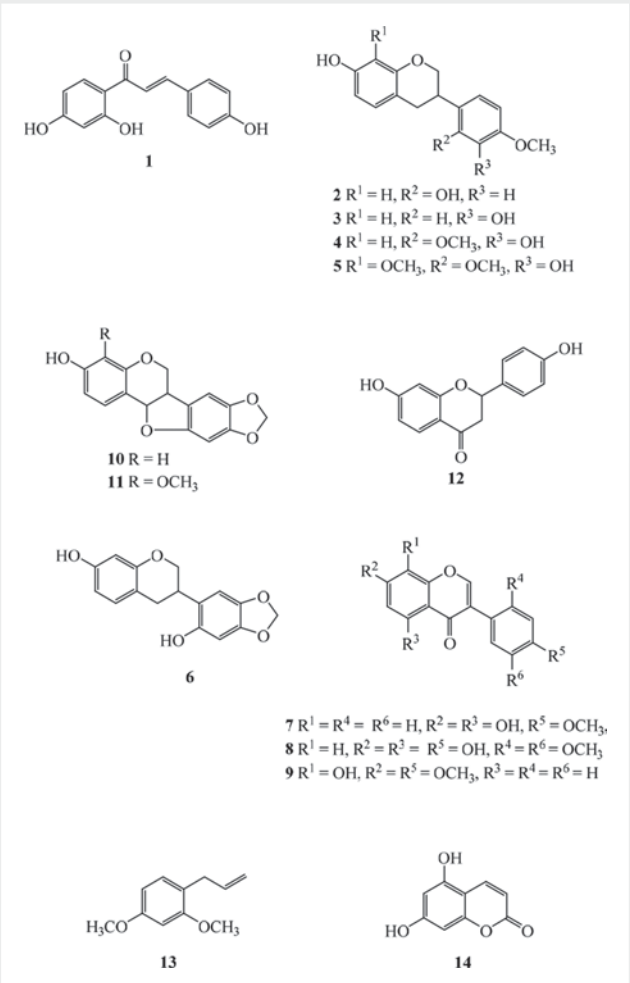
DOI 10.1055/s-0039-3399935



▶**Fig. 1**

Fourteen known compounds, including a chalcone(**1**), eight isoflavonoids(**2-9**), two pterocarpans(**10-11**), a flavonoid(**12**), a arylpropene(**13**) and a coumarin(**14**), were isolated from the stems of *Dalbergia stipulacea*[1][2]. The chemical structures of all isolated compounds were elucidated by using spectroscopic methods including 1D and 2D-NMR, MS and IR data. Antifungal activity against *Pythium insidiosum* is under investigated.

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P-226 Three new secoiridoid derivatives from *Olea europaea* flowers: isolation, identification and biological evaluation

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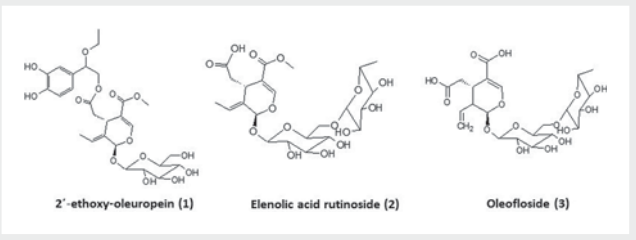
Olea europaea L. is historically one of the most important trees of the Mediterranean countries. The scientific literature contains plenary of phytochemical and biological works regarding its fruits, leaves and olive oil in contrast to olive flowers were only few data are available. The main goal of the current work was to elaborate a phytochemical study of olive flowers for the isolation and identification of their major secondary metabolites.

The analysis was contacted in hydroalkoholic extract and was achieved in two steps. Initially the extract was fractionated by step-gradient CPC and subsequently the chosen fractions were further purified using preparative HPLC. The result of our study was the isolation of nine natural compounds, two of which are flavonoids (rutin, quercetin-3-O-sophoroside), two triterpenic acids (oleanolic acid, maslinic acid) and five secoiridoid derivatives (oleuropein, 2′-hydroxy-oleuropein, 2′-ethoxy-oleuropein, elenolic acid rutinoside and oleofloside). It is important to say that the compounds 2′-ethoxy-oleuropein (1), elenolic acid rutinoside (2) and oleofloside (3) are described for the first time and thus are considered new natural products.

The structural elucidation of isolated compounds was confirmed by NMR and HRMS/MS spectroscopy. In parallel, the hydroalcoholic extract and the isolated secoiridoid derivatives were evaluated for their ability to inhibit tyrosinase, elastase and collagenase activity. The results showed that both extract and the isolated compounds exhibit remarkable biological activity in inhibiting the collagenase enzyme with 2'-ethyloxy-oleuropein and 2'-hydroxy-oleuropein being the most active. In contrast, there was no activity observed, as far as the inhibition of the enzymes tyrosinase and elastase.



▶**Fig. 1** The structures of the new secoiridoid derivatives isolated from olive flowers.

P-227 Transferring petrochemical methods to pharmacognosy: a novel screening system for oligomeric proanthocyanidins using Kendrick mass defect

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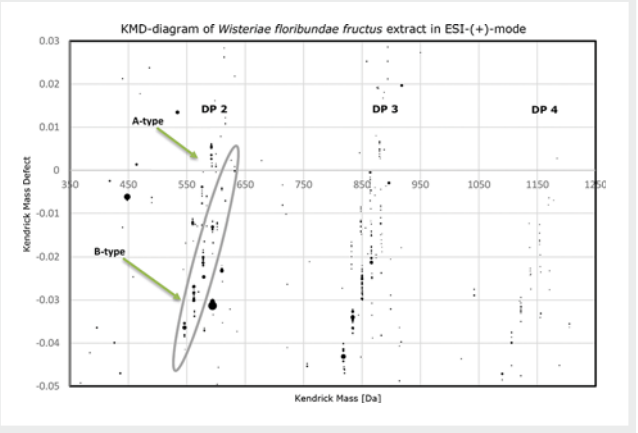
DOI 10.1055/s-0039-3399937

Proanthocyanidins (PACs) are complex polyphenolic substances composed of flavan-3-ol building blocks, existing in numerous plants. They occur as oligomeric and polymeric PACs, depending on the degree of polymerisation (DP). Whereas analytical methodology for PACs with low DP is well established, analysis of higher oligomers and polymers is still challenging, since structural diversity of the polymer strands is increasing with higher DP. Typical modifications are related to building blocks with different hydroxylation pattern or to additional substitution e.g., galloylation. Furthermore, two types of interflavan linkages - B-type (4→6 or 4→8) or A-type (4→8/2→7) - are possible [1]. In this study, Kendrick Mass Defect (KMD), which is a common methodology to characterize hydrocarbon polymers in petrochemistry since the 1980's, is transferred to PACs, based on LC-(+)ESI-qTOF-MS data obtained from extracts of model herbal material *Tiliae flos, Crataegi folium cum flore* and *Wisteriae floribundae fructus*. Catechin, the predominant flavan-3-ol unit in PACs, serves as a reference unit, instead of commonly used CH_2-blocks. With the help of a KMD-diagram extracts can be screened quickly for detailed PAC-composition. This method provides information regarding the different DP clusters within the extract as well as detailed composition of each DP cluster, especially in respect of building blocks. KMD-diagram of *Tiliae flos* revealed oligomeric A-type and B-type PACs composed of (epi)catechin units up to DP 8, which was affirmed by MS^2 fragments. In *Wisteriae floribundae fructus* PACs consisting of three different types of building blocks - (epi)afzelechin, (epi)catechin and (epi) gallocatechin - can be detected.



▶ **Fig. 1**

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P-228 Unlocking secrets of congolese biodiversity: the chemistry of *Croton haumanianus* (Euphorbiaceae) J. Léonard

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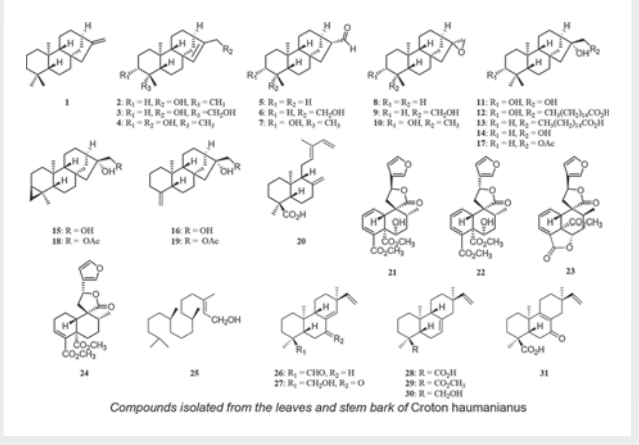
DOI 10.1055/s-0039-3399938

Croton haumanianus J. Léonard (Euphorbiaceae) is a tree occurring mainly in Congo, Democratic Republic of Congo, Equatorial Guinea and Southern Cameroon. *Croton* species have produced two marketed drugs, plaunotol from *C. stellatopilosus*, used to treat ulcers, and Sangre De Drago (or Dragon's blood) from *C. lechleri*, which is used in the treatment of inflammation and dysentery. The stem bark of *C. haumanianus* has been reported to contain two diterpenoids, crotocorylifuran, a clerodane-type, and crotohaumanoxide, a crotofolane-type, and lupeol, a triterpenoid.[1] In the Democratic Republic of Congo, a decoction of the stem bark is used in the treatment of gonorrhea and rheumatism, and as an arrow poison.[2] This research is part of the ongoing project at the University of Surrey investigating the Chemistry of African *Croton* species. Thirty-two diterpenoids, two sesquiterpenoids, two triterpenoids, and a steroid, syringaldehyde, octyl-*trans*-ferulate and phaeophytin-b have been isolated from the CH_2Cl_2 and MeOH extracts of the leaves and stem bark of *C. haumanianus*. The structures of these compounds, shown below, were determined using NMR spectroscopy, mass spectrometry, and infrared and circular dichroism spectroscopy. Eighteen previously unreported diterpenoids, twelve *ent*-kauranes, **4-5, 7, 9-10, 12-13**, & **15-19**, and three *ent*-clerodanes, **21-23**, were obtained along with three *ent*-isopimaranes, **26, 29**, & **31**, and the known compounds, **1-3, 6, 8, 11, 14, 20, 24, 25, 27-28** & **30**. Compounds **11, 12, 13, 15** showed selective activity against three of the NCI 60 cancer cell lines, the colon (HCT-116), the melanoma (M14) and the renal (786-0) cancer cell lines at a concentration of 10^{-5} M.



▶ **Fig. 1** *Compounds isolated from the leaves and stem bark of* Croton haumanianus

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P-229 Unprecedented isoflavanoid-neoflavonoid analogues isolated from Brazilian red propolis

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Propolis is a natural resinous product collected by honeybees from the buds and exudates of various plant sources, which has been used as a traditional remedy in folk medicine for centuries. Among Brazilian propolis varieties, Brazilian red propolis (BRP) is isoflavonoid-rich. Bees producing red propolis feed upon *Dalbergia ecastophyllum* [1–4] In this investigation, BRP was extracted with 80% EtOH shaken at 70°C for 30 min. After extraction, the mixture was centrifuged. The supernatant was evaporated to dryness to give a EtOH extract of Brazilian red propolis (EERP) [1,2]

The EERP extract was fractionated by normal phase silica gel column eluted with a linear gradient of $CHCl_3$/EtOAc. Fraction 5 was separated by C_{18} RPCC and eluted with a linear gradient of MeOH in H_2O. Fractions collected were evaporated to dryness, resulting in thirty fractions. After successive fractionations, eight structurally novel isoflavanoid-neoflavonoid analogues were obtained and identified by extensive analysis of spectroscopic data. The cytotoxic and anti-inflammatory activities of isoflavanoid-neoflavonoid analogues are under evaluation.

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Poster Session 2

P-230 A biochemometric approach for the identification of anti-inflammatory coumarines from *Peucedanum ostruthium*

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Peucedanum ostruthium (L.) Koch has a longstanding history as herbal remedy in the Alpine region of Austria, where the rhizomes (= Radix Imperatoriae) are traditionally used to treat disorders of the gastro-intestinal and respiratory tract as well as the cardiovascular system [1]. A recent ethnopharmacological study on 71 Austrian traditional herbal drugs revealed a distinct anti-inflammatory activity by Radix Imperatoriae [2]. In the present work, the aim was to unravel the constituents responsible for the observed anti-inflammatory activity of a dichloromethane-methanol extract prior to isolation. Therefore, a recently developed biochemometric approach named ELINA (Eliciting Nature's Activities) [3] was applied. Here, the objective was to simplify the crude extract by generating micro-fractions with quantitative variances of constituents over several consecutive fractions. This was achieved via an optimized high-performance countercurrent chromatographic (HPCCC) fractionation. After this single fractionation step, ^{1}H-NMR data and bioactivity data from an *in vitro* assay on the cell adhesion molecules E-selectin and VCAM-1 were collected for all 31 micro-fractions. In parallel to a quantitative variance of ^{1}H-NMR signals over consecutive fractions, bioactivity patterns relating to this variation were obtained. To reveal chemical features crucial for the observed activities, statistical heterocovariance analyses (HetCA) [4] were performed. In addition, LC-MS-CAD data were used to facilitate the identification of the bioactive constituents. As a result, we identified two coumarins (Imperatorin and Ostruthol) and one chromone (Peucenin) responsible for the anti-inflammatory activities. The applied HetCA approach enables an early identification and dereplication of even minor bioactives prior to any isolation without wasting resources.

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P-231 A -enriched fraction of *Bryophyllum pinnatum* inhibits human myometrial contractility *in vitro*

Authors Santos S[1,2], Haslinger C[1], Kalic K[1], Faleschini MT[2], Mennet M[3], Potterat O[2], von Mandach U[1], Hamburger M[2], Simões-Wüst AP[1]

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Preparations containing *Bryophyllum pinnatum* are used in the treatment of premature labour, but the compounds responsible for the activity are not known [1]. We investigated the effects on human myometrial contractility *in vitro* of *B. pinnatum* juice (BPJ), a flavonoid-enriched fraction (FEF), the corresponding flavonoid aglycon mixture (A-Mix), and a bufadienolide-enriched fraction (BEF) [2].

Myometrial biopsies were collected during elective Caesarean sections. Strips of tissue were mounted in an organ bath system (myograph), and spontaneous contractions were recorded. Aliquots of stock solutions of FEF, A-Mix, BEF and BPJ were repeatedly added at intervals of 20 min, and strength (AUC and amplitude) and frequency of contractions were recorded for each interval. After a washout period, vitality of strips was observed. Cell viability assays were performed with two human myometrial cell lines.

Repeated addition of FEF, A-Mix, BEF or BPJ led to a progressive decrease of contraction strength in a concentration-dependent manner (in all cases,

p<0.05). BEF was the most active test substance, since 1 µg/mL BEF lowered AUC to 40.1 ± 11.8% of initial, whereas 150 µg/mL FEF, 6.2 µg/mL A-Mix, and 10 µg/mL BPJ (i.e. 1%) were required to achieve comparable inhibition. Test substances decreased myometrial cell viability only at concentrations higher than those used in the myometrium experiments.

All test substances inhibited myometrial contractility without affecting viability. Given the concentrations of flavonoids in FEF and BPJ, and of bufadienolides in BEF and BPJ, it appears that bufadienolides are mainly responsible for the relaxant effect.

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P-232 A multi-compound extract of *Rhodiola rosea* reduced acute mild stress-induced corticosterone release in mice more than salidroside alone

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DOI 10.1055/s-0039-3399942

We have previously shown that a multi-component hydroethanolic root extract (HRE) of *Rhodiola rosea* (patented process Phytostandard®; EPS Rhodiole, PiLeJe Laboratoire) induces a decrease in corticosterone secretion and a faster return to normal plasma corticosterone levels than the vehicle in mice subjected to acute mild stress. In this study, we determined the phytochemical profile of the HRE (HPTLC, HPLC, LC-MS and MS/MS) and compared its effect to one of its active compound, salidroside. After daily gavage with the HRE (240 mg of dry material/kg) or salidroside (equivalence: 3.6 mg/kg) during 2 weeks, mice were subjected or not to acute mild stress (open-field + elevated plus-maze tests). Corticosterone was measured in plasma collected from the mandibular vein at 0, 30, 60 and 90 min after stress initiation. Salidroside, rosavin, various flavonoids, monoterpene glycosides and several phenylpropanoid derivatives were identified in the HRE. Mice supplemented with the HRE presented a dampening of corticosterone secretion compared to salidroside-fed mice at the different time points. No modulation of stress-responsive gene expression was observed in our conditions. These results highlight the advantage of extracts with a composition close to that of the plant *totum* (i.e. all the active compounds of the plant) compared to a single compound since mice supplemented with the HRE presented a decrease in stress reactivity in an acute mild stress situation which could be associated to a better resilience to stress.

P-233 A new semi-synthetic sapogenol derivative inducing regulated necrosis

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DOI 10.1055/s-0039-3399943

Since saponin's antitumor potency is relatively weak, researchers focus on their semi-synthetic modification to obtain structures with higher potencies. With the same motivation, we prepared a cytotoxic sapogenol derivative (AG-08) from cycloastragenol. Our preliminary studies revealed that AG-08 induced primarily necrotic cell death along with autophagic inhibition. Furthermore, immunoblotting experiments demonstrated that AG-08 promoted cleavage of various proteins such as ATGs, p62, and PARP-1.

The main goals of this study were to verify anti-cancer potential of AG-08 and investigate its possible mechanism(s). Firstly, cytotoxicity of AG-08 on 13 human cell lines was examined and IC_{50} values were found to be between 2.3 ±0.035 to 10.18±0.509 µM with no selectivity towards cancer and normal cells. Since AG-08 induced cleavage of various proteins, we investigated the effect of several protease inhibitors on the cell death. Inhibitors of calpain-1, general caspases, cathepsin B/L/S, and caspase 8 partially alleviated cell death with 1.52, 1.55, 1.38 and 1.24-fold, respectively, whereas cathepsin D/E inhibitors did not cause any significant change. Our results from autophagy and lysosomal proteases studies prompted us to evaluate the integrity of lysosomes in live cells. Our lysotracker staining data suggested that AG-08 may be an inducer of lysosomal membrane permeabilization. In conclusion, AG-08 is a potent cytotoxic agent possessing necrotic cell death and autophagy inhibitory properties. Further studies are in progress to clarify complete mechanism of AG-08.

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P-234 The ripening stage of ginseng (*Panax ginseng* C.A. Meyer) berry influences its phytochemical compositions and their bioavailability

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Ginseng root is the most popular medicinal herb in the world, whereas the berry has been considered a useless by-product. However, the presence of large amounts of bioactive compounds such as saponins and flavonoids in ginseng (Panax ginseng) berry suggests its potential as a functional resource for the food and medical industries. In this study, we evaluated the pharmaceutical value of ginseng berry by analyzing its antioxidant activity and anti-melanogenic effects and analyzed the variation in bioactivities of ginseng berry during the ripening process. As a result, the highest level of antioxidant and anti-melanogenic activities was observed in fully ripe berry extracts (Go-S3). Phytochemical screening showed that antioxidant and whitening activities mediated by polyphenolic compounds such as delphinidin-3-glucoside (1.1 ± 0.1 to 541.8 ± 5.4 µg/g of extract) and cyanidin-3-glucoside (0.5 ± 0.2 to 462.8 ± 11.7 µg/g of extract) increased during the ripening process. Furthermore, results obtained by quantitative real-time PCR, western blot, tyrosinase inhibition assay and molecular docking analysis suggested that Go-S3 probably inhibits tyrosinase activity by interacting with copper-coordinating histidines and second shell residues of tyrosinase, resulting in the reduction of melanin production in α-MSH-stimulated B16F10 cells. Taken together, these finding suggest the potential of ginseng berry as a resource for functional applications in the phytocosmetic industries and demonstrate that fruit ripening stages will be useful for further studies on ginseng berry for its applications in pharmaceutical industries.

P-235 Activity of three South African plants on phytopathogenic bacteria and fungi of tomatoes and chemical profiling of the extracts

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Soil-borne pathogens in the fungal and bacterial kingdom cause destruction of large fields of agricultural crops, with great economic loss worldwide. The aim of this study was to evaluate three South African plant extracts for activity against phytopathogenic bacteria, fungi and to profile their chemical constituents. A serial microplate dilution method was used to determine the minimum inhibitory concentration (MIC) of *Leonotis leonurus, Clausena anisata* and *Lantana rugosa* crude extracts and fractions prepared using solvent-solvent fractionation. Antibacterial activity was determined against *Xanthomonas perforans, Xanthomonas vesicatoria, Ralstonia solanacearum, Ralstonia pseudosolanacearum* and *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) while antifungal activity was evaluated against *Fusarium oxysporum* f. sp. *lycopersici*. Gas chromatography-mass spectrometry (GC-MS) was used for profiling the phytochemicals from the acetone and dichloromethane/methanol extracts. *L. leonurus* and *L. rugosa* extracts had moderate to weak activity with MIC values ranging between 0.156 to 2.5 mg/mL. *L. rugosa* fractions were more active with MIC values ranging between 0.078 to 0.156 mg/mL against most phytopathogenic bacteria, followed by dichloromethane and ethyl acetate fractions of *L. leonurus*. All extracts and fractions were inactive against *Fusarium spp.* except the water extract of *L. leonurus* with MIC of 0.156 mg/mL. The selectivity index indicated that *L. rugosa* was unsafe. The GC-MS analysis of *L. leonurus* dichloromethane/methanol extract revealed a high quantity (32%) of 9,12-octadecadienoyl chloride, (Z,Z)-. This was followed by 9-octadecenamide, (Z)- with 20%, and this compound is known to have antibacterial activity. This study supports further investigation of *L. leonurus* for management of pest diseases of tomatoes.

P-238 Age-related chemical profiles and osteoblast differentiation activity of 18-month old *Cissus quadrangularis* L.

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Herbal product uniformity is a big challenge as constituents are affected by many factors. To produce high quality herbal products, the consistent supply of high quality herbal raw materials is necessary which will result in the development of the standard cultivation and harvesting process that controls the phytochemical-related factors for each plant [1]. This study defined those factors needed to achieve this outcome. *Cissus quadrangularis* L. (CQ) stems have been used in many Asian traditional preparations—e.g., for healing of bone fracture in Ayurvedic medicine [2]. The CQ harvesting time is highly varied from 6 months to 3 years and no study has indicated the cultivating factors that affect quality. Therefore, we study the activity-related chemical profiles of CQ stems during age change. CQ was cultivated separately in six rows in the same area in Prachinburi province, Thailand. The new-growth stems were labelled every month until 18 months old before collecting separately. TLC fingerprints of CQ hexane extracts—a bone-formation active extract [3,4]—were established with two-mobile phase systems. The stem chemical profiles showed differences between young shoots (<3 months), mature (4–15 months) and old woody stems (16–18 months); moreover, differences between the stems, leaves and inflorescence were observed. The CQ extracts stimulated activities on osteoblast differentiation *in vitro* [3] and the activity-chemical profile relation was analyzed by multivariate analysis. The results will be discussed for further development of the standard production process of consistent high-quality CQ raw materials.

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P-239 AGEs (advanced glycation end products): an advanced method for an advanced problem

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Advanced glycation end products (AGEs) are heterogeneous, sugar-derived protein modifications (▶**Fig.1**) that have received attention in various research fields, such as food preparation, the normal aging process and pathophysiological conditions in the human body. [1]
Several *in vitro* experiments are reported to identify AGEs inhibitors, for instance: measuring glycation of bovine serum albumin, determination of fructosamine adducts and measuring α-dicarbonyl compounds. [2] However, the latter methods suffer from various drawbacks like a complex formation between test products and the protein can result in quenching of the fluorescence signal and misinterpretation of the data. Therefore, it was of utmost importance to develop and validate a reliable and universal method to circumvent these problems. Subsequently, several classes of natural products will be tested in the new method for their AGEs inhibition properties.
In this research, the precise quantification of *N*(6)-carboxymethyllysine (CML), one of the most abundant AGEs [3], was achieved through HILIC Ultra-Performance Liquid Chromatography coupled to a Xevo G2-XS QTof MS system, using d_2-CML as an internal standard. CML was generated by incubation of GK-peptide and ribose. The sample preparation included a hydrolysis step with hydrochloric acid and a clean up step with P3 (Protein Precipitation Plate) cartridges. The method was validated following the FDA guidelines of Bioanalytical Method Validation [4] regarding linearity, LLOQ, precision and quality controls.
Overall, advanced glycation is a leading cause for the progression and pathogenesis of many chronic diseases (e.g. diabetes mellitus). Specifically, this validated method can contribute to the unambiguous discovery of new potential anti-AGEs agents.

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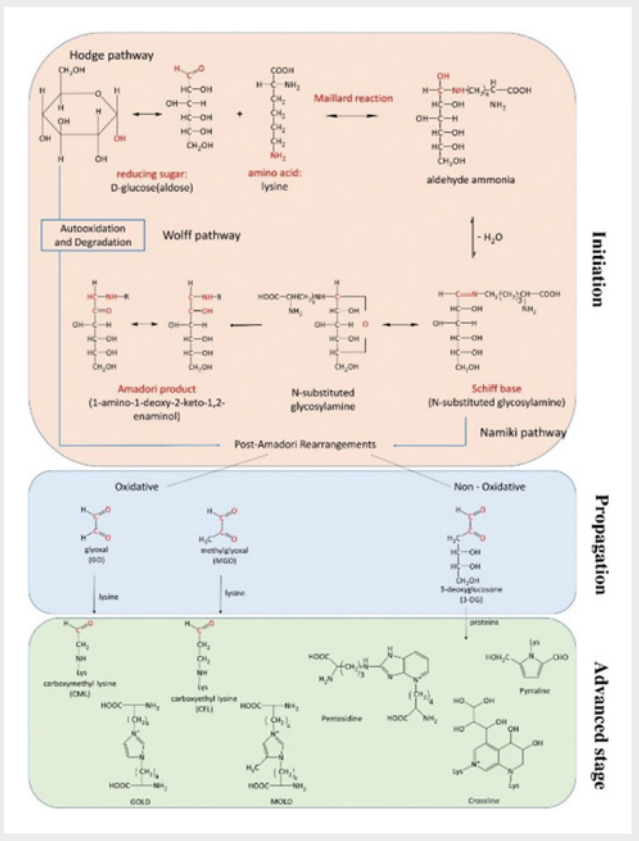

▶ **Fig. 1** General scheme of AGEs formation.

P-240 An integrated strategy to characterize new anti-inflammatory lead compounds derived from Filipendula ulmaria (meadowsweet)

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Since many NSAID show severe gastrointestinal side effects, there is a high need for new drugs. [1] An integrated strategy, based on natural pro-drugs and their metabolites, is developed to characterize new anti-inflammatory lead compounds derived from *Filipendula ulmaria*.

Firstly, the phytochemical composition was explored in a comprehensive manner using UPLC-DAD-HRMS. Next to salicylates like salicylic acid, an active *in vivo* metabolite, a rich diversity of phenolic constituents was identified. [2,3]

Extensive biotransformation after oral intake can be expected. This urges the need for identification and activity profiling of the intestinal and hepatic metabolites. Therefore, an *in vitro* gastrointestinal biotransformation model (GIBM) was used, which mimics the gastric, intestinal and colonic phase, including fecal fermentation. [4] These metabolites subsequently undergo *in vitro* hepatic biotransformation via human S9 fraction. Salicin, an inactive pro-drug in *F. ulmaria*, was tested as proof-of-concept: a time-dependent hydrolysis of salicin to saligenin in the GIBM was observed. Saligenin was further biotransformed to salicylic acid by the S9 fraction.

As a last step, biotransformed samples will be evaluated with *in vitro* anti-inflammatory assays, focusing on cyclooxygenase (COX). [5] A non-biotransformed extract of *F. ulmaria* (20 µg/mL) was tested in a cell-based COX-2 gene expression assay. No inhibition was observed. However, the same extract (50 µg/mL) showed an inhibition of 76.6 ± 2.4% on the COX-1 and 43.7 ± 9.9% on the COX-2 enzyme.

The combination of these analytical and *in vitro* methods, results in an innovative concept to identify and further develop new leads for drugs.

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P-241 Analysis of bioactive constituents of Hardy Kiwi (*Actinidia arguta*)

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Hardy kiwi (*Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq.) is an indigenous fruit tree in Korea. Compared to other kiwifruits, Hardy kiwifruit is smaller, however, sweeter and is able to be eaten without peeling. Hardy kiwifruits have high contents of vitamin C, lutein, phenolic compounds and several minerals. In order to use other parts of Hardy kiwi, for example, roots or leaves as well as fruits, antioxidant effects and pancreatic lipase inhibitory effects in each part were measured. Moreover, bioactive constituents that are highly related to both effects were investigated. Antioxidant effects and pancreatic lipase inhibitory effects were higher in roots and leaves than in fruits. In those parts, contents of phenolic compound, flavonoid and triterpenoid were five to ten times higher than in fruits. As bioactive constituents, quinic acid derivatives conjugated with phenolic acids were isolated from Hardy kiwifruits. In addition, triterpene derivatives and catechin derivatives were isolated from leaves and roots, respectively. Among these components of Hardy kiwi, quinic acid derivatives and triterpene derivatives effectively suppressed NF-kB activation stimulated by lipopolysaccharide in RAW 264.7 macrophage cells. Conclusively, large amount of sugars and organic acids were contained in Hardy kiwifruits in order to reserve nutrients. On the other hand, a variety of bioactive constituents for growth and defense to disease and stress were included in roots and leaves.

P-243 Antiadhesive activity of the phtalide Sedanenolide from *Apium graveolens* fruits (Celery) against uropathogenic *E. coli*

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Celery fruits (Apium graveolens L.) are used traditionally for treatment of uncomplicated urinary tract infections. The experiments aimed to identify potential antiadhesive compounds from celery extract to provide strategies for improved standardization of the herbal material. Decoction, hydroalcoholic and acetone extracts were prepared from celery fruits. Bioassay-guided fractionation was performed by Fast Centrifugal Partition Chromatography and preparative HPLC, followed by LC-MS and NMR investigations. The antiadhesive activity of extracts, fractions and purified compounds was assessed by flow cytometry, evaluating the adhesion of fluorescent-labelled uropathogenic bacteria (UPEC NU14) to T24 bladder cells; mannose served as positive control. Influence of the extract on gene expression of selected adhesins and fitness genes was monitored by qPCR. Concentration-dependent antiadhesive activity was found for the hydroalcoholic and even more for the acetone extract AE (IC_{50} 85 µg/mL). Bioassay-guided fractionation revealed the presence of the phthalides senkyunolide (inactive) and sedanenolide (IC_{50} 790 µM), which is assessed as the main antiadhesive compound, and accounts for 4.0% in the water extract, for 18% in the hydroethanolic extract and for 71% in AE. Additionally, a similar phthalide, Z-ligustilide, was shown to exert an IC_{50} of 611 µM. Furthermore, AE caused a significant upregulation of fimH and sfaG in free floating, non-attached UPEC and significantly down-regulated these genes in adherent bacteria. Summarizing, phthalides were identified as the main active compounds in polar and semi-polar extracts, which exert strong antiadhesive activity against UPEC and support the traditional use in phytotherapy.

P-244 Antiausterity activity against pancreatic cancer cells and antiplasmodial properties of naphthylisoquinoline alkaloids and their analogues

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Cancer and malaria are causes for concern within the global health sector both accounting for millions of fatalities annually. Contributing to this is the reduced survival rate (< 5%) of patients diagnosed with pancreatic cancer and drug resistance in malaria which threatens to further exacerbate the already worrisome situation. These emphasize the need to develop new drugs to combat these diseases. Inspired by the fact that natural products have served as a source of unique privileged chemical scaffolds, we have screened a small library of naphthylisoquinoline alkaloids isolated from *Ancistrocladus* species from the D.R. Congo for their antiausterity activity against human PANC-1 pancreatic cancer cells and antiplasmodial activity against both intra-erythrocytic asexual and sexual *Plasmodium* parasites. The compounds showed moderate to strong antiausterity activities at a concentration-dependent manner with their PC_{50} values ranging from 7.6 to 67.8 µM. New compounds ancistroyafungines B and D were the most potent representatives within this series[1]. Eleven out of 30 compounds investigated for their antiplasmodial activity showed good growth-inhibitory effects (IC_{50} < 1 µM) against both drug sensitive and resistant intra-erythrocytic asexual *P. falciparum* parasite strains with most promising minimal (10 > R.I. >1) to no cross resistance (R.I. < 1) to chloroquine with selectivity indices of > 10. Jozimine A_2 was the most potent agent against *P. falciparum* gametocyte stages and in blocking exflagellation of microgametes. Our study provides encouraging data for the continued interrogation of naphthylisoquinoline alkaloids in search of novel drugs for the treatment of both cancer and malaria.

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P-245 Antibacterial activity of abietane diterpenes from the roots and hairy roots of *Salvia corrugata* Vahl.

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The major compounds of the surface extract of the aerial parts of *Salvia corrugata* Vahl., demethylfruticuline A (**1**) and fruticuline A (**2**), showed significant antibacterial activity [1] and inhibited *in vitro* the synthesis of biofilm of multi-resistant clinical strains of *Staphylococcus* and *Enterococcus* [2]. 7β-acetoxy-20-hydroxy-19,20-epoxyroyleanone and 7β-ethoxy-6β,20:19,20-diepoxyroyleanone, isolated from *in vitro* shoots [3] were active at various degrees against the same bacterial genera.

The methanolic extract of the roots and the twelve semi-purified fractions obtained by its chromatographic separation were tested against several multi-drug resistant clinical strains also of marine origin (*S. aureus, S. epidermidis, S. capitis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. simulans, S. warneri, E. faecalis, E. faecium, E. gallolyticus, E. durans, E. avium*) displaying MIC values ranging from 4 to >128 µg/mL. Horminone (**3**), 7-*O*-acetylhorminone (**4**) and 7-*O*-methylhorminone were isolated. **3** and **4**, obtained in suitable amounts, showed MIC values ranging from 4 to 64 µg/mL against the same bacterial strains.

Hairy roots were induced from the leaves of *S. corrugata*, obtained through *in vitro* culture of nodal segments of wild plants, transformed with *Agrobacterium rhizogenes* strain (MTCC532). The methanolic extract did not contain **1** and **2** and it was moderately active against multi-resistant clinical strains of *E. faecalis* and *E. faecium* displaying MIC values ranging from 64 to 128 µg/mL. Four semi-purified fractions obtained by chromatographic separation showed MIC values ranging from 4 to 16 µg/mL. The fractions showed lower activity against Staphylococci. Ferruginol and the new 19[4→3]abeo-*O*-demethyl-14-hydroxy-cryptojaponol (**5**) were isolated from fraction III. **5** showed a wide range of activity against Enterococci (MIC values 1 - 128 µg/mL).

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P-246 Antibacterial activity of p-menthanes against bacteria causing respiratory infections in vapour phase

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The relationship between the chemical structure of a molecule and its antimicrobial activity is a promising concept in the discovery of new anti-infective agents. Due to their typical physico-chemical properties, the potential of volatile antimicrobials lies especially in the inhalation therapies for treatment of respiratory infections. Although there were some attempts to determine the antimicrobial effect of certain classes of plant-derived volatiles such as oxygenated monoterpenes [1], acyclic, bicyclic and p-menthane terpenoids [2] in liquid phase, the effect in volatile phase is still not well understood.

In this study, the growth-inhibitory effect of certain representatives of p-menthanes was tested using broth microdilution volatilisation method in liquid and vapour phase [3] against standard strains of bacterial pathogens causing respiratory infections such as *Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae*, and *Streptococcus pyogenes*.

As a result, thymoquinone produced the strongest antibacterial activity against all strains tested with MICs ranging from 2 to 32 µg/mL in liquid phase and from 4 to 64 µg/mL vapour phase. Thymol and carvacrol have an antimicrobial activity varying in ranges of 64 to 256 µg/mL both in vapour and liquid phase. 1,8-cineol and p-cymene have no relevant antimicrobial activity neither in liquid, nor vapour phase. The results suggest that quinone structure contribute significantly to the antibacterial action of p-menthanes in vapour phase. In addition, presence of hydroxyl group on the p-cymene skeleton increases its antibacterial action. In comparison to previous experiments, this study was performed with broader spectrum of bacteria and the activity of tested compounds was compared with other groups of monoterpenes (e.g. pinanes). The results can lead to the development of new medicinal products being used as inhalation therapy acting directly in the respiratory tract.

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P-247 Antibacterial activity of wood vinegars from *Tamarindus indica, Mangifera indica* and *Azadirachta indica*

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The finding of new antibacterial agents for an alternative treatment is an urgent issue. Wood vinegar is a condensed acidic liquid, which obtained during the processing of wood charcoal production and used to control animal diseases. Three wood vinegars from each plant including *Tamarindus indica, Mangifera indica* and *Azadirachta indica* was evaluated for the antibacterial activities using agar disc diffusion and broth microdilution methods and the mode of action of each wood vinegar determined by scanning electron microscope. Three wood vinegars showed broad-spectrum antibacterial activity against both gram-positive and gram-negative pathogenic bacteria. The minimum inhibitory concentration and minimum bactericidal concentration values were similar with ranging from 1.25-6.25 %v/v. The electron micrographs of cells treated with each wood vinegar showed the similar activity by the disruption of cell membrane in both gram-positive and gram-negative pathogenic bacteria. Microstructural observations showed that wood vinegars depleted of the content of bacterial cells, indicating that the cell structures of treated bacteria were severely affected and damaged by the antibacterial agents. The treated cells showed an incomplete and deformed shape of the cells when compared with the untreated cells. A pH value in culture condition may affect the conformation of the bacterial cell driven by the ATPase energy-consuming pump in bacterial cell membrane [1,2,3]. Moreover, wood vinegar exhibited a pH in acidic value. Wood vinegar may target on a bacterial cell membrane. It revealed that *T. indica, M. indica* and *A. indica* wood vinegars may be developed for an alternative antibacterial agent to control pathogenic bacteria.

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P-249 Anti-cancer and anti-gout potential of some Indonesian and Malaysian medicinal plants

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Medicinal plants have undoubtedly been considered by human beings since ancient times as they have been shown to have therapeutic effects on various diseases including cancer and gout. Hence, this study aimed to evaluate the potential of some Indonesian and Malaysian plants as anti-cancer and anti-gout agents. In this study, Annona muricata (soursop) and Hedyotis corymbosa (pearl grass) leaves were investigated for their anti-cancer activity as well as their bioactive compounds which contributed to this activity. Meanwhile, Orthosiphon stamineus (java tea) leaves were investigated for the anti-gout properties using xanthine oxidase inhibition assay. For the anti-cancer activity, both plants showed potent anti-cancer activity against human breast cancer cell line (MCF7) in vitro. The isolation of the pure compounds from the methanol extract of A. murita and H. corymbosa qualitatively revealed the presence of non-phenolic compounds and steroids; respectively. For the anti-gout activity, the aqueous extract of O. stamineus displayed moderate xanthine oxidase inhibitory activity with IC50 value of 156.8 µg/ml. However, allopurinol, the standard drug used for the gout treatment exhibited higher anti-gout activity with IC50 value of 5.35 µg/ml. Therefore, this study demonstrated the anti-cancer and anti-

gout properties of some Malaysian and Indonesian medicinal plants which can be a great potential for use in complementary and alternative medicines, although further studies are necessary to corroborate the reported results.

P-250 Anticholinesterase activity of an endemic Atlantic rain forest bamboo species

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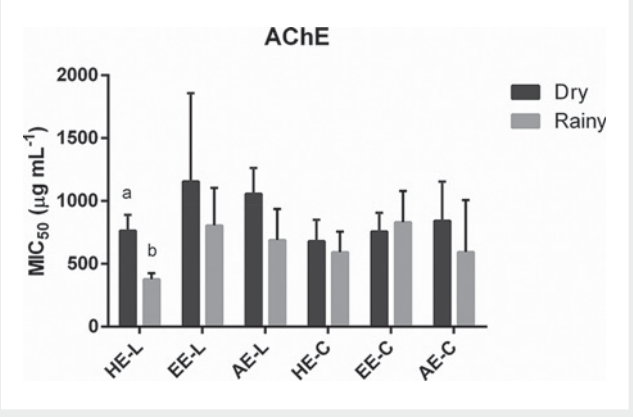
DOI 10.1055/s-0039-3399956

Alzheimer's disease is characterized by cognitive and memory disturbances. It is associated with a reduction of acetylcholine levels. Thus, it is interesting to find drugs that inhibit the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [1]. Asian bamboos are reported to improve spatial memory capacity and to reduce AChE activity [2]. Therefore, this study aimed to evaluate the anticholinesterase potential of *Merostachys neesii* Rupr., an endemic Brazilian woody bamboo. Leaves and culms of *M. neesii* (*n*=3) were collected during the dry (August/2016) and rainy (February/2017) seasons. Plant material was submitted to two extraction processes: serial maceration using hexane and 70% ethanol; and infusion. AChE and BChE inhibitory activity was evaluated by Ellman's colorimetric method using a microplate reader. Results were expressed as minimal inhibitory concentration to reach 50% inhibition (MIC_{50}). MIC_{50} values were compared by the *t*-test or Wilcoxon test for two samples (non-parametric data) using R software to verify if there was a significant difference (p 0.05) caused by seasonality. In general, hexane extract of leaves (HE-L: 376.92 ± 48.14 µg mL^{-1}) and culms (HE-C: 592.24 ± 164.14 µg mL^{-1}) from the rainy season showed the lowest MIC_{50}, but still higher than that of neostigmine (33.84 ng mL^{-1}). AChE inhibition has also been reported for *M. magellanica* [3]. Also, HE-L showed a significant difference between the seasons, with lower MIC_{50} value in the rainy season (▶**Fig. 1**). No inhibition of BChE was observed. In conclusion, seasonality influenced the AChE inhibitory activity of HE-L; however, no extract showed promising anticholinesterase activity.



▶**Fig. 1** Seasonality effect on inhibition of acetylcholinesterase (AChE) by *Merostachys neesii* Rupr. Data are presented as the mean ± standard deviation (n=3). Means were compared between seasons of the same extract. Different letters represent significantly different means (p 0.05). MIC_{50}: minimal inhibitory concentration to reach 50% inhibition. HE-L: hexane extract of leaves; EE-L: ethanolic extract of leaves; AE-L: aqueous extract of leaves; HE-C: hexane extract of culms; EE-C: ethanolic extract of culms; AE-C: aqueous extract of culms.

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P-253 Anti-inflammatory activity and ROS regulation of sinapaldehyde in LPS-stimulated RAW 264.7 macrophages

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Sinapaldehyde is a member of the class of compounds known as methoxyphenols. Sinapaldehyde can be found in plant extracts such as *Senra incana* and *Ailanthus altissima* Swingle [1,2]. This study evaluated the anti-inflammatory effect of sinapaldehyde in LPS-stimulated RAW 264.7 macrophages using nitric oxide (NO) assay, cytokine ELISA, and reverse transcription polymerase chain reaction analyses. Sinapaldehyde exhibited inhibitory activity on the production of NO, ROS, and cytokines such as TNF-α and IL-6. Sinapaldehyde (100 µM) significantly inhibited total NO and ROS inhibitory activity at 93% ($p < 0.001$) and 34% ($p < 0.05$), respectively, when compared with LPS control. Moreover, sinapaldehyde significantly ($p < 0.001$) down-regulated the mRNA expression of inflammatory marker genes like TNF-α, IL-6, and iNOS against LPS stimulation. Sinapaldehyde did not show any cytotoxicity at different concentrations (3 - 100 µM). Our results demonstrate that sinapaldehyde is able to show anti-inflammatory effects by suppression of ROS and NO. It could be used as a new natural product for the effective treatment of inflammatory diseases.

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P-254 Anti-inflammatory and barrier stabilising effects of myrrh, coffee charcoal and chamomile flower extract in a multicomponent-cell-model of the intestinal mucosa

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The combination of myrrh (*Commiphora molmol E.*), coffee charcoal (*Coffea Arabica L.*) and chamomile flower dry extract (*Matricaria chamomilla L.*), known as Myrrhinil-Intest® has a long tradition in the treatment of gastrointestinal disorders in Germany. Recent clinical evidence suggests its efficacy in the therapy of inflammatory bowel diseases (IBD)[1]. However, the mechanisms of action remain to be fully elucidated.

The present study aimed to evaluate the effects of myrrh, coffee charcoal and chamomile flower extract on pro-inflammatory communication between immune and epithelial cells and the resulting barrier dysfunction. A complex co-culture-cell-model allowed the simultaneous investigation of these two IBD-characteristics.

To model the intestinal mucosa a monolayer of 90 % Caco-2 and 10 % HT29-MTX cells was differentiated on transwell-inserts for 21 days. Co-cultivation with LPS-activated THP-1 macrophages over 48 h served as inflammatory stimulus. Concomitantly the cells were treated with various concentrations of

plant extracts. Cytokine (IL6, TNF) and chemokine (IL8, MCP-1) release into the supernatant were quantified by ELISA. The transepithelial electrical resistance (TEER) was measured to evaluate effects on the barrier function.
Myrrh, coffee charcoal and chamomile flower showed concentration-dependant effects on the release of pro-inflammatory mediators to varying extent. In addition a TEER-increase of inflamed monolayers could be observed with higher concentrations of myrrh and coffee charcoal. The respective IC_{50} and EC_{50} values are presented in table 1.
All three plant extracts exhibited anti-inflammatory properties. A barrier stabilising effect could be shown for myrrh and coffee charcoal. In both fields myrrh displayed the most distinct pharmacological activity.

▶**Tab. 1** Summary of the half maximal effective and inhibitory concentrations (EC_{50} and IC_{50}) of the plant extracts on the TEER and the release of cytokines and chemokines

	EC_{50} - TEER increase	IC_{50} – inhibition of mediator release			
		IL6	TNF	IL8	MCP-1
Myrrh extract (*Commiphora molmol E.*)	67 µg/ml	9 µg/ml	13 µg/ml	34 µg/ml	36 µg/ml
Coffee charcoal extract (*Coffea Arabica L.*)	108 µg/ml	129 µg/ml	–	62 µg/ml	132 µg/ml
Chamomile flower dry extract (*Matricaria chamomilla L.*)	–	–	35 µg/ml	62 µg/ml	–

References [1] Langhorst J et al. Alimentar Pharmacol Ther 2013; 38(5): 490–500

P-258 Antimicrobial and anti-inflammatory potentials of *Aidia genipiflora* (DC.) Dandy (Rubiaceae)

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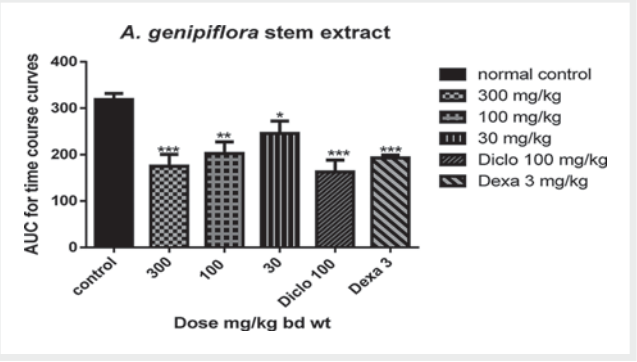
An increased resistance to current antimicrobial therapy has informed the search for alternative antimicrobial agents. *Aidia genipiflora* is used by traditional medicine practitioners to treat infectious diseases and inflammatory conditions. However, there is no scientific evidence to support its biological activity. The present study investigated the antimicrobial and anti-inflammatory potential of whole extract (AG) and its petroleum ether (AGPE), ethyl acetate (AGEt) and methanol (AGM) fractions. Using the High-throughput culture growth inhibition assay (HT-SPOTi) for the *in-vitro* antimicrobial effect against clinically significant microbes, the whole extract showed activity against *Escherichia coli* and *Proteus mirabilis* at MICs of 250 µg/mL and against *Streptococcus pyogenes* and *Enterococcus faecalis* at MICs of 500 µg/mL. The ethyl acetate fraction showed activity against *Proteus mirabilis* and *Kleibsiella pneumonia* at MICs of 250 µg/mL and against *Pseudomonas aeruginosa* and *Salmonella typhi* at 500 µg/mL. The methanol fraction and petroleum ether fraction showed activity at MICs of 250 µg/mL against *Proteus mirabilis* and *Staphylococcus aureus* respectively. All extracts showed activity against *Vibrio cholerae* at MICs of 500 µg/mL. The whole extract, methanol and ethyl acetate fractions showed dose dependent anti-inflammatory activity in the carrageenan induced footpad oedema model with respective percentage inhibition of oedema of 45.11±3.41, 31.12±3.42 and 29.28±3.58 ($p < 0.001$) at the highest dose of 300 mg/kg (figure 1). Diclofenac, used as reference drug, gave % inhibition of 48.94±3.58. The results of this study has demonstrated that *Aidia genipiflora* could be an alternative source of antimicrobial and anti-inflammatory agents.



▶**Fig. 1** Anti-inflammatory activity

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P-259 Antimicrobial properties of tannin extracts as animal feed

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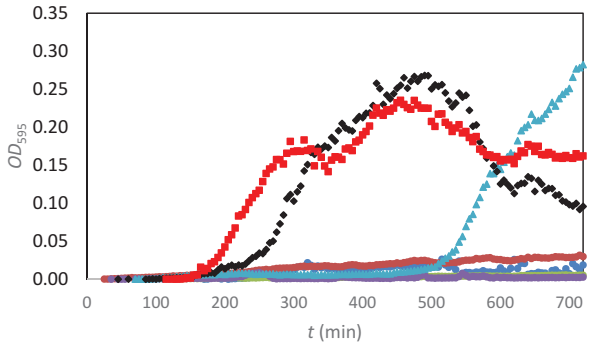
For many years, antibiotics were added to animal feed as additives to improve growth rates and reduce costs [1]. That caused, together with antibiotic over prescription, overuse and missuses in human medicine [2], an increase in antimicrobial resistance. The use of antimicrobial compounds as growth-promoting factors after 2006 has been forbidden by the European commission with the Regulation of the European Parliament and of the Council (EC) No 1831/2003 [3]. Without the use of antibiotics, the incidence of infectious diseases is increasing, and alternative means to control bacteria growth are necessary [1]. Tannins are natural compounds with proven antimicrobial properties and as such represent suitable alternatives [4]. The aim of our study was to determine the *in vitro* minimal inhibitory concentration (MIC) of tannins against *Escherichia coli* under different circumstances. We studied several pure compounds (vescalagin, castalagin, gallic acid and tannic acid) as well as various commercial plant extracts. We determined the minimal inhibitory concentrations of these compounds using two complementary methods (broth microdilution method by measuring optical density and by adding INT

dye) that provided similar results. We also changed the composition of the media. Our results showed that minimal inhibitory concentration (MIC) highly depends on both the selected compound and media composition. Gallic acid has the highest MIC while tannic acid provides the best results (lowest MIC).



▶**Fig. 1** Growth curves for the bacteria E. coli in media with increasing concentration of tannins. Elongation of lag phase can be observed at tannin concentration just below MIC (blue triangles).

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P-261 Antimycobacterial screening and safety evaluation of *Tithonia rotundifolia*, a southern Africa alien invasive weed

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Tuberculosis remains a global threat and a leading cause of mortality. Multidrug-Resistant (MDR) and Extensively-Drug Resistant (XDR) tuberculosis are major challenges, especially in Africa. Considering this, the investigation of medicinal plants as a source of treatment is advocated. *Tithonia rotundifolia*, an invasive plant with negative ecological impacts in Africa, was screened against non-pathogenic *Mycobacterium aurum, M. fortuitum, M. smegmatis* and pathogenic *M. bovis and M. tuberculosis* H37RV. The serial microdilution assay was used to determine antimycobacterial activity of acetone, dichloromethane and hot water extracts of the weed plant. Cytotoxicity of the extracts against African Vero monkey kidney, human colon Caco-2 and C3A liver cells was studied using a tetrazolium-based colorimetric assay while genotoxicity tests were conducted against *Salmonella* strains TA98 and TA100 using the Ames test. The tested extracts were inhibitory against both the pathogenic and non-pathogenic *Mycobacterium* strains. Better activity was displayed against the non-pathogenic *M. aurum, M. fortuitum* and *M. smegmatis* with minimum inhibitory concentration values ranging between 0.04 and 0.08 mg/ml. Little cytotoxicity of the extracts was noticed with LC_{50} values against Vero Monkey kidney cells between 0.78 and 0.96 mg/ml (selectivity index, SI = 0.38 to 24.05), Caco-2 between 0.198 and 0.32 mg/ml (SI = 0.05 to 5.84) and C3A between 0.67 and 0.88 mg/ml (SI = 0.29 to 22.56) respectively. No genotoxicity was detected against the *Salmonella* strains TA98 and TA100. The results from this study motivate further investigation of *T. rotundifolia* for possible development of effective antimycobacterial treatments.

P-263 Antioxidant and anti-inflammatory potential of *Aloe vera* and *Punica granatum:* onconutraceutical potential in intestinal epithelial cells

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Intestinal epithelial cells play a pivotal role in maintaining intestinal homeostasis. Different noxious agents can damage the intestinal epithelial integrity. This damage is also associated with anticancer therapies resulting in reactive oxygen species (ROS) and pro-inflammatory factors overproduction, also at gastrointestinal level. Gastrointestinal mucositis is a frequent and severe side effect of chemotherapy and radiotherapy in cancer patients, affecting approximately 50 to 80% of patients [1,2]. Currently, no effective treatment exists for chemotherapy-induced mucositis, prompting the need to develop an anti-mucositis agents for use in clinics.

Our study focused on the effect of *Aloe barbadensis* and *Punica granatum* combination in intestinal epithelial cells (IEC-6) during oxidative stress and inflammatory conditions. Oxidative stress and inflammatory mediators such as ROS release, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), heme oxygenase 1 (HO-1) and NAD(P)H Quinone Dehydrogenase 1 (NQO1) expression, were evaluated by cytofluorimetric tecniques. Tumor necrosis factor-α (TNF-α) levels were evaluated by ELISA assay.

Aloe barbadensis inhibited ROS production both during oxidative stress and in inflammatory conditions as well as COX-2 and iNOS expression, at all tested concentrations, during inflammation in IEC-6. In the same experimental conditions, *Aloe barbadensis* also increased the cytoprotective enzymes HO-1 and NQO1 expression and reduced TNF-α release. The treatment of IEC-6 with *Aloe barbadensis* plus *Punica granatum* (9:1 ratio) significantly increases the activity of *Aloe barbadensis* alone.

Our results indicate that *Aloe barbadensis* and *Punica granatum* combination could be useful to reduce the oxidative stress and inflammatory-mediated complications, also associated to chemotherapy and radiotherapy, at intestinal level.

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P-265 Antipruritic effects of the aerial part of *Oxalis corniculata*

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DOI 10.1055/s-0039-3399963

Background: The discovery of antipruritic substances is expected to help patients with chronic and severe pruritus, such as atopic dermatitis. We previously searched for natural products with antipruritic activity using *in vivo* assay systems, through evaluation of the inhibitory effect on scratching behavior induced by compound 48/80 (COM, a mast cell degranulation agent) in a mouse model of chronic itch, which was developed originally to investigate blood stasis and stress. The result of our search revealed the antipruritic activity of the fresh aerial part of *Oxalis corniculate* (FAOC). Dried whole plants of *O. corniculata* have been used in Chinese herbal medicine for their effects on pyresis, urticaria and sedation. Several flavonoid derivatives have been isolated from the whole plants, but their antipruritic activity is unknown.

Aim: To determine the antipruritic effects of the FAOC and it's active compounds.

Methods: The activity were measured as previously described. The extracts (100 mg/kg) were administered *p.o.* 1hr before injection of COM. The structures of compounds were identified with authentic samples by comparing the LC-MS data.

Results: A MeOH extract of the FAOC significantly inhibited COM-induced scratching behavior. Bioassay-guided fractionation of MeOH extract led to the identification of isoorientin, orientin, isovitexin and swertisin. The antipruritic effects of the compounds and the identification of other active compounds are presently under investigation.

Conclution: Our findings showed that the FAOC may provide new leads for the discovery of antipruritic substances for the treatment of the itching sensation that accompanies allergic reactions.

P-266 Antispasmodic phenolic compounds isolated from *Morus nigra* root bark

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Natural antispasmodic agents may have a potential for the treatment of asthma or gastrointestinal disorders including irritable bowel syndrome. *Morus nigra* has been valued as an antispasmodic traditional medicine but no related studies are available on its isolated constituents [1].

In this study, our aim was to investigate the antispasmodic potential of phenolic compounds of the root bark of *M. nigra*.

Seven compounds were isolated through the consecutive use of various preparative chromatographic techniques. These compounds were identified as morusin (**1**), kuwanon U (**2**), kuwanon E (**3**), moracin P (**4**), moracin O (**5**), albanol A (**6**), and albanol B (**7**). Their *ex vivo* antispasmodic activity was tested on isolated rat ileal and tracheal smooth muscles. Compound **2**, **5** and **7** exerted strong activity on both models, while compound **3** (a methoxy analog of **2**) was inactive. EC_{50} values and maximum effects for compounds **2** and **7** showed them equipotent with papaverin. Moracin O (**5**) exerted antispasmodic activity on ileal and tracheal muscles with EC_{50} values of 1.1 µM and 62 nM, respectively, and showed maximum effects on both muscles significantly higher than those of papavarine. Our findings suggest moracin O (**5**) as a new antispasmodic lead compound.

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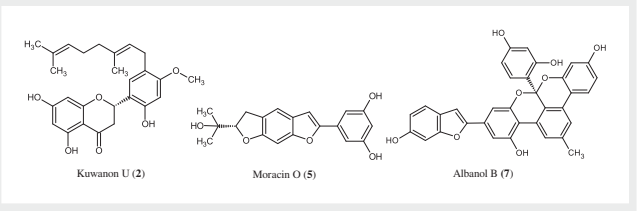

►**Fig. 1**

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P-267 Anti-staphylococcal activity of *Myristica hypargyraea* and *Myristica inutilis* from Samoa

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Staphylococcus aureus is a human pathogen colonizing especially skin and respiratory tract. This bacteria has a harmful potential to cause a variety of community and hospital-acquired infections. Over the years, *S. aureus* strains resistant to commonly used antibiotics have been reported, which complicates the staphylococcal infections' treatment [1]. Various plant-derived products, such as *Melaleuca alternifolia* essential oil, are used for elimination of *S. aureus* in clinical practice [2]. Therefore, plant compounds and their mixtures (e.g. extracts) are still perspective materials for the development of new anti-staphylococcal agents. Since the antimicrobial properties of some *Myristica* sp. have already been reported [3], we decided to evaluate growth-inhibitory effects of seed and aril hexane extracts from two Samoan indigenous plant species *Myristica hypargyraea* A.Gray and *Myristica inutilis* Rich ex A.Gray, against twelve *S. aureus* strains including antibiotic-resistant forms using the broth microdilution method [4].

In this study, all plant extracts tested showed a certain degree of anti-staphylococcal effect with minimum inhibitory concentrations (MICs) ranging from 8 to 1,024 µg/mL. The aril extract of *M. hypargyraea* possessed the highest antibacterial activity against *S. aureus* ATCC 33591 and three clinical isolates with a MIC value of 8 µg/mL.

Our results suggest potent antimicrobial properties of seed and aril extracts from *M. hypargyraea* and *M. inutilis* that could be used by the pharmaceutical industry for the treatment of skin and respiratory infections caused by *S. aureus*. However, further research focused on chemical analysis, cytotoxic effects and *in vivo* evaluation is necessary to be carried out.

Acknowledgments This research was financially supported by the Czech University of Life Sciences Prague (projects IGA 20195003)

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P-268 Antiviral screening and bioautographic assessment of radical scavenging, estrogenic and AchE-inhibitoric activity of *Sideritis* species

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Sideritis L. (Lamiaceae) is a genus represented by more than 150 species [1] and is known in folk medicine to treat respiratory diseases [2], geriatric disorders [3], enhance memorizing skills [4,5] and possess antioxidant properties [6].

In this work, the species *S.clandestina* (Bory&Chaub.)Hayek; *S.endressii* Willk.; *S.glacialis* Boiss.; *S.hirsuta* L.; *S.italica* (Mill.)Greuter&Burdet; *S.dichotoma* Huter; *S.raeseri* Boiss.&Heldr.; *S.scardica* Griseb.; *S.syriaca* L. and *S.hyssopifolia* L. were screened by planar DPPH, planar Acetylcholinesterase (AchE) & its microtiter format assay [7] and planar Yeast Estrogen Screen (YES) assay on the developed HPTLC plate (Silica 60-mobile phase: dichloromethane/methanol/water, for DPPH with acetic acid).

Selected extracts were tested against different infectious viruses: None of the extracts had an effect against the human corona virus (HCoV-229E). *S.hirsuata* showed a clear effect at concentration range 6-100 µg/ml on Influenza H3N2 virus.

All species except *S.italica* and *S.clandestina* exhibited AchE inhibition on HPTLC plate, some of them were elucidated as artefacts in a control assay. In microtiter plate, all extracts (10 mg/ml) demonstrate enzyme inhibition: *S. scardica* the strongest (70%) and *S.italica* the lowest (25%) effect. *S.clandestina* shows one spot with clear estrogenic activity. For *S.dichotoma* and *S.glacialis* only weak estrogenic zones were observed. In planar DPPH assay, all species show radical scavenging capacity at 75 µg/µl.

In conclusion, the screening indicates that not only the *Sideritis* species currently recognized by HMPC [8] might be of interest for health related treatments and further research is desirable, especially for antiviral activity, which is reported here the first time.

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P-271 Bactericidal property of myrrh oil and two formulations against standard bacterial strains and multidrug-resistant clinical isolates with GC/MS chemical profiling

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Myrrh is the resinous exudate obtained by the incision of *Commiphora molmol* trees (Family Burseraceae). The bactericidal activity of its total oil (extracted by hexane) was compared to its essential oil (MEO, 5% v/v) using viable count technique against *Staphylococcus aureus* (*S. aureus* ATCC 6538) and *Pseudomonas aeruginosa* (*Ps. aeruginosa* ATCC 9027). MEO exhibited a better activity with >99.999% killing of both tested strains after 2h contact time. MEO (5% v/v) was tested using the same technique against four multidrug resistant isolates; *S. aureus* (MRSA, sputum), *Escherichia coli (E. coli*, urine), *Ps. aeruginosa* (wound) and *Klebsiella pneumonia (K. pneumonia*, sputum). Highest bactericidal activity was observed against *Ps. aeruginosa* and least activity was against *K. pneumonia* (99.59 and 54.04% killing, respectively after 2h contact time). A cream and mouthwash were formulated using 5% v/v MEO. The cream showed a better activity against *Ps. aeruginosa* than *S. aureus* (86.68 and 51.11 % killing, respectively after 2h contact time). A 75% reduction in oral aerobic bacteria was observed after gargling the mouthwash for 2 min. This reduction was sustained for 20-30 min. followed by slow gradual increase in the number of recovered bacteria. GC/MS analysis allowed the identification of 17 and 9 compounds representing 92.01 and 97.99% of the total and essential oil, respectively. Furano-eudesma-1,3-diene (15.99%) and 2-acetoxy-furano-diene (26.82%) were the major identified compounds in the total and essential oil, respectively. These results indicate that Myrrh essential oil is a promising antibacterial agent that can be formulated against multidrug resistant bacterial strains.

P-272 Benzoxazinoids in human diet: an anti-cancer agent?

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Benzoxazinoids (BXs) are phytochemicals present in selected cereal crops and medicinal plants. Effects of BXs as plant defense and allelopathic agents have been widely studied [1,2], while the knowledge on effects in mammals is limited. Potential therapeutic effects of BXs in humans [3], and presence of BXs in prostate cancer tissue in prostate cancer patients fed with a diet of rye, which was rich in BXs, [4], motivated us towards exploring the effects of BXs in prostate cancer cells.

The effects were studied by exposing prostate cancer cell lines LNCap, DU145, PC3, and C4, and primary prostate cancer cells to selected BXs. Agluconic forms of BXs, which were reported to be the most active form of the compounds, were tested. DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) and DIMBOA (2,4-

dihydroxy-7-methoxy-1,4-benzoxazin-3-one) caused a decrease in cell viability across all the cell lines tested. The IC_{50} of the compounds ranged from 40 to 200 µM across different cell lines. The results are encouraging and further experiments will be carried out using RNA sequencing. The ultimate goal is to understand the mechanism behind the potential anti-cancer effect of BXs and to elucidate whether they can be developed into therapeutic agents.

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P-273 Bioactivity-guided fractionation of extracts using mushroom tyrosinase – friend or foe?

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Tyrosinase (Tyr) catalyzes the rate limiting step of melanogenesis in human skin and is thus the main target for the pharmacological treatment of pigmentation disorders. This has led to an increased research interest in Tyr inhibitors during the last decades, often pointing to polyphenols as active compounds with polyphenols occurring particularly frequent. However, in the early stages of drug discovery, it is nowadays common practice to avoid the high costs of human tyrosinase by using the more economic mushroom tyrosinase (mh-Tyr). Since some polyphenols are accepted as substrates by mh-Tyr, the present study aims to investigate this enzyme's substrate specificity towards common polyphenols, and to discuss its significance in the context of bioactivity-guided fractionation.

A dataset of 56 natural products was assembled and classified into assay interferers and non-interferers, using spectrophotometric and LC-ESI-HRMS assays. Based on these experimental findings, SARs defining for AIs were deduced and implemented into an *in silico* tool that will allow for rapid prescreening in the future.

Polyphenols that fulfill specific structural criteria are converted by mh-Tyr to the respective o-quinones, which are likely to form further oxidation products with different absorption maxima. Mh-Tyr substrates can thus change the sample color during an inhibition assay, leading to falsified inhibition constants or to the discontinuation of a bioactivity-guided fractionation campaign.

Therefore, Mh-Tyr based enzyme inhibition assays are not suitable for bioactivity-guided fractionation of extracts and fractions. Inhibition constants determined with such assays for pure polyphenols have to be critically evaluated.

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P-274 Bioassay guided isolation of naphthoquinones from *Onosma aksoyii*, investigation of their cytotoxic properties

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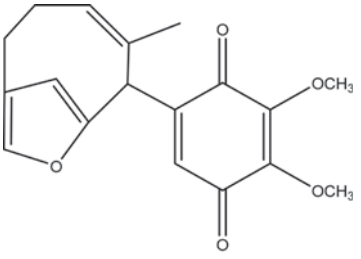
The genus *Onosma* L. (Boraginaceae) includes about 230 species, distributed mainly in the Mediterranean region and Central Asia. Major constituents of *Onosma* species are alkaloids, naphthoquinones, polyphenols, phytosterols, terpenoids and fatty acids [1, 2].

Naphthoquinones are naturally widespread secondary metabolites deriving from some higher plants, fungi and bacteria. They exhibit significant biological activities such as cytotoxicity, antimalarial, antibacterial, antifungal and wound healing [2, 3]. Recently naphthoquinone derivatives have also been recognized as potent topoisomerase inhibitors [4].

As part of our ongoing studies performed on Turkish *Onosma* species, we carried out cytotoxicity guided isolation studies to identify and characterize new naphthoquinone-type constituents from *Onosma aksoyii*, Aytaç&Türkmen a recently determined endemic species.

As a result, four compounds, one of which was new (**1**), were isolated from *O. aksoyii*, and their structures were elucidated by spectral methods (NMR and MS). According to the cytotoxicity screening results, IC_{50} values of these compounds were ranging between 6.485 µM and 32 µM. Further studies are in progress to determine DNA topoisomerase inhibitory effects of the isolated compounds.



►**Fig. 1** Structure of compound **1**

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P-275 Bio-guided fractionation of essential oils looking for plant bioactive secondary metabolites with potential hypoglycemic activity

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Diabetes mellitus is a chronic metabolic disorder characterized by a difficulty in blood glycaemia maintenance. People in the world with diabetes have increased dramatically over recent years and still are increasing. A possible therapeutic approach for treating diabetes mellitus is to decrease postprandial hyperglycemia, by inhibiting the carbohydrate hydrolyzing enzymes, such as α-amylase. Thanks to its inhibition, carbohydrate digestion is stopped, and as a consequence glycaemia reduces. Examples of such inhibitors used in the clinical practice for treating diabetes are acarbose, miglitol and voglibose [1]. However, these drugs are known to be associated with various gastrointestinal side effects, among others diarrhea [2,3].

The aim of this study is the research for new α-amylase inhibitors deriving from plant secondary metabolism. A bio-guided fractionation approach, based on an *in vitro* α-amylase inhibition assay, was adopted to isolate and identify the active fractions/compounds in different essential oils. Eighty-four essential oils obtained by distillation from different plant species and botanical families were submitted to the enzymatic assay.

Three essential oils resulted particularly active (*Eucalyptus radiata* A.Cunn. ex DC., *Laurus nobilis* L. and *Myristica fragrans* Houtt.) with an inhibitory capacity comparable or slightly higher than acarbose, chosen as positive control. The obtained results showed that all essential oil components seem to play a synergic effect. Moreover, an interesting number of both hydrocarbon and oxygenated compounds were characterized by a good α-amylase inhibition, around 30%. These preliminary results demonstrate that essential oils may represent a promising source of potential α-amylase inhibitors.

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P-277 Biotransformation of the promising neuroregenerative hop chalcone Xanthohumol C

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The prenylated chalcone xanthohumol C from *Humulus lupulus* L. -hops- was identified as an inducer of neuronal lineage-specific differentiation of neuronal stem cells [1]. Furthermore, a structure-activity study revealed that a pyrano ring is a structural characteristic responsible for the neuro-differentiation effect and that the unknown targets respond to structural changes [2]. Since the induction of neuronal-differentiation is the key mechanism concerning a therapy for neurodegenerative diseases using the body's own regeneration mechanism, xanthohumol C, is a promising candidate for further pharmaceutical evaluation.

To evaluate the potential of xanthohumol C as a drug candidate, studies concerning absorption, distribution and metabolism are needed. Recently, a formulation of xanthohumol C in cyclodextrin was characterized and enhanced water solubility was determined. Furthermore, a small animal study showed that the compound is absorbed in this formulation and reaches the brain as target [3]. To the best of our knowledge, there is only little known about biotransformation of this promising candidate. Accordingly, human microsomes were used to metabolize xanthohumol C and HPLC/MS analysis showed that xanthohumol C was transformed into four main metabolites by cytochrome monooxygenases p450 (CYPs). Using mostly selective inhibitors and a synthesized isotope labelled internal standard, the CYPs 2C8, 2C19 and 2D6 were identified as possible main metabolizers.

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[3] in review

P-279 Branched-chain amino acids (BCAAs) promotes liver regeneration by decreasing SOCS3 expression to enhance IL-6/STAT3 proliferative signals

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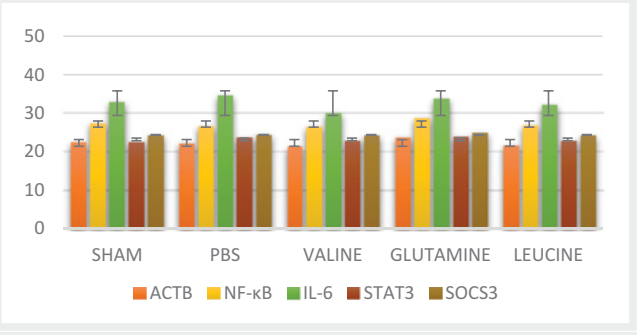
Liver regeneration is one of the primary clinical importance in the setting of liver injury, resection, and transplantation [1,2]. Although some amino acids are recognized to have favorable effects on the liver regeneration after partial hepatectomy (PH), molecular mechanisms underlying these effects are barely known [3].

We aimed to investigate the effects of valine, glutamine, and leucine amino acids on PH-induced NF-κB signal pathway.

Expressions of 8 genes involving in the NF-κB signal pathway was examined by an RT-PCR method in the liver tissue specimen. In our study, the effect of Leucine amino acid, a kind of Branched Chain Amino Acid (BCAA) on liver regeneration after PH in rats, was determined by RT-PCR, Western blot, and PCNA. Results showed that significantly decreased BCAA-induced upregulation of STAT-3, SOCS3, and NF-κB. However, IL-6 levels significantly increased in all groups.

In summary, demonstrating that BCAA enhances IL-6 proliferative signals in hepatocytes through down-regulation of SOCS3 while decreasing IL-6 levels and reducing its pro-inflammatory signals, all of which optimize liver regeneration. SOCS3 plays a dual role in modulating the rate of hepatocyte proliferation. In particular, this is the first demonstration of an endogenous mechanism to limit hepatocyte proliferation after injury.



▶ **Fig. 1**

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P-280 *Bryophyllum pinnatum* fractions inhibit the oxytocin-induced increase of intracellular calcium concentration in human myometrial cells

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Bryophyllum pinnatum is used in the treatment of premature labour [1]. Previous work with myometrial cells showed that *B. pinnatum* juice (BPJ) inhibits the increase of intracellular calcium concentration ($[Ca^{2+}]_i$) induced by oxytocin (OT) [2]. We here compared the effects on this increase of BPJ, a bufadienolide-enriched fraction (BEF [3]), a flavonoid-enriched fraction (FEF [3]), the corresponding flavonoid aglycon mixture (A-Mix [3]), bersaldegenin-1,3,5-orthoacetate (BO), and the combination of BEF and FEF.

hTERT-C3 and PHM1-41 cells loaded with a calcium specific fluorescent probe were pre-incubated with test substances and stimulated with OT. $[Ca^{2+}]_i$ was measured by real-time fluorescence spectrophotometry. Atosiban was used as a positive control.

BPJ led to concentration-dependent decrease of the OT-induced increase of $[Ca^{2+}]_i$ in both cell lines ($p<0.0001$), achieving ca. 75% inhibition at 20 µg/mL concentration. BEF, FEF, BO, A-Mix, and the combination of BEF and FEF led to a concentration-dependent decrease of the OT-induced increase of $[Ca^{2+}]_i$ in hTERT-C3 cells ($p<0.05$). BEF (2.2 µg/mL), FEF (17.4 µg/mL), BO (0.04 µg/mL), and A-Mix (0.7 µg/mL), at concentrations corresponding to 20 µg/mL BPJ led to ca. 25% decrease of the OT-induced increase of $[Ca^{2+}]_i$. The combination of BEF plus FEF led to a decrease of 55.3%.

In conclusion, the data confirm previous observations showing that BPJ promotes a specific and concentration-dependent effect on the OT signalling pathway. Compounds present in BEF and in FEF seem to have a synergistic effect on the inhibition of the oxytocin-induced increase of $[Ca^{2+}]_i$, which is similar to the effect of BPJ.

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P-281 Bufadienolides and anti-angiogenic homoisoflavonoids from *Rhodocodon cryptopodus*, *Rhodocodon rotundus* and *Rhodocodon cyathiformis*

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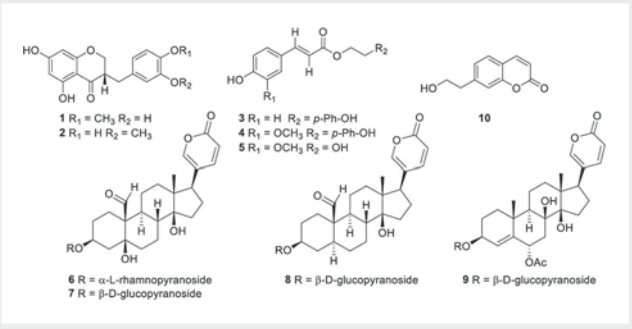
Abnormal retinal vascularization is the leading cause of conditions associated with vision loss in developed countries, such as age-related macular degeneration (AMD) or diabetic retinopathy. [1] The current approach for their treatment is based on inhibitors of the vascular endothelial growth factor (VEGF). However, up to 30% of patients are non-responsive to these drugs, which are also linked to ocular and systemic side effects. [2] For that reason, there is an urgent need for small antiangiogenic molecule leads to supplement the existing biologics. Homoisoflavonoids have previously shown potent antiangiogenic activity *in vitro* and *in vivo* in animal models of ocular neovascularisation, together with promising synergistic effects with existing VEGF inhibitors.[3,4] In this work, the phytochemistry of three species of *Rhodocodon* (Scilloideaea subfamily of the Asparagaceae family), endemic to Madagascar, *R. cryptodus, R. rotundus* and *R. cyathiformis*, was investigated. Two homoisoflavonoids 3S-5,7-dihydroxy-(3'-hydroxy-4'-methoxybenzyl)-4- chromanone **1** and 3S-5,7-dihydroxy-(4'-hydroxy-3'-methoxybenzyl)-4-chromanone **2** were isolated, together with three cinnamic acid derivatives **3-5**, four bufadienolides **6-9** and a coumarin **10** (*Fig. 1*). The antiangiogenic activity of the two homoisoflavonoids was tested against human retinal microvascular endothelial cells (HRECs), giving excellent GI50 results of 0.13 µM and 0.49 µM respectively. Moreover, compound **2** showed a 100-fold specificity for HRECs over other tested cell lines. Its high antiangiogenic activity and promising specificity make compound **2** a suitable candidate in the development of new treatments against ocular neovascularization.



►**Fig. 1** Compounds isolated from R. cryptodus, R. rotundus and R. cyathiformis.

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P-282 *Caenorhabditis elegans* as model to study natural products affecting metabolism and lifespan

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Current drug discovery efforts are mainly focused on target directed approaches, which have certain limitations owing to the complexities of biological systems and disease pathophysiology. Still a major part of new drug entities is discovered by phenotype directed approaches in cells and animals [1]. The screening of natural products in phenotypic rodent models is however hampered by several disadvantages, e.g. financial efforts, legal and ethical considerations, large quantities of test materials, in particular pure isolates and a challenging target deconvolution afterwards. Many of these problems can be avoided by using the simple roundworm *Caenorhabditis elegans* which serves as a convenient and proficient addition to the set of current preclinical model organisms [2]. We recently established a robust *C. elegans* screening platform using 96-well plates for medium throughput screening of extracts and constituents thereof for the discovery of natural products beneficial to the metabolic syndrome. Herein we present approaches and methods for *C. elegans* based preclinical screening using a combination of (i) optimized extract preparation, (ii) lifespan assay and (iii) fat accumulation assay.

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P-283 *Camelina sativa* glucosinolate fraction: NMR characterization and effect on human colon cell lines

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Glucosinolates (GLs) are trending molecules in vegetable-based supplements and in recent years they gained importance in diet due to their antioxidant and anticarcinogenic properties. Usually species belonging to Brassicaceae contain high concentrations of one or a few GLs; among these plants *Camelina sativa* is known for its specific GLs, namely glucocamelinin and glucoarabinin. The aim of this work was to valorize oil-cakes, a by-product derived from the pressing process of *C. sativa* seeds for food applications, given the presence of GLs, a class of metabolites known to be active against tumor cells. Selective extraction for GLs was performed by methanolic and aqueous extractions through maceration processes. After purification through Solid Phase Extraction columns, HPLC analysis was performed on all samples. GLs were the most abundant molecules in the extracts (1.5 mg/mL) as shown by NMR analysis, with small traces of residual lipids; proteins were not found by Bradford assay. Human colon cell lines (healthy CCD841, cancer E705 and CaCo2) were chosen to test the effects of GLs on viability through the MTT assay. First results did not show a considerable effect, but a higher concentration of GLs induced a noticeable selective effect on viability between healthy and cancer cells. Activity of enzymes involved in glutathione metabolism such as glutathione *S*-transferase, glutathione peroxidase, glutathione reductase and enzymes responsible for reactive oxygen species detoxification, such as catalase and superoxide dismutase will be performed through spectrophotometric assays, to evaluate cellular changes in response to a stress that does not induce significant alteration in the cellular viability.

P-284 Cannabidiol-enriched *Cannabis sativa* L. extract modulates inflammatory-induced human peripheral mononuclear cells response

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Recent studies propose non-psychotropic *Cannabis sativa* L. as a candidate drug having a role in the pathogenic mechanisms involved in inflammation [1]. In order to evaluate the biological effect of a chemically standardized extract of *C. sativa* var. *carmagnola* dried female inflorescences (CSE) and its main constituents, the purpose of this study was to investigate the modulation of cannabinoid receptors (CBr) and pro-inflammatory cytokines in an acute inflammatory stress *in vitro* model. CSE was chemically characterized by HPLC-DAD and GC. The CSE biological effect was investigated on human peripheral blood mononuclear cells (PBMC) firstly exposed to the endotoxin LPS (2, 6, 24 hours) in order to evaluate CBr and cytokines regulation. Then, cells were pre-treated with CSE and its main components at the concentration of 1 µg/ml, followed by a 2 hours stimulation with the endotoxin LPS.
CSE was found to contain cannabidiol (CBD) >20%, THC <0.6% and β-caryophyllene as principal sesquiterpene; flavonoids were found only <0.1%. Short term exposure to LPS significantly downregulated CB1r and CB2r gene expression and induced IL-1β, IL-6 and TNF-α release. CBr transcription resulted attenuat by pre-treatment with CSE, and more with CBD. Moreover, the LPS-induced release of the pro-inflammatory cytokine IL-6 was attenuated by CSE and CBD treatment.
C. sativa extract and its main constituent CBD were able to regulate the LPS-induced inflammatory PBMC response through the modulation of CBr expression. These results contribute to support the role of the non-psychotropic cannabis compounds in the management of the inflammatory mechanisms.

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P-285 CBD-A and THC-A content in different hemp varieties

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Hemp (*Cannabis sativa* L.) is one of the oldest cultivated plants that has been used mostly as a source of fibre, oil, food and medicine. It contains many secondary metabolites. The most studied are cannabinoids, especially THC (delta-9-tetrahydrocannabinol) which is psychoactive and CBD (cannabidiol) which is non-psychoactive [1]. Hemp varieties have

generally low content of cannabinoids in comparison with medical marijuana, but in hemp varieties the ratio between CBD and THC could be still 10:1 [2]. The highest content of cannabinoids is in inflorescences, especially in flower trichomes, while seeds do not contain any cannabinoids [3]. The aim of our study was to compare content of cannabinoids CBD-A and THC-A, precursors of CBD and THC in 15 hemp varieties grown in years 2017 and 2018 on two different locations in Slovenia, Žalec and Gornja Radgona. The highest content of CBD-A was achieved in variety 'Antal' (2017) and in variety 'Helena' (2018) and the lowest content in variety 'Santhica' (2017 and 2018). The content of THC-A was the highest in variety 'Tiborszallasi' (2017) and in variety 'Antal' (2018), while the lowest content was determined in variety 'Santhica' (2017 and 2018). Based on this study, farmers will be able to grow hemp varieties with higher content of CBD-A which could be used for pharmaceutical purposes.

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P-286 Challenges in the discovery of natural products effective against acute respiratory infections

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According to the WHO, lower respiratory tract infections are the fourth most common cause of death globally and the first cause in low-income economies. Mainly caused by influenza viruses and rhinoviruses, lethality is heavily boosted by co-infection with *S. pneumoniae* and *S. aureus*. The efficacy of antiviral drugs (e.g. neuraminidase inhibitors, NAIs) and antibiotics is hampered by resistance issues. Remarkably, herbal remedies have been successfully used to reduce respiratory symptoms throughout history.

In a recently finalized project, we used this empirical knowledge for the selection of natural materials for antimicrobial studies, optimized our extraction protocol to avoid testing of promiscuous compounds, and explored the strengths and limitations of cell-based, target-based, and/or *in silico* strategies to evaluate the antimicrobial potential of 162 natural extracts.

As a result, we have established activity/cytotoxicity thresholds and a protocol to discriminate between antiviral and dual-active agents and to characterize their mechanism of action [1, 2]. Further, a screening platform as tool for the identification of novel NAIs and their antimicrobial profiling was established [3]. Our workflow applying a previously adapted extraction protocol for extracts and isolated natural compounds uses a sophisticated set of different assays, which enables us to (i) recognize caveats, (ii) circumvent pitfalls related to assay interferences, and thus to (iii) generate reliable data on natural products targeting acute respiratory infections.

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P-287 Chemical composition and antimicrobial activities of two *Mentha* species essential oils

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Species belonging to the *Mentha* genus have many pharmacological and nutritional properties, with great economic importance in terms of the presence of essential oils (Bhat et al., 2002; Elmasta, 2006; Yadegarinia, 2006).

The purpose of this paper is to analyze the chemical composition of essential oils of *M. spicata* L. and *M. x piperita* L. in order to highlight their antimicrobial activities. The plant material was collected at two different stages of development (vegetative and anthesis). The essential oils were extracted by hydrodistillation according to European Pharmacopoeia standards. The separation and the identification of the components were carried out using GC-MS.

In order to evaluate the antimicrobial activity of the volatile oils, two different methods were used: microplate method and the Kirby-Bauer diffusion method. Chemical analysis of essential oils led to the identification of 52 terpenes, among which linalool, carvone and *p*-menthan-1-ol appeared as major compounds. Following testing of essential oils on *Staphylococcus aureus* and *Escherichia coli* it was contingent that all the analyzed oils showed antimicrobial activity, the minimum inhibitory concentration being of 0.1 % for all tested samples. The diffusion method showed an evident inhibitory action for *E. coli* (40 mm) in the case of *M. x piperita* volatile oil (anthesis stage) versus 19 mm, in *S. aureus* species at the same sample.

It can be concluded that essential oils of *Mentha* species possess great antimicrobial potential and could be used in pharmaceuticals and natural therapies of infectious diseases for humans or management of plant diseases.

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P-288 Abstract see SL AR-02

Abstract see on page 1388

P-289 Chemical composition, direct and indirect antibacterial activity against multidrug resistant bacteria and toxicity assessment of essential oil of *Cymbopogon giganteus* leaves

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Because of antibioresistance rising [1], the search of new alternative strategies by combining classic antibiotics and essentials oils to restore antibiotics efficacy may be a promising approach [2]. The aim of this work was to study the direct and indirect antimicrobial activity of *Cymbopogon giganteus* essential oil from Benin (EOCG) on multidrug resistant bacteria, its chemical composition and its oral acute toxicity. Direct antimicrobial activity was tested by determination of Minimal Inhibitory Concentration (MIC), and indirect activity, by calculating the Fractional Inhibitory Concentration Index using checkerboard (FICI; synergy: FICI ≤ 0.5; additivity: 0.5<FICI≤1) on reference but also on multidrug resistant clinical isolates. Composition was determined by GC-MS and GC-FID. Cytotoxicity was evaluated *in vitro* against human non-cancer fibroblast cell line (WI38) by MTT assay and oral acute toxicity by determination of the "limit dose test" at 2000mg/kg [3]. Limonene (12.07%) and p-menthane derivatives (54.87%) were the major components. Our results confirmed the direct antimicrobial activity of EOCG, but here on clinical resistant strains (MIC from 0.125%v/v to 0.5%v/v). We also observed, for the first time, the synergistic effects between EOCG and amoxicillin with FICI between 0.12-0.5 against two *Escherichia coli* amoxicillin-resistant clinical strains, synergistic to additive effects between EOCG and colistin or oxacillin/ampicillin respectively against *Pseudomonas aeruginosa* PA544 and *Staphylococcus epidermidis* SE361 (two multiresistant clinical isolates). EOCG had a low cytotoxicity (IC50: 67.06±2.69 µg/ml) and no acute toxicity at the dose of 2000mg/kg per os. This is the first report of oral acute toxicity assessment of this essential oil.

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P-290 Inhibition of heat shock protein 90 (Hsp90) by diterpenoids from roots of *Zhumeria majdae*

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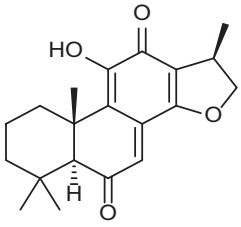
Zhumeria majdae Rech.f. & Wendelbo is an endemic plant in Iran. and an unique member of Zhumeria genus. The constituents of this genus are rarely studied. Roots of *Z. majdae* were extracted with *n*-hexane, ethyl acetate, and methanol, respectively. The phytochemical investigation of the *n*-hexane extract by different chromatographic techniques, such as Silica gel and Sephadex LH-20 chromatography, and RP-HPLC, led to the isolation and identification of two new abietane diterpenes, 1-hydroxysahandone and 1-hydroxy-11-demethylsahandone, and 18 known abietane derivatives [1, 2]. The structures of the isolated compounds were elucidated by 1D and 2D NMR and MS techniques and absolute configurations were established by circular dichroism spectroscopy. A surface plasmon resonance analysis (SPR), MTT assay (Hela & MCF 7 cancer cell lines) western blot (WB) analysis on HSP-90 and several of its client proteins, including HSP-70, p-Akt, Akt, Cyclin A, p-Erk1 and Erk1, along with ATPase activity were implemented to screen a small diterpene library towards HSP90. This chaperone is involved in the turnover, trafficking, and folding of a large number of proteins, including oncoproteins. Thus, there is an interest in the development of new anticancer drugs targeting HSP90 [3]. Results showed that among all isolated diterpenes, lanugon Q showed higher affinity towards HSP90, with SPR KD of 2.98±1.97 nM. The results of WB and MTT assays were in agreement with SPR analysis. However, in the ATPase assay, lanugon Q did not show any inhibition.



lanugon Q

▶ **Fig. 1**

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P-291 Clove oil reduces the aminoglycoside resistance of *Pseudomonas aeruginosa*

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The worldwide emergence of multi-drug resistance (MDR) in clinical *Pseudomonas aeruginosa* strains necessitates the development of novel therapeutic strategies and fuels the investigation of alternative treatment options.

Given that natural substances in essential oils and extracts from plants are ethnopharmaceutically well documented and have long been known to exert antimicrobial activity in traditional medicine [1], we here surveyed the synergistic antimicrobial effects of clove oil (*Syzygium aromaticum* (L.) MEER. & L. M. PERRY) with the aminoglucoside antibiotic gentamicin against distinct clinical MDR *P. aeruginosa* isolates.

The results from determinations of the minimal inhibitory concentrations (MIC) in geometric dilution series and subsequent checkerboard used for calculations of the fractionated inhibitory concentration index (FICI) revealed that clove oil significantly lowered the gentamicin MIC from 8192 mg/l to ≤256. The significance of synergy was confirmed by FIC index values of ≤ 0.0625. Notably, the concentrations of clove oil that increase the antibiotic susceptibility significantly were with 0,25 % far below the MIC of >8% and so did not inhibit the growth of MDR *P. aeruginosa* strains under investigation.

In future investigations further aminoglycoside antibiotic compounds will be analyzed in combination with cloveoil for potential synergistic antimicrobial effects in more clinical isolates of MDR *P. aeruginosa*. In addition, the molecular main components of clove oil such as eugenol and essential oils from other plants will be examined for synergy with other antibiotics.

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P-292 Combination effects of essential oils with antimicrobials

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Antimicrobal resistance is a leading cause of treatment failure for infectious diseases, resulting in increased mortality and morbidity. Several attempts have been made to combine for example essential oils, which are known to have antimicrobial effects, with conventional antibiotics in order to achieve synergistic activity [1].

The antibacterial effects of pine oil (*Pinus sylvestris*), peppermint oil (*Mentha piperita*) and thyme oil (*Thymus vulgaris*) were tested individually and in combinations with rifampicin and tetracycline in a preliminary agar diffusion assay. Thyme oil and pine oil were further investigated. A MIC determining assay was conducted with serial dilutions in 96-well plates. Fractional inhibitory concentration values were calculated and presented as isobolograms.

Synergy was detected in one combination of rifampicin and peppermint oil, whilst several other combinations presented additive results. However, the main part of the tested combinations demonstrated strong antagonism. Thyme oil demonstrated the strongest antimicrobial effect on its own whilst peppermint oil contributed mostly to synergistic interactions.

The results demonstrate that the combination of antibiotics with essential oils can be a promising approach to increase the effectivity of antibiotics, although further studies must be conducted to determine the adequate antibiotic-to-oil ratios.

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P-293 Combinatory effect of plant compounds and their derivatives with conventional antibiotics on diarrhoea causing bacteria

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Diarrhoeal infections are major cause of morbidity and mortality of children globally [1]. Moreover, emerging antibiotic resistance complicates diarrhoea treatment. Combination of compounds and antibiotics is considered as a new prospective strategy for overcoming bacterial resistance. For example, augmentin (amoxicillin and clavulanic acid) is used to treat certain bacteria-caused diseases such as urinary tract infections. In long time practice, various plant-derived compounds such as berberine sulphate and berberine hydrochloride are useful for diarrhoea control [2]. However, their combinatory effect with conventional antibiotics against diarrhoea causing bacteria has only poorly been investigated.

In this study, we tested *in vitro* combinations of plant compounds (e.g. berberine chloride, tannic acid and sanguinarine) and their derivatives (e.g. 4, 8-hydroxy-5-nitroquinoline and zinc pyrithione) with selected conventional antibiotics (e.g. ciprofloxacin and tetracycline) against standard strains of diarrheagenic bacteria (e.g. *Escherichia coli* and *Shigella* sp.). Minimum inhibitory concentrations of each agent and antibiotics were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute [3] guidelines, whereas the combinatory effect was evaluated according to the sum of fractional inhibitory concentration (ΣFIC) indices obtained by chequerboard method [4].

Several combinations (e.g. sanguinarine with tetracycline) showed additive effects against the majority of bacterial strains tested with ∑FICI ranging from 0.507 to 0.531, whereas the synergistic activity exhibited combination of sanguinarine with ciprofloxacin (∑FICI 0.281 - 0.375) against *Vibrio parahaemolyticus* and *Shigella flexneri*.

The results can be used by food and pharmaceutical industries for development of new herbal-based food and pharmaceutical preparations.

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P-294 Complexity – another bit of the puzzle in the standardization of testing methods to determine efficacy of natural products

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Both EUCAST and CLSI specify Mueller Hinton agar for evaluating antimicrobial susceptibility using the Kirby-Bauer disc-diffusion method [1, 2]. While a number of factors can affect the results obtained (for example, how the bacteria is applied to the agar or depth of agar) neither organization recommends a particular manufacturer of the Mueller Hinton agar (M-H). Antimicrobial susceptibility is evaluated in terms of specific breakpoints (MIC and inhibition diameter) for antimicrobial agents (antibiotics) at specified dosages.

This research investigated any variability in results of antimicrobial susceptibility testing of natural products. M-H from three different manufacturers was investigated using two control strains of *Staphylococcus aureus,* namely ATCC 29213 and ATCC 25923 using the well-diffusion method.

The results showed that the source of the M-H had significant effects on the antimicrobial bioassay.

In conclusion, the source of the Mueller Hinton agar used in antimicrobial susceptibility testing would appear to be another variable that needs to be considered when investigating the antimicrobial properties of natural products.

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P-295 Computational investigation of multi-target effects in the arachidonic acid cascade on the example of potent natural 5-LO inhibitor garcinoic acid

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Background Secondary plant metabolites often exert their effects by affecting not only one primary target but by interacting with a network of related proteins. The arachidonic acid (AA) cascade is a key pathway in inflammation, which metabolizes AA to a variety of pro and anti-inflammatory mediators. Due to the similarity of the lipid mediator substrates in the pathway, many involved enzymes share common features in their binding sites, and dual or multiple inhibition of the AA cascade is not uncommon [1].

Aims: This work aims to elucidate the similarities between the binding pockets of several AA cascade enzymes, showing why multi-target interaction among these targets is prevalent in many anti-inflammatory natural products. For this purpose, garcinoic acid, a derivative of vitamin E was docked into the enzyme structures from the AA cascade to investigate common binding patterns. Garcinoic acid inhibits 5-lipoxygenase in the nanomolar range (IC50 = 35 nM) and also shows weaker effect on other investigated enzymes: cyclooxygenase 1 in human platelets, IC50 = 6.9 µM, human recombinant leukotriene C4 synthase synthase, IC50 = 9.9 µM, and microsomal prostaglandin E2 synthase, IC50 = 8.8 µM [2].

Results Docking studies revealed an alternative binding site on 5-LO, which was shown to be similar to the binding site of mPGES-1. Overlapping binding site interaction patterns could also be identified with LTC4S.

Conclusion Multi-target effects on related enzymes can be computationally rationalized by binding site comparison, giving us the tools to anticipate such effects and to intentionally search favourable target combinations.

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P-296 Cryptotanshinone from *Salvia miltiorriza* roots reduces Cytokeratin 1/10 expression in keratinocytes by activation of peptidyl-prolyl-*cis-trans*-isomerase FKBPO1A

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Cryptotanshinone CTS (1 µM) from the roots of *Salvia miltiorrhiza* exerts a strong influence on the terminal differentiation of human keratinocytes (HaCaT cell line, primary natural human keratinocytes) and downregulates the expression of differentiation-specific cytokeratins CK1 and CK10 on protein and gene level. Other differentiation specific proteins such as involucrin, filaggrin, loricrin and transglutaminase were not affected to a high extend. CTS (1 µM) did not influence the cell viability and the proliferation of keratinocytes. Using a combination of Drug Affinity Response Target Stability Assay in combination with a proteomic approach and multivariate statistics for target elucidation, peptidyl-prolyl-*cis-trans*-isomerase FKBP1A (known target of inhibitors such as tacrolimus or rapamycin) was addressed as a potential molecular target of CTS. The interaction of CTS with FKBP1A was additionally shown by thermal shift and enzymatic activity assays. Interestingly, CTS served as an activator of FKBP1A, which led to a reduced activity of the TGFβ receptor pathway and therefore to diminished CK1 and CK10 expression. The combination of the FKBP1A activator CTS with the inhibitor tacrolimus neutralized the effects of both compounds. From these data, a potential dermatological use of CTS and CTS-containing plant extracts (e.g., hydroalcoholic extract from the roots *of S. miltiorrhiza*) for keratinopathic ichtyosis, a disease characterized by overexpression of CK1 and CK10, is suggested. This study displays an experimental strategy for combining phytochemical aspects on active natural products with systematic identification of molecular targets on the gene, protein and cell level.

P-298 *Cystoseira barbata* C. Agardh from Romanian Black Sea coast: phlorotannins profile, *in vitro* antioxidant and antiproliferative potential

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The Romanian Black Sea has abundant seaweed resources, but little effort has been done to screen their biological potential. The aim of the present study was to assess the *in vitro* antioxidant and antiproliferative activities of *Cystoseira barbata*Sargassaceae a brown alga inhbiting the costal line of Romanian Black Sea. 70% acetone, methanol and water were used for the extraction of the algal material. The antioxidant activity of *C. barbata* extracts was assessed by DPPH and ABTS radicals scavenging assays, reducing power and 15-lipoxygenase inhibition assays. Cytotoxic activity of the extracts was determined by MTT viability assay using three adenocarcinoma cell lines (mammary MCF-7, alveolar A549 and colorectal HT-29). The effect of the extracts in cell cycle distribution was evaluated using flow cytometry (FACS) [1,2]. 70% acetone extract exhibited the highest antioxidant activities in all assays, with IC_{50} values comparable or higher than those of known antioxidants. The antitumor tests showed that 70% acetone extract had the most potent antiproliferative effects. MCF-7 was the most sensitive of all cell lines examined. FACS analysis revealed that the 70% acetone extract induced a significant

increase in the subG1 fraction in MCF-7 and A549 cells, indicative of apoptosis induction. From these findings, the 70% acetone extract was investigated by HPLC-DAD-QTOF-ESI-MS/MS [3]; phlorotannins with molecular weights ranging from 375 to 870 Da were tentatively identified on the basis of their characteristic MS/MS fragmentation pattern. In conclusion, *Cystoseira barbata* represents an important source of bioactive compounds, with potential use in food and pharmaceutical industries.

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P-299 Cytotoxic activities of new water-soluble polysaccharides from *Ornithugalum bungei*

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The importance of various polysaccharides as an anti-tumor agent has a long historical background [1]. *Ornithogalum bungei* Boiss. (Hyacinthaceae), is an Iranian native plant distributed in Golestan province [2]. The initial screening of cytotoxic activities of the extracts from different parts of the plant showed the promising result for the bulbs. Therefor in the present study detailed investigation of cytotoxic effects of the bulb extracts on human hepatocarcinoma cell line (HepG2), prostate cancer cell line (PC3) and Human myelogenous leukemia (K562) were carried out as well as isolation and structure elucidation the active compounds. The method was based on bio-assay guided fractionation. AF3400024ter each step of chromatography all fractions were assayed on the mentioned cells. Finally two new water-soluble polysaccharides, OBP_1 and OBP_2 with molecular weights of 56.2 kDa and 97.9 kDa respectively, were isolated. Purification of the polysaccharides was carried out using hot water extraction and further purification methods such as DEAE-cellulose A52 and Sephadex G-100 columns. The linkages of polysaccharides were determined by using methylation method. OBP_1 was composed of glucose (Glc), galactose (Gal), arabinose (Ara) and mannose (Man) in a molar ratio of 10.8:3.52:3.72:1.97 and OBP_2 was composed of glucose (Glc), galactose (Gal), arabinose (Ara), mannose (Man) and glucoronic acid (GlcA) in a molar ratio of 7.48:4.01:3.09:4.13:5.05. The uronic acid content of OBP_2 was about 20%. Two polysaccharides exhibited significant cytotoxic activity in a concentration- independent manner against HepG2, K562 and PC3 cells. Results suggested that these polysaccharides could be a potential natural cytotoxic agents.

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P-300 Dietary polyphenols and their immunomodulating effects: implications during parasite-induced inflammation in the gut

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Polyphenols are a group of intensively studied compounds and their diverse effects on the immune system have encouraged a multitude of interdisciplinary research to study them. We are investigating proanthocyanidins, which are among the most common dietary polyphenols, to assess their impact on gut inflammation caused by a helminth infection.

In this study, were purified from cocoa, grape seeds and alpine currant, by series of extractions, ephadex separation (19 samples), and semi-preparative liquid chromatography (152 samples). The samples were analyzed by Ultra igh Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS), and their mean degree of polymerization (mDP) and procyanidin/prodelphinidin ratios were assessed.

In order to identify the most active compounds, each ephadex sample was initially tested in-vitro on murine RAW 264.7 macrophages, to assess impact on cytokine secretion. Seven of the eight grape seed fractions reduced IL-6 secretion, and we found a correlation between high mDPs and high cytokine secretion for the fractions of alpine currant. The cocoa fractions had low mDPs and showed limited IL-6 suppression.

Following further in-vitro investigations, active samples will be selected to assess their effects on mucosal immune responses to parasitic infection in a mouse model.

P-301 *Dischidia nummularia* – a potential plant for cytotoxicity and anti-proliferative activity

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Cancer treatment is one of the biggest challenges of the current time as it has high morbidity and mortality rates worldwide, with more than 18 million new cases and above 9 million deaths in year 2018, according to last statistics [1]. Natural sources have enormous potential for the discovery of new drugs against cancer, since 93 drugs approved for the treatment of cancer in the last forty years were from these sources [2]. An epiphytic plant found in north Sumatra Indonesia, *Dischidia nummularia* R.Br. (Apocynaceae) is used traditionally used for the treatment of cancer [3].

The aim of this study is to find out the relevant constituents of this plant, which are responsible for this activity. Activity-guided isolation of the different extracts (n-hexane, dichloromethane and methanol) of the plant was carried out using human cancer cell lines and different chromatographic and spectroscopic techniques (LC, LC-DAD-MS, GCMS and NMR) for bioassay, isolation and analysis of the chemical constituents, respectively. The *n*-hexane and dichloromethane extracts of the plant showed strong activity against various cancer cell lines (MDA-MB-231 and CCRF-CEM). 2H-Chromene was isolated for the first time from the *n*-hexane extract of this plant. In addition, a large number of fatty acids were isolated from the plant along with glycerol-mono-stearate that was also found to be moderately cytotoxic against the cancer cell lines. Isolation of further active compounds is in progress.

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P-302 Discovery of $GABA_A$ receptor modulators of natural origin – validation of a FLIPR assay for screening and HPLC-based activity profiling

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Gamma-aminobutyric acid type A ($GABA_A$) receptor modulators are used to treat epilepsy, insomnia, anxiety, and mood disorders. However, currently used drugs lack receptor subtype selectivity and, therefore exhibit various side effects. Moreover, the scAF3400027fold diversity of synthetic drugs and experimental compounds targeting $GABA_A$ receptors is limited. Natural products such as piperine have been reported as allosteric $GABA_A$ receptor modulators interacting with a benzodiazepine-independent binding site. For screening of large extract libraries and efficient localization of active compounds via HPLC-based activity profiling we established a Fluorometric Imaging Plate Reader (FLIPR) membrane potential assay utilizing stably transfected Chinese Hamster Ovary (CHO) cells expressing $GABA_A$ receptors of $\alpha_1\beta_2\gamma_2$ subunit composition. Assay protocols for rapid screening of plant extract libraries and localization of active compounds in extracts were validated with known GABAergic natural products. An HPLC-based activity profiling protocol was developed. Extract separations (0.4 to 1.2 mg) on an analytical HPLC column were sufficient for the sensitivity of the bioassay. The protocol successfully localized the activity of magnolol in *Magnolia officinalis*, valerenic acid in *Valeriana officinalis*, and piperine in *Piper nigrum* extracts. EC_{50} values of compounds (magnolol: 4.81 ± 1.0 µM, valerenic acid: 12.56 ± 1.2 µM and piperine: 5.76 ± 0.7 µM) were found to be comparable or lower than those reported using *Xenopus* oocyte assays. The FLIPR assay is now used for the screening of a large extract library and identification of new GABAergic natural products via HPLC-based activity profiling.

P-304 Effects of *different* essential oils on HaCaT keratinocytes against hydrogen peroxide induced oxidative stress

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Many essential oils have found applications in pharmaceutical and cosmetic products. They are frequently used as antibacterial, antifungal and antiviral and antioxidant compounds. Essential oils may exhibit cytotoxic effects on skin cells. They can sometime cause skin irritations and allergic reactions. Citronellol, citral, cinnamal, geraniol, linalool, d-limonene, eugenol, hydroxyl-citronellol are some essential oils compounds with well-known allergenic properties.

The effect of different concentrations (0.001, 0.01, 0.1 and 1% (v/v)) of common juniper (*Juniperus communis),* oregano (*Origanum vulgare),* laurel *(Laurus nobilis),* lavender *(Lavandula officinalis), sage (Salvia officinalis)* and myrtle (*Myrtus communis) essential oils* on human skin cells HaCaT in vitro was explored. Essential oils effect on hydrogen peroxide (H_2O_2)-treated keratinocyte HaCaT cells were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Essential oils were extracted with hydrodistillation.

This study has demonstrated that all essential oils were cytotoxic to human keratinocytes *in vitro* at a concentration of 1% (V/V). At a concentration of 0.1%, all essential oils, with the exception of *Laurus nobilis L.*, were still irritating. At lower concentrations all essential oils showed no toxicity and no protective effect on HaCaT human keratinocytes from oxidative stress induced by exposure to hydrogen peroxide.

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P-305 Effects of oral co-administration of captopril and *Hibiscus sabdariffa* on blood pressure and histological changes on 2K1C animal model

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The Two Kidney One Clip (2K1C) animal models are commonly used during preclinical studies related to renovascular hypertension [1]. *Hibiscus sabdariffa* (HS) aqueous extract has angiotensin converting enzyme (ACE) inhibitory activities and is often co-administered with captopril (CAP) during management of hypertension therapy. [2] In this study, we compared the effect of CAP plus HS aqueous extract co-administration to CAP and HS administration alone on blood pressure and histological changes in clipped kidney of 2K1C animal models. The 2K1C Sprague dawley rats were randomly divided into 4 groups i.e. negative control (no treatment), positive control (4.5 mg captopril/200 g BW), HS alone (30 mg/200 g BW), and coadministration group (30/200 g BW HS and 4.5 mg/200 g BW CAP). The CAP and HS extract were given by oral gavage for two weeks.

All treatment groups showed significant blood pressure reduction compared to negative control. There is no significant difference ($p > 0.05$) between measured blood pressure of rats in CAP alone group and CAP plus HS co-administration group. The size of clipped kidney was significantly reduced compared to unclipped kidney. Clipped kidney of 2K1C groups showed the symptom of nephritis.Treatment with HS alone and co-administration of CAP plus HS could not stop the mononuclear infiltration and necrosis progress of clipped kidney. Kidney's specimen of CAP alone showed a normal tubules profiles however the abnormal accumulation of mononuclear cells was similar with the 2K1C groups. Co-administration of CAP plus HS didn't give both additional blood pressure reduction and significant effect on histological changes in 2K1C animal models, therefore co-administration of *H. sabdariffa* L. aqueous extract with captopril should be reconsidered.

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P-306 Effects of *Oroxylum indicum* (L.) Kurz extract on lipid profile of high fat diet-induced mice

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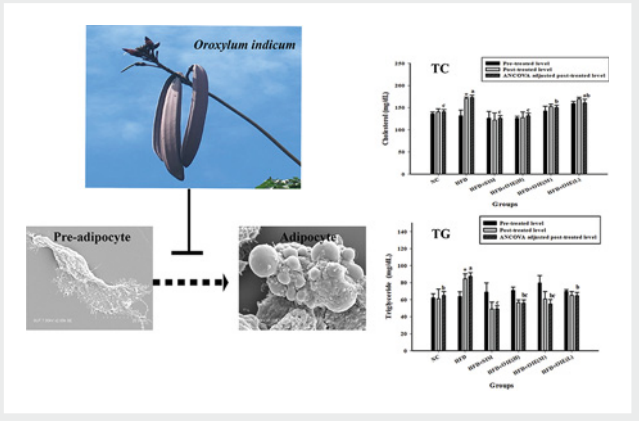
Oroxylum indicum has been known as traditional herbal medicine, its widely found in Asian countries [1]. Our previous study showed that the fruits of *O. indicum* extract (OIE) were remarkably reduced the lipid accumulation in 3T3-L1 adipocytes (*In vitro*) [2]. This study was aimed to assess the effect of the OIE on the lipid level in high fat diet-induced mice. Forty-eight of male mice were divided into 6 groups; (1) Normal control (NC); (2) High fat diet (HFD); (3) High fat diet treated with 25 mg/kg/day simvastatin (positive control); (4) High fat diet treated with 100, 200, and 300 mg/kg/day of OIE, respectively. At the end of the experiment (4 weeks), mice were fasted overnight, and blood samples were collected to measure total serum cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). During the study, food intake, body weight, and toxicity signs were measured. The results demonstrated that OIE did not show any visible signs of toxicity. Food intake and body weight were also no significant difference between HFD and OIE treated group ($P < 0.05$). The TC and TG of OIE treated groups were significantly lower than the HFD group in a dose-dependent manner ($P < 0.05$), while LDL and HDL levels showed no significantly different. In conclusion, the OIE has potential on decreased of lipid profiles in hyperlipidemic mice. Thus, it could be a benefit for further long term investigation to prevent hyperlipidemia.



▶ **Fig.1**

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P-307 Effects of *Vernonia cinerea* L. on uterine contraction in gestational diabetic rats

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Gestational diabetes is the serious metabolic disorder, which causes maternal and fetal disability and may associate with poor myometrial contractility. *Vernonia cinerea* L. is widely used in Thai traditional medicine with an anti-hyperglycemic property. The effects of *V.cinerea* L. on pregnant diabetes have never been investigated. This study was to evaluate the effects of V.cinerea L. crude extract on myometrial contraction in diabetic pregnant rats. After mating, the animals were divided into two group (five for each); non-gestational diabetic rats (non-GD) and gestational diabetic rats (GD). At day 5 of pregnancy, diabetes was induced by streptozotocin (60 mg/kg) i.p. At day 20 of pregnancy, both groups of animals were sacrificed. Longitudinal muscular strips were dissected from uterus and placed in the organ bath containing Krebs'solution for tension measurement. Control period was taken in stable spontaneous contraction 30 minutes before the exposure to high K^+ depolarization (KCl), oxytocin (OT), or *V.cinerea* L. crude extract. There were significantly decreased frequency, amplitude and area under the contraction (AUC) with prolonged duration in GD. When exposure to KCl and OT, the responses were significantly reduced in GD compared with non-GD. However, *V.cinerea* L. crude extract was significantly increased the frequency and AUC in both non-GD and GD. Impaired uterine contractility was found in gestational diabetes. The application of *V.cinerea* L. crude extract stimulated uterine contraction in both non-GD and GD. The treatment of *V. cinerea* L. crude extract may be helpful for preventing miscarriage or preterm birth, which is commonly found in diabetes pregnancy.

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P-309 Enzyme inhibitory, antioxidant activities and phytochemical studies on *Juniperus macrocarpa* & *Juniperus excels*

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Diabetes mellitus and obesity are metabolic disorders that are spreading day by day. Some species belonging to *Juniperus* are used ethnopharmacologically for the treatment of diabetes mellitus [1]. The main aim of this study is to determine the inhibitory activity of branches, fruits, and leaves of *J. macrocarpa* Sibt. & Sm. and *J. excelsa* Bieb. (Cupressaceae) against α-glucosidase, α-amylase, and pancreatic lipase *in vitro*. For the determination of the activities, ethyl acetate, methanol, and aqueous extracts were prepared for each part of the plants. The antioxidant activities of the extracts were also examined with different assays. Additionally, the amounts of amentoflavone, agathisflavone and umbelliferone were analyzed with HPLC-UV.
All extracts showed high and dose-dependent inhibitory effect on α-glucosidase enzyme. *J. macrocarpa* branch methanol extract (99.78 ± 0.03%), was more effective than acarbose at 1 mg/ml. When the α-amylase inhibitor effects of the extracts were examined, the highest effect was observed in *J. excelsa* leaf methanol extract at a concentration of 1 mg/ml (65.27 ± 1.90%). At 3 mg/ml dose of *J. macrocarpa* branch ethyl acetate extract (77.37 ± 7.75%) displayed potent pancreatic lipase enzyme inhibitor activity. As a result of the HPLC study, the amounts of amentoflavone were found to be higher than the other compounds in the extracts. The highest amount of amentoflavone was calculated as 0.77 ± 0.00% in *J. macrocarpa* leaf ethyl acetate extract. Consequently, *J. macrocarpa* and *J. excelsa* may be considered as

a source of isolation of novel compounds having antidiabetic and antioxidant activity. The *in-vivo* studies would be performed in further studies.

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P-310 Eupatoriopicrin enhance the phagocytosis through modulation of *S.aureus* uptake and killing by THP-1 cells

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Phagocytes play an essential role in the host defense against pathogens. The recognition of *S.aureus* can be promoted either by opsonins (opsonic phagocytosis), or by binding to surface receptors (nonopsonic phagocytosis). The ability of macrophages to directly interact with nonopsonized bacteria depends on the recognition by Toll-like receptors (TLRs) [1]. Following uptake, the killing of *S.aureus* is closely related with MAP kinases promoting of trafficking the bacteria into mature phagolysosomes [2].

The treatment of *S.aureus* infections is still poorly ensured and searching new drugs is highly needed. We tested the hypothesis that eupatoriopicrin through enhancement of phagocytosis suppress growth of *S.aureus* in phagocytes (THP-1 cells). To verify this hypothesis we evaluated influence of eupatoriopicrin tested at concentration range 2.5–0.5 µM on (I) uptake of *S.aureus* by THP-1 cells performed by flow cytometry; (II) killing ability of THP-1 performed by CFU analysis; (III) MAP kinases modulation analyzed by westernblotting; (IV) cytokines release performed by ELISA tests and (V) morphology of THP-1 analyzed by confocal microscopy.

Our observations showed that eupatoriopicrin-treatment enhanced the phagocytosis through significant increase of the number of phagocyted bacteria. We noticed correlation between modulation of MAP kinases signaling and suppression of *S.aureus* growth which may be relate with promotion of phagolysosomal acidification. The modulation of MAP kinases also resulted in inhibition of IL-1β, TNFα and IL-8 release.

Our observations indicate the promising benefits for enhancing the canonical killing of pathogens through phagocytosis using plant-derived compounds.

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P-311 Eupatoriopicrin may modulate lipopolysaccharide-induced inflammation in human respiratory epithelium

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Lungs represent the largest epithelial surface in the body and is a major portal of entry for pathogens. Lipopolysaccharide (LPS) is an important antigenic component of Gram-negative bacteria, and is a potent stimulus to local and systemic immune responses. The stimulation of Toll-like receptor 4 (TLR4) by LPS activates Myd88 pathway, what leads to release of proinflammatory cytokines, and, consequently, increase chemotaxis of leukocytes. Prolonged exposure to LPS significantly decrease cell viability of epithelial cells and intensify the necrosis.

Therefore we tested the hypothesis that eupatoriopicrin may modulate LPS-induced inflammatory response. To verify this hypothesis we used lung adenocarcinoma cells (A549) and normal bronchial epithelial cells (NHBE) and verified the influence of eupatoriopicrin (tested at 0.25–2.5 µM) on: (I) integrity of cellular membrane using propidium iodide staining, (II) expression of adhesive molecules analyzed by flow cytometry, (III) modulation of TLR4-Myd88 pathway on epithelium using flow cytometry and immunoblotting analysis, (IV) cytokine release performed by ELISA tests and (V) apoptosis of epithelium analyzed by flow cytometry.

Our observations showed that eupatoriopicrin-treated cells significantly decreased TLR4 expression resulted in Myd88 phosphorylation level decrease in A549 cells. We noticed suppression of the release of IL-6, IL-8 and TNFα in both epithelial models. Interestingly, we observed that eupatoriopicrin decreased the number of necrotic cells and increased apoptosis.

Eupatoriopicrin through modulation of the LPS-induced signal transduction is interesting candidate for further evaluation as potential therapeutic agents in a treatment of inflammation-based pulmonary diseases.

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P-313 Evaluation of onconutraceutical potential and chemical characterization of vegetable smoothies

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The research towards onconutraceutical products not only for cancer prevention but also as a valid support to the pharmacological therapies is of growing interest. Doxorubicin is one of the most potent and widely used chemotherapeutic agents for various tumors, as breast cancer. However, doxorubicin clinical application is limited by cumulative and dose-related cardiotoxicity, which may lead to congestive heart failure [1]. Thus, this study aims to identify possible nutraceutical matrices able to reduce the doxorubicin toxicity without modifying its antineoplastic activity on breast cancer cells.

We evaluated the onconutraceutical potential of smoothies polyphenolic extracts from orange (*Citrus sinensis*) and red grape (*Vitis vinifera*), and 3 different mixes, composed by different ratios of the two matrices, on embryonic rat heart-derived cells (H9c2) and human breast adenocarcinoma cells (MCF-7), also in presence of doxorubicin.

The tested extracts, as well as the relative mixes, don't exhibit a significant antiproliferative activity on H9c2 and MCF-7. In doxorubicin-treated cells, the orange and the grapes extracts, and the 3 mixes, reduce the antiproliferative activity induced by doxorubicin on H9c2. Interestingly, the doxorubicin antiproliferative activity on MCF-7 cells was unaltered, in presence of the tested extracts. In particular, the more effective smoties' mix, 1:1 ratio, was able to reduce the doxorubicin-induced reactive oxygen species release, and to increase the expression of antioxidant cytoprotective enzymes in cardiomyocytes.

Our results indicate that the smoothies polyphenolic extracts could be usefull as onconutraceutics, in order to reduce the some doxorubicin-induced side effects.

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P-314 Exploitation of genome mining tools for accelerated natural product discovery

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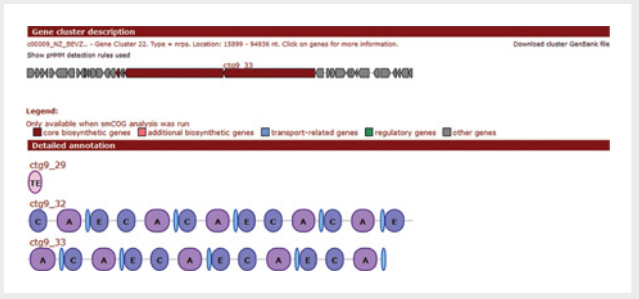
Streptomycetes are rich sources of new bioactive natural products. Their genomes typically contain over twenty biosynthetic gene clusters encoding for secondary metabolite pathways [1]. One of those are nonribosomally produced peptides (NRP). They represent a structurally diverse family of natural products with a broad spectrum of biological activities, e. g. antibiotic, anti-cancer and immunosupressive [2, 3]. Clusters encoding for nonribosomal peptide synthetases (NRPS) can be easily identified by genome mining, i. e. screening and identification of these clusters employing bioinformatics [4].

Aim of the project is to identify novel gene clusters by genome mining and to isolate the predicted products of these clusters as well as to evaluate their bioactivity. An interesting candidate is *Streptomyces fragilis* which is predicted to produce an NRP by two NRPS (▶**Fig. 1**).

In order to isolate the predicted NRP, the substrate specificity of the adenylation domains of the two NRPS were determined by cloning, overexpression and γ-$^{18}O_4$-ATP-pyrophosphate exchange assay [5]. l-Valine was found to be the predominant substrate for six out of nine tested adenylation domains. To identify a valine-rich natural product produced by *S. fragilis*, ^{14}C-valine was fed to a 25 ml culture. Culture extracts were analyzed by radio-HPLC and radio-TLC for radioactive compounds. One radioactive product was detected. Its identification is in process.

Taken together, we have identified a cluster encoding for two NRPS with unusual substrate specificity which predicts the ability of this two NRPS to produce a new secondary metabolite.



▶**Fig. 1** A gene cluster encoding two NRPS, which are predicted to produce an interesting NRP [4].

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P-315 Exploration of α-Pyridone-containing compounds as multitarget antifungals

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There is an endless search for more reliable and effective antifungals. α-Pyridone-containing compounds can be thus considered as an important moiety for their development [1], possessing multitarget action. Thus, as part of our research on antifungal agents, an *in-house* collection of natural and synthetic α-pyridone-containing analogs (n = 68) were studied by molecular docking and molecular dynamics simulations within the active site of 65 fungal enzymes, along with broth microdilution test on *Candida albicans* and *Fusarium oxysporum*. Results indicated that tri and tetracyclic-containing α-pyridone analogs were found to exhibit the best docking scores and interaction profile with *N*-myristoyltransferase (NMT), lanosterol 14α-demethylase (LDM) and the respective Upc2 transcription factor as well as *in vitro* antifungal effect. Resulting docking scores were appropriately correlated with experimental inhibition against fungi using supervised statistics, and this antifungal dataset correlated very-well with molecular interaction fields provided by Comparative Molecular Field Analysis (r^2 > 0.9, q^2 > 0.7). 3-ethoxycarbonyl-1-(3,4-methylendioxybenzyl)-2-methyl-α-pyridone, a very antifungal compound (IC_{50} < 0.1 μM), was found to interact with NMT, LDM and Upc2TF according to the *in-silico* performance. Computational protein:ligand interaction profiles exhibited a common receptor contact which contributes to the non-polar stabilization. The hits and structural requirements can be used in the further development of antifungals based on α-pyridone analogs, being an excellent starting point for future studies on structural optimization of this kind of analogs towards development of multitarget antifungal drugs

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P-316 Exploring the traditional medicine of Atacama people from Northern Chile as in inestimable source of bioactive compounds

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Background Traditional medicines from Northern Chile have not been extensively studied [1]. Nevertheless, they should be considered as a potential source of bioactive compounds, especially in the fields of infectious and neglected tropical diseases, chronic inflammation and multiple drug resistance.

Aim Identification of bioactive compounds isolated from plants used in the medicine of Atacama people, following traditional indications.

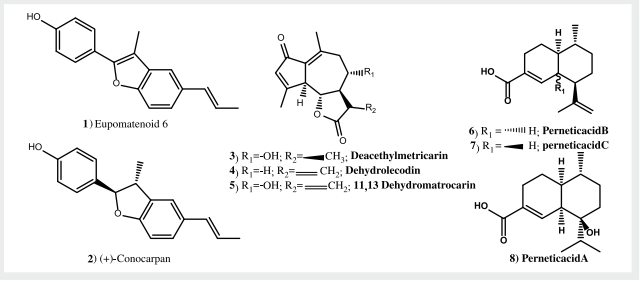
Results A collaborative work within the Atacama community of Taira (Calama, Chile) has led to the collection of 18 medicinal plant species based on their traditional medicinal use.

Anti-bacterial screening has resulted in the identification of *Aloysia deserticola* and *Krameria lappacea* EtOAc extracts being the most active against the growth of Gram-positive bacteria strains (5-100 µg/mL). After bioguided fractionation, the neolignan (+)-conocarpan from *K. lappacea* has demonstrated the lowest MICs values (1-5 µg/mL).

Regarding the anti-parasitic activity, *Artemisia copa* and *Azorella atacamensis* showed IC_{50} values between 1-10 µg/mL against Plasmodium, Leishmania and Trypanosoma strains. Several sesquiterpene lactones and diterpenes bearing an endoperoxide group were isolated, their biological evaluations are under progress.

The EtOAc extract of *Fabiana denudata* has shown a strong anti-inflammatory effect: it has inhibited 70% of TNFα production at 1 µg/mL. Polymethoxyflavones and sesquiterpenes were isolated, their evaluation is undergoing.

Conclusion The Taira community traditional medicine is rich of bioactive compounds, such as neolignans and endoperoxide diterpenes active against the growth of pathogenic bacteria and parasites, respectively, and sesquiterpenes with anti-inflammatory potential. These encouraging results will be completed with deeper mechanistic studies, as well as synergistic evaluation with reference antibiotics.



▶**Fig. 1** Some examples of isolated compounds from active extracts presenting different biological activities.

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P-317 Further insights in the antiproliferative activity of cardenolides from the aerial parts of *Pergularia tomentosa*

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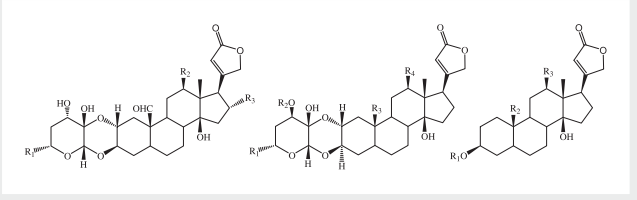
Pergularia tomentosa (Asclepiadaceae) is a milkweed tropical plant used in traditional medicine for the treatment of several diseases such as bronchitis, constipation and skin diseases [1-3]. Previously we reported the occurrence of cardiac glycosides with *trans* fused A/B rings of the steroidal skeleton and with a single sugar, linked to the 2α- and 3β-positions of the aglycone by hemiketal and acetal functions respectively [1,3,4]. Isolated cardenolides inhibited cell viability of PC3, Hela, Calu-1, MCF-7, and U251MG cell lines exhibiting IC_{50} values ranging from 0.2 to 8.0 µM [4]. Herein, the ability of the isolated compounds to inhibit cell viability of Hepg2 (liver hepatocellular carcinoma) cell line has been evaluated. Of all tested compounds, calotropin and calactin showed the highest activity with IC_{50} values of 0.83 µM and 0.11 µM, respectively. Chemically, these compounds differ in the stereochemistry of C-3′ of the sugar linked to the aglycone. With the aim to deeper explore their ability to inhibit cancer progression, the effects on cellular migration and proliferation were also investigated. Our results showed that calotropin and calactin at 1µM inhibited cell migration (wound healing assay) and proliferation (fase S entry) of the Hepg2 cancer cell line. They induced apoptosis associated with activation of caspase-3 and increased p53 expression, more than 35% and 45%, respectively, when compared with staurosporine used as positive control. Moreover reduction of GRP78, a general ER-stress marker was also observed. These findings suggest that selected compounds from *P. tomentosa* are potential leads to be explored as anti-cancer agents.



▶**Fig. 1**

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P-318 Glycerol extracts of *Glycyrrhiza glabra*, *Echinacea purpurea*, *Silybum marianum* and *Berberis vulgaris*: chemical characterisation and cosmeceutical potential

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Natural ingredients in cosmetic products may prevent or even reverse changes caused by ageing such as hyperpigmentation, loss of elasticity or skin hydration. Furthermore, they may hinder inflammatory processes in skin. The aim of this work was to prepare and compare the extracts of four medicinal plants (i.e. *Glycyrrhiza glabra, Echinacea purpurea, Silybum marianum*, and *Berberis vulgaris*) suitable for use in cosmetic products. To achieve this, glycerol – a natural, non-toxic, eco-friendly liquid, capable of acting both as solvent and humectant in cosmetic products – was used. The content of species-specific substances such as isoliquiritigenin, glabridin (*G. glabra*), caftaric and cichoric acid (*E. purpurea*), flavonolignans (*S. marianum*), and berberine (*B. vulgaris*) was determined using RP-HPLC-DAD. In addition, DPPH radical scavenging activity of the extracts, activity in ß-carotene linoleic acid assay, as well as antiinflammatory (ovalbumin coagulation assay) and tyrosinase-inhibiting activity were assessed. The results demonstrated that glycerol efficiently extracted the active components of the selected medicinal plants. Even though all the prepared extracts were capable of slowing down thermally induced ß-carotene-linoleic acid oxidation and scavenged DPPH free radicals, the most active in all the employed assays were *G. glabra* extracts. They were also able to inhibit up to 80% heat-induced albumin coagulation. Furthermore, *G. glabra* extracts inhibited over 80% tyrosinase activity, even when diluted in 1:100 ratio. The results indicate that the glycerol extracts of all the investigated plants and especially of *G. glabra*, are promising ingredients of cosmeticeutical products due to their antioxidant, skin whitening, and antiinflammatory properties.

P-319 Greek mountain tea, a medicinal plant from Mediterranean countries and Balkan

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DOI 10.1055/s-0039-3400041

Greek mountain tea, *Sideritis scardica*, has a long history in traditional medicine. Currently, *S. scardica* is investigated for its pharmacological activity in the central nervous system in which cognition-enhancing and neuroprotective properties have been described [1-2]. Dried leaves of *S. scardica* were extracted with 80 % ethanol, and the extract was suspended in distilled water and successively extracted with solvents of increasing polarity. Selected extracts were fractionated by different chromatographic techniques and isolated compounds identified by NMR spectroscopy. Isolated compounds and extracts were tested in antioxidant and anti-inflammatory *in vitro* systems, as well as in cholinesterase inhibition assays. The 80% ethanol extract was found to contain flavones (glycosides of isoscutellarein and hypolaetin), phenylethanoids (mainly verbascoside) and chlorogenic acid as the main constituents. Antioxidant and anti-inflammatory effects were ascribed to the high content of polyphenols in the 80% ethanol extract. No inhibition of acetylcholine- or butyrylcholinesterases was observed. Antioxidant and anti-inflammatory effects are suggested to play a protective role in the pathogenesis of neurodegenerative diseases. Our findings may seem to be in compliance with previous *in vivo* findings and should be followed up in future studies.

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P-320 High affinity HERG and low affinity Cav1.2 blockers dehydroevodiamine and hortiamine in decoctions of the TCM drug Evodiae fructus

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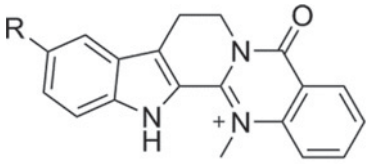
Most herbal drugs used in Traditional Chinese Medicine (TCM) are considered safe based on their use over centuries. However, the major alkaloids dehydroevodiamine (**1**) and hortiamine (**2**) in Evodiae fructus (fruits of *Evodia rutaecarpa*) were recently found to be potent blockers of I_{Kr} (rapid delayed rectifier current) with proarrhythmic effects *in vitro* and *in vivo*.[1] The herbal drug and *Evodia*-containing products are freely available via various distribution channels.

For a better assessment of possible risks associated with the use of *Evodia*, we prepared aqueous decoctions according to TCM procedures from a range of commercially available herbal drug samples. The content in extracted alkaloids was determined by LC-MS. In the decoctions 0.3-5.2 mg of **1** and 0.08-0.39 mg of **2** were found per gram of herbal drug (corresponding to approx. 20% of the alkaloid content in the drugs). Taking into account the dosage recommendations of the Chinese Pharmacopoeia for Evodiae fructus, this would correspond to a daily intake of 0.9-11.7 mg of **1** and 0.11-1.8 mg of **2**. The effect of these decoctions on action potentials in stem-cell derived cardiomyocytes was studied. Additionally, the effects on HERG (IC_{50} of I_{Kr} inhibition <1 µM) and Cav1.2 (IC_{50} of I_{Ca} inhibition >50 µM) channels expressed in HEK-293 cells was determined.

In conclusion, decoctions of Evodiae fructus lead to the intake of significant amounts of I_{Kr} blocking alkaloids **1** and **2**. The comparably low potency inhibition of Cav1.2 suggests a high risk for pro-arrhythmic effects such as Torsade de Pointes.[2]



1 R = H
2 R = OCH_3

▶ **Fig. 1**

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P-321 HPLC-based activity profiling of *Haplophyllum tuberculatum* In vitro activity against *Madurella mycetomatis*

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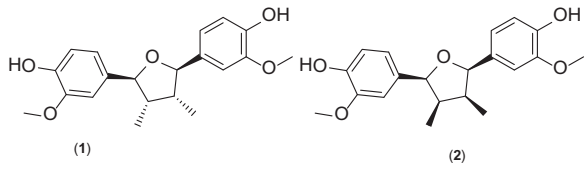
Eumycetoma is a chronic debilitating inflammatory fungal infection caused mainly by *Madurella mycetomatis*. Eumycetoma is endemic in most tropical and subtropical countries and was recently enlisted by the WHO among the neglected tropical diseases (NTDs) [1]. Current treatment includes long-term treatment with itraconazole and surgical intervention in most of the cases. Given limited efficacy, frequent side effects, and high cost of treatment, there is an urgent need for new antimycetomal drugs.

A subset of extracts from a repository of Sudanese medicinal plants traditionally used as anti-infectives was screened in a 96-well microtitre assay for *in vitro* activity against *M. mycetomatis* employing resazurin viability assay [2].

The chloroform extract from roots of *Haplophyllum tuberculatum (Forsskal) A. Juss. (Rutaceae*) was found to be the most active. Activity in the extract was localized by HPLC-based activity profiling, and two lignans, nectandrin B (**1**) and tetrahydrofuroguaiacin B (**2**), were isolated from the active time window.



▶**Fig. 1**

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P-322 Hypoglycemic and antioxidant effects of oral treatment of *Verbesina montanoifolia* extract in diabetic rats.

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DOI 10.1055/s-0039-3400044

Diabetes is a chronic disease associated with high oxidative stress and inflammation due to hyperglycemia[1,2]. Medicinal plants constitute an alternative source of potential therapeutic agents for diabetes. *Verbesina montanoifolia* (VM), a shrub native to Mexico, is used in Mexican traditional medicine to treat different illnesses. However; there are no reports worldwide on the components or the activity of this plant to treat diabetes.

To evaluate the effects of Capitaneja *(Verbesina montanoifolia)* on blood glucose levels and antioxidant enzymes in alloxan-induced hyperglycemic Wistar rats.

Acute administration of VM aqueous extract VMAQE (200 mg/kg) acetone extract (VMAE), and alcoholic extract (VMOHE) had a hypoglycemic effect on the glucose tolerance curve and the fasting glucose curve in Alloxan-induced diabetic male Wistar rats (100 – 150 g). In the sub-chronic administration (8-weeks), VMAQE helped to maintain body weight and showed a significant decrease in glucose levels from the 3rd through to the 8th week. VM extracts contained high levels of flavonoids, phenols, and polyphenols that presented antioxidant effects *in vitro* and improved antioxidant enzymes.

To the best of our knowledge, this is the first study to report that VMAQE ameliorates hyperglycemia and has antioxidant effects in diabetic Wistar rats.

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P-323 Hypothesis: the water-insoluble beta glucans within mushrooms that activate the dectin-1b signaling pathway are derived from colonizing yeast

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DOI 10.1055/s-0039-3400045

Research supports the theory that the microbiome of plants [1-4] and mushrooms [5] produce potent activators of pathogen recognition receptors which are principal contributors to the stimulation of macrophages. We have previously reported [5] that the *in vitro* macrophage stimulatory activity of water-soluble extracts from 13 different types of edible mushrooms is predominantly due to Toll-like receptor agonists originating from the naturally occurring bacterial communities within these materials. The purpose of the current study was to evaluate whether these 13 types of mushrooms contain water-insoluble beta glucans that activate the dectin-1b signaling pathway. Culinary mushrooms (*Agaricus bisporus* varieties) were essentially inactive, whereas most of the medicinal mushrooms (*Lentinula edodes, Grifola frondosa, Hypsizygus marmoreus* varieties, *Flammulina velutipes*) exhibited potent activation of the dectin-1b signaling pathway. *A. bisporus* mushrooms with no detectable dectin-1b-dependent activity had yeast colony forming units that were 687 times lower than *L. edodes* mushrooms exhibiting high activity (half maximal activation values between 6.8 and 11.7 µg/ml). Analysis of 12 commercial batches of *L. edodes* obtained from different sources indicated that 10 batches exhibited moderate variation (half maximal activation values between 6.6 and 23.1 µg/ml) and 2 batches had minimal or no activity. The two batches that lacked activity were sourced from companies that cultivated the mushrooms in controlled laboratory environments that minimize the growth of microbial organisms. These hypothesis-generating data suggest that the dectin-1b-dependent activity exhibited by these mushrooms is due (at least in part) to the insoluble beta glucans derived from the yeast colonizing these natural products.

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P-324 Abstract see SL YRW-02

Abstract see on page 1395

P-325 Abstract see SL AR-03

Abstract see on page 1359

P-326 Identification of the main component of a rambutan leaf extract responsible for the stimulation of collagen 1 on human keratinocytes

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Considering the growing environmental consciousness of consumers in the Cosmetics industry, we have launched a program based on the sustainable development of Rambutan, a well-known tree in Asia. We have developed a bioactive ingredient based on Rambutan leaves to answer one of the major demands of the Cosmetics market: anti-aging. This bioactive ingredient has proven its efficiency on the formation *in vitro* on collagen and elastic fibers.

In our study, we identified the chemical component of the rambutan leaves that may be responsible at least in part for its anti-aging activity.

We observed that Rambutan leaves are rich in polyphenols, and especially in ellagitannins (hydrolysable tannins). Among C-glucosidic ellagitannins, the presence of Corilagin (CAS: 23094-69-1, C27H24O18 [1]) was established by various methods (HPLC PDA, LC MS) and a molecular profile of extract was investigated with an innovative method using a dereplication procedure based on the interpretation of NMR data. To confirm our hypothesis, we tested the Corilagin molecule on normal human keratinocytes *in vitro*. Corilagin stimulated the synthesis of collagen I *in vitro* on cell culture.

Using various analytical methods, we were able to identify the major component of the bioactive that may be responsible for the anti-aging activity.

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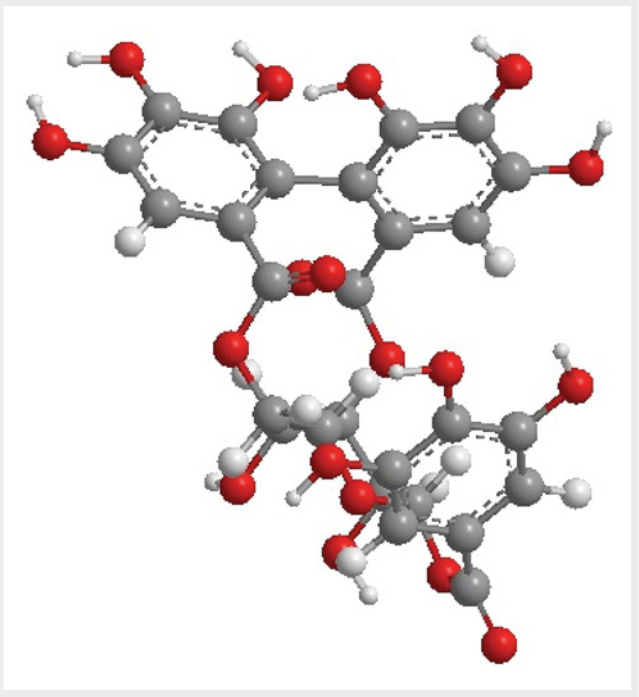

▶**Fig. 1** Chemical structure of Corilagin.

P-327 Immunomodulatory effect of *Acacia nilotica pods* on Leishmania parasitized THP-1 macrophage cells

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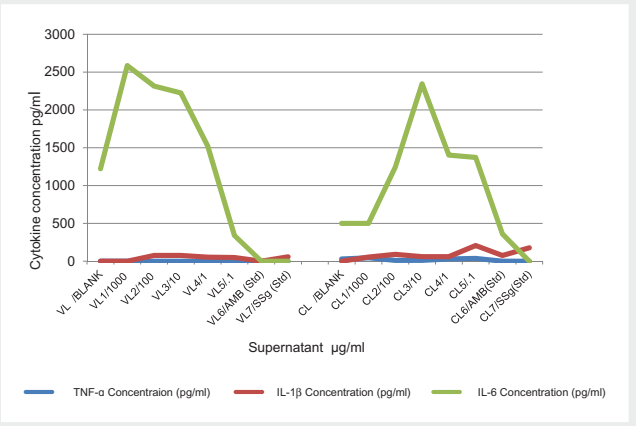
DOI 10.1055/s-0039-3400047

Leishmaniasis is a neglected disease with an estimated 12 million humans infected and there is an urgent need for alternative novel drugs. *Acacia nilotica* (L.) Willd. Ex Del (Fabaceae) is frequently used in treating parasitic disease in Sudan. Analysis of the ethyl acetate fraction of *Acacia nilotica* pods revealed its high contents of both hydrolysable and condensed tannins [1].

The *in vitro* growth inhibition and THP-1 macrophage activation of the ethyl acetate (EtOAc) and chloroform ($CHCl_3$) fractions of *A. nilotica* pods on *L. donovani* and *L. major* were assessed by measuring the production of TNF-α, IL-6, IL-β cytokines and nitric oxide. The EtOAc fraction inhibited significantly ($P < 0.05$) the growth rate of *L. donovani* and *L. major* promastigote with IC_{50} of 40μg/ml and 10μg/ml, respectively.

EtOAc fraction also inhibited the amastigotes in *L. donovani* and *L. major,* in a dose dependent response, with an IC_{50} of 10ug/ml and 100 ug/ml compared with pentostam and amphotericin B, respectively. EtOAc fraction caused significantly higher levels of IL-6 coupled with lowering TNF-α and IL-β levels in the infected macrophages of both *Leishmania species* (▶**Fig. 1**). The nitric oxide exhibited highest level (1000ug/ml) in comparison with the blank. However, the $CHCl_3$ fraction was almost devoid of any significant effect. The standard drugs' IC_{50} were ranging between >24μg/ml and >10μg/ml. The present results demonstrate the viability of THP-1 macrophage as model to study the immune modulatory effects of tannins by macrophage activation *via* extra- and intra-cellular release of cytokines [2].



▶**Fig. 1** TNF-α, IL-β and IL-6 concentrations in supernatants of THP-1 Cell lines infected with *Leishmania donovani* (VL) *and Leishmania major* (CL) and treated with different concentrations of Ethyl acetate fractions of *Acacia nilotica*.

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P-328 Abstract see SL YRW-09

Abstract see on page 1398

P-330 *In vitro* anti-melanogenisis effect of sesquiterpene lactones from roots of *Saussurea lappa* and their analogues

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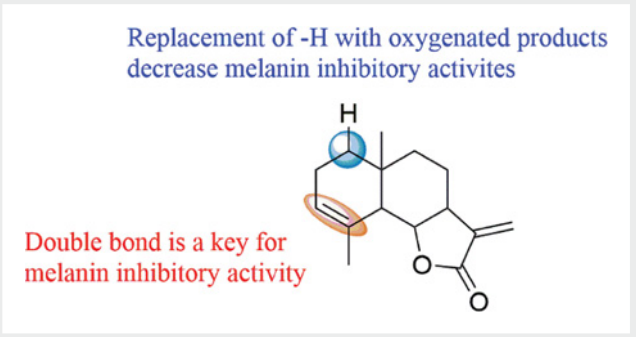
In this study, nine sesquiterpene lactones from *Saussurea lappa* root extracts including costunolide (**1**), α-, β-, γ-cyclocostunolide (**2, 3, 4**), santamarine (**5**), reynosin (**6**), arbuscirin A (**7**), dehydrocostus lactone (**8**), and 11β, 13-dehydrocostus lactone (**9**) were screened on melanin production, which was focused on extracellular melanogenesis in B16 melanoma cells [1]. Among tested compounds, only compound **2** significantly decreased extracellular melanin content in a concentration-dependent manner, with an IC_{50} value of 5.75 µM. Particularly, at 10 µM melanin content is 1.6-fold lower than arbutin as the skin lightening agent at 730 µM. Until now, the structure-activity relationship of sesquiterpene lactones on anti-melanogenesis has not been reported. To clarify their structure-activity relationship (SAR) profiles, 12 sesquiterpene lactone analogues were synthesized and evaluated the potential for melanin production. The results showed that all analogues did not display any significant for this activity. Furthermore, compound **2** had no effect on mushroom tyrosinase inhibitory activity. This indicated that decreasing melanin was not caused by tyrosinase inhibition, which might affect protein expression at a post-translational modification.
These results provide the first SAR profile of sesquiterpene lactones and indicate that a double bond at C3-4 is the most effective position on anti-melanogenesis for eudesmanolide-skeleton. In addition, the C1-substituent with hydroxyl, ketone and ester moieties markedly decreased this activity. For the findings, therefore, suggest that α-cyclocostunolide (**2**) might represent a lead compound in the therapy of skin disorders.



▶**Fig. 1** Summary of SAR of the eudesmanolide skeleton for melanin inhibitory activity

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P-331 *In vitro* anti-staphylococcal combinatory effect of *Cinnamomum cassia* essential oil and 8-hydroxyquinoline evaluated simultaneously in liquid and vapour phase

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Staphylococcal infections are often difficult to treat due to increasing resistance to antibiotics, which is leading to a need for discovering of new drugs and alternative treatments. Plant essential oils are of great potential for the development of new antimicrobial preparations and furthermore, due to their volatility, they are suitable for inhalation therapy, which is an effective way for healing of respiratory diseases. However, compared to well-established methods for testing of antimicrobial combinatory effects in liquid media, there are no standardized assays for determination of interactions between volatile compounds in gaseous phase, although disk volatilization assay is probably the most frequently used method [1, 2]. In this study, we tested *in vitro* antimicrobial combinations of plant volatile compound 8-hydroxyquinoline (8-HQ) with *Cinnamomum cassia* essential oil (CCEO) in liquid and vapour phase by broth volatilization chequerboard method [3] developed in our laboratory based on combination of standard microdilution chequerboard method [4] and broth volatilization method [5]. Combinatory effect against *S. aureus* was evaluated according to means of fractional inhibitory concentration indices (ΣFICI) [4]. Results showed additive effects in both, liquid (broth) and solid (vapour) phases. The best combinatorial effects were obtained for *S. aureus* BAA 976 in vapour at combination of 145.8 µg/mL of CCEO and 8 µg/mL of 8-HQ (ΣFICI=0.672) and in broth for *S. aureus* 29213 at combination of 71.1 µg/mL of CCEO and 1 µg/mL of 8-HQ (ΣFICI=0.503). These results can be potentially applied in development of various pharmaceutical applications that are based on volatile antimicrobials.

Acknowledgements *Czech University of Life Sciences Prague (project IGA.20195003) supported this research.*

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P-333 *In vitro* evaluation of the effect of *Bidens tripartita* extract and its main constituents on the intestine contractility

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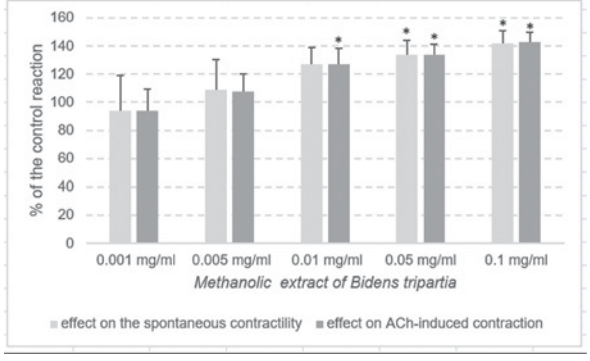
DOI 10.1055/s-0039-3400050

Gastrointestinal (GI) motility disorders include a wide array of signs and symptoms that can occur anywhere throughout the luminal gastrointestinal tract. Motility disorders are often chronic in nature and dramatically affect patients' quality of life. Due to the complex and sometimes unclear etiology of GI diseases, there is grand requirement of new drugs which could bring significant relief and control the severity of clinical symptoms. Thus, the aim of our study was to evaluate the effect of *Bidens tripartita* (Asteraceae) methanolic extract and its main constituents: luteolin (LUT), cynaroside (CYN) and flavanomarein (FLA) on the contractility intestinal smooth muscle specimens. The study was performed on porcine isolated jejunum strips. All experiments were conducted under isometric conditions. The extract was tested at concentrations ranging 0.001 to 0.1 mg/mL, individual phytoconstituents at doses 0.001 to 100 µM. The obtained results revealed that all tested samples induced increased gut contractility in both - spontaneous motoric function and ACh-induced reactivity. Among all tested substances, CYN turned out to be the most potent contractile agent and its prokinetic effect was dose-dependent. FLA and LUT induced only a slight myocontractile effect.



▶ **Fig. 1**

Concluding, on the weight of these findings in the porcine model, *B. tripartita* is unlikely to be successful as a therapeutic agent for diarrhea-inducing gastrointestinal disorders but it may find an application as a pro-kinetic agent for gastrointestinal disorders accompanied by diminished motility or atony.

P-334 *In vitro* hepatotoxicity of *Petasites hybridus* extract (Ze 339) depends on the concentration, intrinsic cytochrome activity and the species used

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Ze 339, a CO_2 extract prepared from the leaves of *Petasites hybridus,* possesses antispasmodic and anti-inflammatory effects and is proven to be effective in the treatment of allergic rhinitis. To study possible hepatotoxic effects of Ze 339, its main constituents and metabolites, a series of *in vitro* investigations were performed. Furthermore, different reconstituted fractions of the extract were examined in three *in vitro* test systems using hepatocytes: Two human cell lines, with lower and higher intrinsic activity of cytochrome P450 enzymes (HepG2, HepaRG) as well as a rodent cell line with high intrinsic activity (H-4-II-E) were used. Metabolic activity, assessed by the WST-1 assay, was chosen as indicator of cytotoxicity. To assess potential bioactivation of Ze 339 compounds, metabolic experiments using S9 fractions from rats, dogs and humans, and isolated cytochromes (human/rat) were performed and the formation of reactive metabolites was assessed by measuring cellular concentrations of glutathione and glutathione disulphide. Apoptotic behavior was examined by determining caspase activity of the extrinsic and the intrinsic pathway. Modification of mRNA expression of genes involved in adaptive, cellular defense mechanisms was examined by quantitative real-time polymerase chain reaction (RT-PCR) in HepaRG cells. Our data revealed that the cytotoxicity of Ze 339, its single constituents and main metabolites depends on the concentration, the intrinsic cytochrome activity of the cell system and the species used (rat > dog > human).

P-335 *In vitro* neuraminidase inhibitory effect and activity against influenza virus A H1N1 of herbal drugs used for common cold

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DOI 10.1055/s-0039-3400052

Influenza viruses are causative agents of common respiratory infections, often underestimated and badly managed. Monomolecular anti-inflammatory and anti-pyretic drugs are extensively used in infection related symptoms, but they do not trigger virus replication. Also herbal drugs are traditionally used [1], although specific anti influenza activities are rarely recorded and little investigated. By means of validated *in vitro* tests, in this study we investigated the effect of ten herbal extracts and poplar propolis commonly used for respiratory infections. We tested their effectiveness against an influenza A H1N1 strain in pre- and post cell infection and neuraminidase activity inhibition. *Camellia sinensis* Kuntze leaves dried extract 60% catechins (CSE), *Pelargonium sidoides* DC. roots standardized d.e. (PS) and *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter & Burdet (formerly *Cistus incanus* L.) aerial parts commercial d.e. 20% polyphenols (CIS) showed a strong anti-neuraminidase effect (IC_{50} <25 µg/ml). Only these extracts were efficacious in *in vitro* inhibiting influenza virus entry and (in a weaker manner) virus replication in MDCK cells; toxic/effective concentration ratio was >4 only for CIS. Chemical analyses showed that galloil derivatives and flavan-3-ols oligomers mainly occur in CIS and,

similarly to PS and CSE, these constituents are likely to contribute more to the antiviral efficacy [2, 3]. Our findings suggest that *C. creticus* subsp. *eriocephalus* is worth to be better investigated for its anti-influenza efficacy and ongoing researches are aiming to investigate molecular mechanisms of CIS constituents on viral targets such as the influenza virus glycoproteins, emoagglutinin and neuraminidase.

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P-336 Inhibition of NFκB-mediated inflammatory response by β-damascenone

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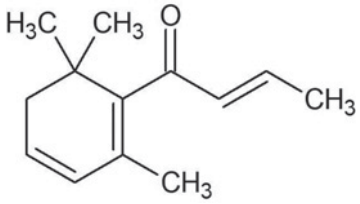
The transcription factor NFκB is one of the central regulators within the immune system. It is activated by various stimuli like cytokines and bacterial products. NFκB controls over 150 target genes, including genes regulating immune response and inflammation [1]. Recently we have found that an active component of *Epipremnum pinnatum*, β-damascenone, was able to inhibit the induction of NFκB-dependent genes in human endothelial and monocyte-like cells stimulated by bacterial lipopolysaccharide (LPS) that activates the NFκB signalling pathway via the Toll-like receptor 4 (TLR4) [2].
The goal of the present work was to examine whether β-damascenone can also inhibit the cellular response to the proinflammatory cytokines TNF-α and IL-1β, which stimulate cells via cytokine receptors. β-Damascenone inhibited E-selectin mRNA expression in HUVECtert cells and TNF-α gene expression in THP-1 cells stimulated by either IL-1β or TNFα. We conclude that β-damascenone acts at the post-receptor level, most likely via inhibiting the NFκB signalling pathway, which is shared by all three receptors. Further investigations to confirm this mechanism are in progress.



▶**Fig. 1** Structure of β-damascenone

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P-337 Inhibition properties of *Actinobacteria* extracts towards β–lactam resistance

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Actinobacteria is one of the most valuable sources of natural products with agricultural, biotechnological, industrial and medicinal importance. They are top producers of antibiotics and have the ability to produce a wide variety of secondary metabolites. The rapid development of resistance bacteria stresses the need to find new adjuvant or resistant modifying agents to combat the growing number of resistant pathogens. In this study, 92 *Actinobacteria* extracts that were grown in ISP 2 medium were studied for their ability to potentiate the activities of ampillicin, methicillin and oxacillin against *Staphylococcus aureus* ATCC 43300, a strain that confers resistance to β–lactam drugs. The anti-*Staphylococcal* activities of the extracts were evaluated using resazurin microtiter-based assay [1] and the minimum inhibitory concentration of the selected antibiotics was initially determined. A 50% sub-inhibitory concentration of the antibiotics was used in combination with the extract to evaluate β–lactamase inhibition potential. The results from this study showed that a number of *Actinobacteria* extracts was able to enhance the activity of ampillicin at a concentration of 1mg/mL. In addition, the active extracts displayed weak inhibitory activities (<20% cell growth inhibition) when tested against the same resistant strain in the absence of the antibiotic. This implied that *Actinobacteria* extracts may contain active metabolites that can act as β–lactamase inhibitor and restore the effectiveness of penicillin derivative antibiotics.

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P-339 Investigation of EPs®7630 in co-culture assays to predict possible drug effects

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EPs®7630 is an herbal drug preparation from the roots of *Pelargonium sidoides* (1:8-10; extraction solvent: ethanol 11% [w/w]) and is used for the treatment of respiratory tract infections. Numerous pharmacological test models have shown that EPs®7630 exerts antibacterial and antiviral activity [1]. To investigate further pharmacological effects of EPs®7630, it was analyzed in the BioMAP® Diversity PLUS Panel assay (provided by Eurofins DiscoverX Corporation) which includes 12 individual human primary cell-based systems predicting potential drug effects in multiple tissues and disease states. With this assay an identification and biological interpretation of relevant biomarker activities and a search for a phenotypically similar compound from a database (> 4,500 reference compounds) is possible. For EPs®7630 (3.3, 10, 30 µg/ml) anti-proliferative activities on endothelial cells and T-cells as well a as inflammation-related, tissue remodeling, hemostasis-related and immunomodulatory activities were found. At a concentration of 30 µg/ml, a strong correlation was found with respect to the activity profile of methimazole, an antithyroid drug, and troglitazone, a ligand of peroxisome proliferator-activated receptors (PPARα and PPARµ). PPARα agonists have been proposed to

play a role in inflammatory lung diseases [2]. At 10 µg/ml, a close EPs®7630 activity resembled that of myricetin, a flavonoid with antioxidant properties. In addition, effects similar to those of LPS, a Toll-like-receptor-4 agonist, and fucoidan, an L-selectin antagonist, were obtained. In summary, EPs®7630 showed amongst other an immunomodulatory potential in the BioMAP® Assay and gives new interesting approaches for further investigations, such as PPAR, TLR-4 and L-selection signaling experiments.

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P-340 Investigation of potential cytotoxicity and mutagenicity of guaianolides isolated from *Chrysophthalmum montanum* (DC.) Boiss

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In research on natural compounds, medicinal plants belonging to the Asteraceae, containing sesquiterpene lactones, are being investigated for their cytotoxic activities leading to the development of new anticancer agents [1]. In this regard, in our previous study we investigated the cytotoxic activity of *Chrysophthalmum montanum* (DC.) Boiss. (Asteraceae) on human cancer cell lines [MCF 7, MDA-MB-231, PC3, and HT-29] using the SRB assay through bioassay-guided fractionation. We revealed that the active chloroform sub-extract led to guaianolide-type sesquiterpene lactones, namely 6α-acetoxy-4α-hydroxy-1βH-guaia-9.11(13)-dien-12.8α-olide (**1**), 6α-acetoxy-4α-hydroxy-9β.10β-epoxy-1βH-guaia-11(13)-en-12.8α-olide (**2**), 4α,6α-dihydroxy-1β,5α,7αH-guaia-9(10),11(13)-dien-12,8α-olide (**3**), and (4α,5α,8β,10β)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide (**4**). The guaianolides (**1-4**) exhibited significant growth inhibition, and **4** possessed strong cytotoxicity against HT-29 with high selectivity between cancer, and normal (BEAS-2B) cell lines [2]. In the continuation of this study, we aimed to evaluate the cytotoxic potential of these guaianolides against different human cancer cell lines, i.e. cervical (HeLa), and lung (A549), and a normal human cell line (BEAS-2B) using the MTT method. In addition, we firstly assessed their mutagenicities *in silico* by combining knowledge-based and statistical-based approaches. Among the tested compounds, **1**, and **2** showed the strongest activities with IC_{50} values of <4 µg/mL on HeLa, A549, and BEAS-2B. We also revealed that **1**, **3**, and **4** were non-mutagenic, while **2** was mutagenic. As a conclusion, this study firstly reported cytotoxicities of the isolated guaianolides against HeLa, and A549 cell lines, as well as their mutagenicities. Our results suggest that **1**, and **2** can be regarded as promising candidates for discovering anticancer drugs, and determination of their cytotoxic mechanisms.

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P-343 Loss of the Transcription Factor FoxM1 in colorectal cancer cells exposed to prenylated xanthones isolated from *Metaxya rostrata*

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Natural compounds and their derivatives are an important source of anticancer agents. The tree fern *Metaxya rostrata* C.Presl (Metaxyaceae) is widespread in the rainforests of Central and South America, where suspensions of the dried rhizome in water are orally administered against intestinal ulcers or tumors. An activity-guided study led to the isolation of structurally related xanthones. Two of them, 2-deprenyl-rheediaxanthone B (XB) and 2-deprenyl-7-hydroxy-rheediaxanthone B (OH-XB) were analysed and showed cytotoxic activity at concentrations < 10 µM. Both induced active cell death by distinct mechanisms [1]. Our study detected suppression of the transcription factor FoxM1 as the common target. Protein as well as mRNA levels were decreased after treatment with both xanthones. Knockdown of FoxM1 by siRNA interference decreased the cytotoxic effect and xanthone-induced caspase activity in SW480 colorectal cancer cells. Comparison with additional cell lines (HCT116, Caco-2, HT29 and DLD1) demonstrated decreased FoxM1 mRNA and protein levels as well as induction of caspase activity in all except HT29. HT29 cells contained no detectable FoxM1 protein and were insensitive to both, XB and OH-XB. There was no difference in the baseline FoxM1 mRNA level between HT29 and the other cell lines. Therefore, ongoing experiments explore differences in FoxM1 protein stability in compound-exposed and control cultures.

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P-344 *Luffa operculata* administration in late pregnancy in Wistar rats impairs behavior and can lead to the development of anxiety

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The dried fruits of *Luffa operculata* (*buchinha-do-norte*) are popularly used to minimize symptoms of sinusitis, but turns out to be abortifacient. Previous studies have shown microscopic and macroscopic alteration in the testis of adult male rats [1]. The objectives of the present study were to verify the behavioral changes in female rats that received 1mg/kg of the aqueous extract of the dried fruits from *buchinha-do-norte* (BNE), that was administered via gavage during gestation for five consecutive days, between the gestational days GD17 to GD 21. The behavioral parameters were evaluated at the 50th day after delivery (PND50) in open field (OF) and light-dark box (LDB) apparatuses. Females were divided into control and experimental groups (CG, EG). All the locomotion, exploratory and anxiety parameters observed in the OF did not show significant differences between EG and CG ($p>0.05$). EG showed an anxiety-like behavior in relation to CG, due to the EG remained more time in the dark side than that in the light side of the box ($p<0.05$). EG showed increase in the number of attempts to enter the light side ($p<0.05$) and an increase in locomotion in the dark side ($p<0.05$). Rats from the EG explored more in the dark side and less in the light side of the box ($p<0.05$). They also remained longer in the dark side of the box and performed less grooming compared to the CG ($p<0.05$). Data found in the present study suggests a possible anxiogenic effect found after administration of BNE.

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P-345 *Luffa operculata* impaired testis relative weight and testosterone concentration in adult Wistar rats in comparison to young adult male rats

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The tea made with the dried fruits of *Luffa operculata*, also known as *buchinha-do-norte*, is popularly drunk or inhaled as a medicine to treat the symptoms of sinusitis and rhinitis. Nonetheless, when drunk, the tea can cause abortion. According to recent literature, adult male rats have undergone microscopically and macroscopically testicular changes after the administration of the aqueous extract of *buchinha-do-norte* (BNE) [1]. The present study aimed to compare how the oral administration of BNE to sexually-experienced adult male rats (150 days-old) and the prenatal exposition (from Gestation-Day 17 to Gestation-Day 21) of sexually-inexperienced young adult rats (60 days-old) to BNE, at a dose of 1mg/kg/5 consecutive days, influenced the testicular macroscopical parameters and the testosterone concentration in the blood. Four groups were then analyzed: control group and experimental group for the adult rats (CGA and EGA), and for the young adult rats (CGY and EGY). Body weight, testis weight, cranial-caudal axis, lateral-lateral axis and testis volume were higher in the older males ($p<0.05$), while the testis relative weight showed to be significantly higher in both CGY and EGY ($p<0.001$). Also, testosterone serum levels were higher in the young adult rats, despite their sex inexperience ($p <0.05$). Present findings suggested that the direct administration of BNE, via gavage, may have caused impairment in the relative weight of testis from the adult rats and provoked impairment in the testosterone levels, despite the sexual experience of the rats.

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P-346 Mechanism for anti-inflammatory effects of Farnesiferol B in ischemia/reperfusion injury of kidney

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Inflammation plays an important role in the pathophysiological progression of ischemia/reperfusion (I/R)-induced kidney injury. TGR5 regulates macrophage reactivity and attenuates inflammation in different disease models. We aimed to investigate the effects and mechaism on *Farnesiferol B* as a TGR5 agonist in renal I/R injury. Results showed that Farnesiferol B-treated mice reduced the tubular injury score by 47%. *Farnesiferol B* reduced renal oxidative stress by H2O2 and NGAL (23% and 218% respectively) and significantly decreased inflammation factors TNF-a (31%) and MCP-1 (52%) compared with I/R groups. Gene expression of IL-6 and Icam was reduced to 0.64 and 0.37 fold of those of I/R groups. In vitro, *Farnesiferol B* treatment alleviated LPS-induced macrophage migration and NF-κB activation through TGR5. In conclusion, *Farnesiferol B* could protect kidney function from I/R-induced damage by attenuating inflammation reaction though activating TGR5 in macrophage. This might be a potent TGR5 ligand for the treatment against I/R-induced renal inflammation.

P-348 Metabolites isolated from inflorescences of *Piper aduncum* L. (Piperaceae) and structure-activity relationship study of chalcones derivatives with anti-Trypanosoma cruzi activity

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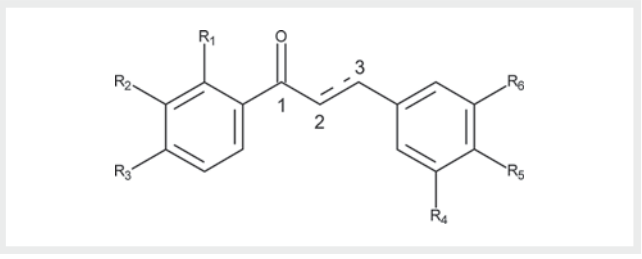
Chagas disease is a neglected disease and affects over 1 million people worldwide. As benznidazole is the only available drug for treatment [1,2,3], searching for prototypes for therapeutical use is crucial. In our work with Brazilian plants, the hexane extract from inflorescences of *Piper aduncum* L. displayed anti-*T. cruzi* activity and after chromatographic procedures, afforded four compounds: dihydroflavokawin B-**I**, nerolidol-**II**, methyl 2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-chromene-6-carboxilate-**III** and 2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-chromene-6-carboxilic acid-**IV**. **I** and **II** showed activity against *T. cruzi* trypomastigotes (EC_{50}=56.1 and 22.9μM, respectively) and reduced cytotoxicity (CC_{50} >200μM). Considering the activity of the dihydrochalcone, 26 related chalcones (▶**Fig. 1**) were synthesized for QSAR studies. The most active possessed phenyl and nitro groups at R^3 (EC_{50}=3.3 and 13.9μM; CC_{50} >200μM and CC_{50}=29.0μM, respectively).Compounds with highest SI were hydrogenated, and became inactive against *T. cruzi*. The implications of structural modifications on antitrypanosomal activity were studied by *in silico* models. The multivariate statistical analysis (MSA) identified promising subunits from the dataset and their statistical weights. The distribution of X variables suggests that substituents like R^1-propenyloxy (VIP=1.8, Coefficient=0.59 to Class A) and methoxyl at R^4, R^5 and R^6 (VIPs=1.22, Coefficient=0.17 to Class A) are essential to antitrypanosomal activity. Furthermore, molecular features were investigated by machine learning techniques (MLT): One-R and J48, both suggesting that R^1-propenyloxy is the main feature for antitrypanosomal activity. In conclusion, chalcones and derivatives provided



▶**Fig. 1** General Structure of Chalcone Derivatives

promising compounds as antitrypanosomal agents. Using MSA and MLT, essential molecular features were identified such as an α,β-unsaturated carbonyl system, methoxyl at R^4, R^5 and R^6, and R^1-propenoxy that improved the antitrypanosomal activity.

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P-349 Molecular networking for the study of antimicrobial activity of *Calophyllum inophyllum* L. oil resin

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Calophyllum inophyllum L. (*Calophyllaceae*) is a tropical tree traditionally used in folk medicine as anti-inflammatory, fungicidal, healing agent and antimicrobial agent. Its properties are explained by a high content in coumarins, xanthones and triterpenes [1]. Particularly, tamanu oil is a traditional Tahitian medicine. This oil is extracted from dried fruits (picture below) and contains a resinous fraction [2] possessing antimicrobial properties. The aim of our study was to investigate the molecular content-activity relationships of the resinous fraction from tamanu oil by aims of molecular networks. Molecular networking is a recent approach which allows to represent LC-MS^2 data according to similarities between fragmentation pathways [3][4]. First of all, five major known specific markers were isolated and identified from the resin: inophyllum E, inophyllum J, tamanolid, inophyllum P and (*E*)-5-methoxy-2,2-dimethyl-6-(2-methylbut-2-enoyl)-10-propyl-2*H*,8*H*-pyrano [2,3-f]chromen-8-one [5], allowing the further annotation of specific clusters in molecular networks. Then, the antimicrobial activity of the crude oil-resin and fractions thereof was evaluated against seventeen bacteria strains and the minimal inhibitory concentration (MIC) measured. Finally, the molecular content of the tested samples including resin and its fractions was explored by UHPLC-$HRMS^2$ analysis and a molecular network using a "fraction layout" was built. The relationship between antimicrobial activity of fractions and the presence of coumarins and acid chromanones will be discussed.

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►**Fig. 1** Picture of dried tamanu fruits (J.B Friday)

P-350 Multiple pharmacognostic evaluation of *Crocus sativus* L byproducts as innovative sources of active principles: focus on anther biological activity principles

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Multiple studies revealed the potential application of high quality saffron byproducts as cheap sources of bioactive compounds endowed with antioxidant activity. In the present study, we analyzed the total fatty acids of the anthers, and explored the pharmacological and toxicological potential of anthers, by evaluating genotoxic and protective effects in multiple cell lines, brine shrimps and isolated rat tissues.

The phytochemical analyses showed that anthers are rich in long chain fatty acids most of which are unsaturated (80.51%). Particularly, anther water extract revealed to be well tolerated by multiple cell lines, and able to modulate reactive oxygen species (ROS) levels, without exerting either genotoxic or cytotoxic effects. The same extract was also able to blunt lipopolysaccharide (LPS)-induced nitrite and malondialdehyde (MDA) in isolated rat tissues. On the other hand, considering the concomitant null effect on HCT116 cell migration, in wound healing experimental paradigm, our findings suggest the efficacy of water anther extract as protective agent without any direct reverting effects on lesioned tissues.

Concluding, the promising results, deriving from the pharmacological and toxicological evaluations, support the valorization of saffron anthers as a strategy to optimize and develop the productive chain of Abruzzo saffron (Italy).

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P-352 Natural products as modifiers of antibiotic resistance

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The resistance of 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 production [1]. 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 antibiotics [2]. 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 combined with ampicillin, piperacillin, imipenem, vancomycin, gentamicin and aztreonam and the effects were investigated with the chequerboard method. We found 29 combinations that worked synergistically, mainly with ampicillin and gentamicin on the gram positive strains. 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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P-353 Neuroprotective extract fractions and single substances from *Sideritis scardica* in the nematode *Caenorhabditis elegans*, an *in-vivo* model for neurodegenerative diseases

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Hydroethanolic extracts of *Sideritis scardica* Griseb. (Lamiaceae), widely known as Greek mountain tea, have repeatedly shown CNS-associated activities, such as enhancement of cognitive performance [1] and ameliorating effects on pathomechanisms of neurodegenerative diseases [2-4]. To investigate the active principle in terms of neuroprotection, we chose *Caenorhabditis elegans* to examine the *in-vivo* activity of six extract fractions (prepared by liquid-liquid extraction, re-precipitation in ethanol and solid-liquid separation of a 40% ethanolic primary extract of aerial parts) as well as seven single compounds occurring in *S. scardica*. The transgenic *C. elegans* strain CL2355 expresses pan-neuronal human amyloid-β, leading to a degeneration of nerve cells involved in chemotactic behavior. The chemotaxis index of the worms significantly increased when treated with the polar-lipophilic fractions or polar, phenolic compounds belonging to the class of phenylethanoid glycosides, including acteoside. Exactly the same pattern was observed in two further assays: These fractions and substances also protected dopaminergic neurons of strain BZ555 from 6-OHDA-induced degeneration (quantifiable through fluorescence intensity) and they reduced the number of aggregates of Q40::YFP, a polyglutamine peptide, in strain AM141. The investigated mechanisms are linked to neurodegeneration in Alzheimer's, Parkinson's, and Huntington's disease, respectively, and were positively influenced by neuroprotective properties of polar-lipophilic *S. scardica* extract fractions and phenylethanoids. In each case, the measured significant effects did not exceed the activity of the primary extract itself. That indicates a relevant contribution to the overall efficacy of crude, hydroethanolic extracts, which themselves, representing multi-substance mixtures, we regard as the active principle of *Sideritis scardica*.

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P-354 Neuroprotective potential of hydroalcoholic extracts of *Juniperus* species

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The genus *Juniperus* (Cupressaceae), one of the most diverse of the conifers, comprises approximately 75 species of evergreen aromatic shrubs or trees native to northern hemisphere. *Juniperus* species have been widely used as herbal medicine from ancient time [1,2]. The present study aimed to evaluate antioxidant and acetylcholinesterase (AChE) inhibitory activities as well as *ex vivo* neuroprotective **effect** of hydroalcoholic extracts of leaves and branches of three *Juniperus* species (*J. communis* L., *J. oxycedrus* L. and *J. phoenicea* L.) growing wild in Croatia. All tested *Juniperus* extracts were rich in polyphenols (3.0-3.9%). They possessed DPPH (IC_{50}: 3-6 µg/mL) and nitric oxide (IC_{50}: 115-186 µg/mL) free radical scavenging activities, ferric reducing properties (IC_{50}: 14-22 µg/mL) and ability to inhibit lipid peroxidation (IC_{50}: 51-60 µg/mL). The most potent inhibitory activity against AChE showed *J. phoenicea* and *J. oxycedrus* (IC_{50}: 42 µg/mL and 72 µg/mL, respectively). Neuroprotection induced by extract treatment was evaluated in a model of cortical spreading depression described as a potential triggering mechanism in migraine. The extracts of *J. communis* and *J. oxycedrus* (800 µg/mL) significantly blunted K^+ 60mM-induced decrease of serotonin turnover, evaluated as 5-HIAA/5-HT ratio in isolated rat cortex. The brine shrimp lethality assay showed that *Juniperus* extracts at the concentrations up to 800 µg/mL have no toxicity. Our results highlighted the potential for further *in vivo* research in order to confirm the protective effects of *Juniperus* species in neurological diseases characterized by increased burden of oxidative stress.

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P-355 New abietane-type diterpenes from *Perovskia abrotanoides* and their anti-inflammatory activity

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Perovskia is a small genus in the Lamiaceae family, which is represented in Iran by three species: *P. abrotanoides* Karel., *P. atriplicifolia* Benth., and *P. artemisoides* Boiss [1]. *P. abrotanoides* is locally used for the treatment of cutaneous leishmaniosis, gonorrhea, typhoid, headache, toothache, motion, vomiting, cardiovascular diseases, atherosclerosis, liver fibrosis, and cough [2-4]. It has been reported that tanshinones with a *nor*-abietane skeleton are the most abundant and important bioactive compounds obtained from the roots of this species [2].

In the present work, we have undertaken a phytochemical investigation of the ethyl acetate extract from the plant root. Preparative isolation by a combination of silica gel column chromatography and HPLC afforded 15 abietane-type diterpenes, including six new compounds, 15,16-dehydrolatifolionol, 1-hydroxyneocryptotanshinone, 1β-hydroxycryptotanshinone, 8β-hydroxy-9(11),13(14),15(16)-abietatrien-12-one-4,16-olide, 1-oxo-neocryptotanshinone, and 3-oxo-1,2-en-1-deoxoarucadiol. Their structures were established using comprehensive spectroscopic data analysis, 1D and 2D-NMR, HRMS and comparison with the literature data.

Since tanshinone derivatives demonstrated to have anti-inflammatory activity [5], the isolated compounds (50-12.5 μM) were subjected to bioassays, indicating that all of them were able to significantly inhibit both nitric oxide release and inducible nitric oxide synthase expression in J774A.1 macrophages, during inflammatory conditions. In particular, among the tested compounds, 8β-hydroxy-9(11),13(14),15(16)-abietatrien-12-one-14,16-olide, 15,16-dehydrolatifolionol and 1β-hydroxycryptotanshinone showed to have the best activity in affecting both the pro-inflammatory parameters.

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P-356 Novel tissue engineering scaffolds as wound dressings loaded with Alkannins/Shikonins as active ingredients

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Alkannin and Shikonin (A/S) are bioactive natural products biosynthesized in the roots of some Boraginaceous plants. They have been primarily well-known for their strong wound healing and regenerative activities [1]. A/S comprise the active ingredients of approved -by the National Organization for Medicines- wound healing pharmaceutical preparations (HELIXDERM®, Histoplastin Red®) invented by Prof. Papageorgiou.

This work is a continuation of our previous studies [2] on fabricating electrospun scaffolds impregnated with a mixture of A/S pigments. Specifically, several biodegradable polymers were used [cellulose acetate (CA), poly(l-lactide) (PLLA), polycaprolactone (PCL) and polycaprolactone/polyethylene glycol (PCL/PEG)] and scaffolds were assessed for the first time for their biocompatibility upon cell seeding.

A/S-loaded and non-loaded scaffolds were produced as previously reported [2]. The scaffolds were cut in 8 mm discs, seeded with human foreskin fibroblast cells (Hs27) and cultured for up to 7 days. Several physicochemical and biological characteristics were evaluated: drug content, *in vitro* drug release profile, scaffolds morphology by SEM, cell viability at different time points by MTT assay and cell infiltration by confocal microscopy.

All A/S-loaded scaffolds displayed a favorable matrix to cell attachment. After 7 days of culturing, the fiber mats demonstrated adequate cell viability with PCL-A/S and PEGylated PCL-A/S showing increased proliferation compared to non-loaded PCL and PCL/PEG scaffolds.

This study was the first attempt to shed light on the biological potential of Alkannins/Shikonins combined with electrospun polymeric scaffolds to serve as medical devices for repairing and regenerating damaged skin tissue.

▶ **Fig. 1**

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P-357 On the trail of fungal defense strategies – Employing a special workflow to spot photoactivity

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Sunlight is not only a key factor for photosynthesis – the process enabling plants to convert solar energy into chemical energy [1] – but can also provide the basis for a winning defense strategy. Some plants, which are generally unable to actively flee from threats, use photons to fend off predators [2]. In detail, light-activated defense is based on the ability of certain pigments/

photosensitizers, to produce reactive oxygen species (e.g. $^1O_2$) after being exposed to light of a specific wavelength [3]. This led to the following hypothesis that fungi – another kingdom with "immobile" reproducing structures – might also possess highly photoactive compounds. To test this hypothesis, a previously established workflow [4] was used to rank the PDT-activity of several basidiomycetes.

While fungal extracts with pigments derived from the shikimate-chorismate pathway or the mevalonate pathway exhibited no significant activity, those containing dyes from the acetate-malonate pathway and nitrogen heterocycles were characterized by promising $^1O_2$-producing activities. Nevertheless, the obtained results pointed out that not all photoactive pigments are able to induce a photo-activated cytotoxic effect *in vitro*.

The hypothesis of a photochemical defense mechanism in the kingdom Fungi was tested. By investigating a set of diverse basidiomycetes, we were able to highlight the fact that pigments derived from the acetate-malonate pathway are promising photosensitizers.

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P-358 Permeability of lichen compounds through the blood brain barrier as an important argument for their potential neuroprotective action

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Lichens are an association of fungi and algae or cyanobacteria. Lichens contain many compounds with multidirectional activities (e.g. antiinflammatory, antibacterial or neuroprotective) [1, 2, 3]. In order to define a pharmacological activity also in central nervous system, the permeability of selected lichen compounds through the blood-brain barrier was assessed.

Blood brain barrier permeability studies were conducted using PAMPA-BBB (the blood-brain barrier specific parallel artificial membrane permeability assay). Acetone extracts from the thallus of *Hypogymnia physodes, Parmelia sulcata, Evernia prunatri, Cladonia uncialis* were produced. The concentration of lichen acids was determined using the HPLC-DAD method.

A gradient HPLC method with UV detection and the Kinetex C18 column (100 × 2.1 mm, 5 µm) was used. The mobile phase consisted of acetonitrile and 0.5 % formic acid with a flow rate of 0.3 mL/min. The detection wavelength was 254 nm and column. temperature was set at 40°C. The method meets all required validation parameters (selectivity, linearity, precision, accuracy).The concentrations of lichen compounds in fluids simulating blood and brain fluids were expressed as apparent permeability coefficients (P_e).

The results showed that the high permeability for physodic acid (P_e = 5.6 cm s^{-1}), evernic acid (P_e = 4.7 cm s^{-1}), (-)-usnic acid (P_e = 36.1 cm s^{-1}) was confirmed. The permeability of salazinic acid was determined to be low (P_e = 0.5 cm s^{-1}). Reference value is $P_e < 2*10^{-6}$ cm s^{-1} [4].

This results show that for the examined lichen compounds, pharmacological effects within the central nervous system are possible.

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P-360 Abstract see SL YRW-07

Abstract see on page 1397

P-361 Phytochemical and pharmacological profile of *Origanum sipyleum* extracts: exploring novel sources for potential pharmaceutical, food, and cosmetic applications

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Origanum sipyleum L., an endemic plant of Western Anatolia has been used as a medicinal tea, food additive, and for the production of essential oil. In this study, the biological potential of three extracts (ethyl acetate, methanol, and aqueous) of *O. sipyleum* was assessed based on antioxidant activity against key enzymes of clinical relvance. The chemical profile of the plant was assessed using spectrophotometric and LC-MS techniques. Additionally, we explored potential antioxidant and anti-inflammatory effects duced by the extracts in an experimental model of ulcerative colitis induced by LPS challenging. LC-MS analysis revealed that the extracts contained different classes of phenolics, such as rosmarinic acid, phlorizin and gallic acid. We found that the aqueous extract was the most effective antioxidant, displaying the highest DPPH and ABTS scavenging, FRAP, CUPRAC, molybdenum(VI) reducing, and metal chelating effect. The aqueous extract howed the strongest acetylcholinesterase (AChE) inhibition; the methanol extract showed the highest α-glucosidase inhibition, while the ethyl acetate extract was the most effective on butyrylcholinesterase (BChE), tyrosinase, and α-amylase. The total flavonoid content was highest in the aqueous and ethyl acetate extract, respectively. Finally, we found that all extracts were effective in reducing LPS-induced activity of pro-oxidant and pro-inflammatory biomarkers including nitrites, LDH, PGE2 and 5-HT, in rat colon, with the best activity showed by ethyl acetate extract. Our results indicated that the three solvent extracts varied in their chemical and biological profiles, but overall, *O. sipyleum* showed promising therapeutic properties, nonetheless, need to be further validated in *in vivo* models.

P-362 Abstract see SL J-02

Abstract see on page 1421

P-363 Phytotherapy in children: data from the PhytoVIS study, a NIS in 20,870 users of herbal medicinal products

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Herbal medicinal products are frequently used in the paediatric population. Since the number of clinical studies is limited, pharmaco-epidemiological research can be an important source of information. To gain insight into the use of herbal medicinal products, data from the PhytoVIS study, the world's largest pharmaco-epidemiological study on the use of herbal medicinal products [1], were evaluated focusing on the paediatric population.

The PhytoVIS data set has been captured in doctor´s offices and pharmacies in compliance to the ENCePP Code of Conduct [2].

2063 data sets from paediatric patients were evaluated, thereof 254 from patients below 2 years, 473 from patients age 2-5, 551 from age 6-11 and 785 from age 12-17. The majority of patients (67.7%) was treated because of common cold and fever, other were gastrointestinal disorders, injuries and pain among others. Co-medication was documented in 24,9% of patients. The efficacy of the therapy was rated very good in 48,4%, good to moderate in 36.8%, missing in 4.0%. 93.7% experienced no adverse events at all, only 0.8% felt a marked impairment due to side effects. Neither the efficacy nor the tolerability seemed to differ within age groups.

The results shed light to a field of pharmacotherapy and give a picture of the use of herbal medicines in an unselected cohort of paediatric patients. They clearly show the safety and therapeutic usefulness in children. They offer good background information for therapeutic decisions based on overall tolerability and perceived effect.

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P-364 Polyphenolic derivatives from propolis attenuates hypertension-induced heart failure

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Propolis is a honeybee product with a broad spectrum of biological properties, such as anti-oxidant, anti-inflammatory, antimicrobial, and antitumor. Polyphenolic derivatives (PPDV) are major constituents of propolis contributing to the broad biological activities. Chronic high blood pressure may result in heart disease. Oxidative stress is the disturbance of the redox homeostasis and produces excessive levels of reactive oxygen species, which plays a central role on hypertension-induced heart failure. We examined the effect of PPDV on hypertension-induced heart disease. Mice were infused with angiotensin II (Ang II) to induce hypertension, leading to heart failure. In the treated group, PPDV was treated in mice daily with Ang II administration. After 14 days of Ang II infusion, cardiac hypertrophy and fibrosis were found in mice. PPDV treatment attenuated Ang II-induced cardiac hypertrophy and fibrosis. In addition, PPDV inhibited Ang II-induced α-SMA (a fibrotic marker) and NADPH oxidase (an enzyme produced superoxide) expression. These all contributed to the improvement of cardiac function of PPDV. Our results provide new insights regarding the cardioprotective effects of PPDV, which provides an efficient therapeutic strategy to attenuate heart disease.

P-365 Preliminary results: essential oils from *Hypericum* spp. growing wild in Greece and their wound healing effects

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Hypericum has been used as wound healing agent since classical Antiquity. The most common preparation for the treatment of wounds and skin inflammations is the infused oil obtained from the aerial parts, containing naphthodianthrones, phloroglucinols and essential oil (EO); however, there is much controversy in the scientific community regarding the composition and stability of this formulation [1]. Extensive literature survey shows, that *Hypericum* EO not yet been evaluated regarding the wound healing efficacy, although many studies report antimicrobial, antioxidant, antiangiogenic, gastroprotective activities [2].

In the present study, *Hypericum* spp. growing wild in Greece (*H. perforatum*-HP, *H. empetrifolium*-HE, *H. amblycalyx, H. jovis, H. triquetrifolium*-HT) were subjected in hydro-distillation to obtain their EOs. GC-MS analyses revealed the presence of 137 individual compounds representing 87.8-96.8% of the total EOs. *Hypericum* L. is generally classified as EO-poor genus; only three taxa (HP, HE, HT) yielded enough EO for in vivo tests, which were conducted in hairless SKH mice. Measurements such as transepidermal water loss, hydration, redness, thickness and elasticity were performed. Statistical analysis interestingly showed some significant wound healing properties ($p<0.05$) of HT and HE in low concentration (0.05%). This is an ongoing project and the results will be further evaluated by photodocumentation and histopathological examination.

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P-366 Preliminary studies of the chemical composition and cytotoxic activity of *Hottonia palustris* herb

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Hottonia palustris L. (Primulaceae) (syn.: featherfoil, water violet, Wasserprimel) is a relatively undemanding semi-aquatic plant, widely distributed throughout lowlands of Western Europe and northern Asia. Up to date little is known about the chemical composition and pharmacological activity of aerial parts of *H. palustris*. It has been used in folk medicine for treating diseases, including heart problems [1]. The aim of our work was a preliminary assessment of the chemical composition by LC/MS technique and the cytotoxic potential of an alcoholic extract from above-ground parts of this plant. The cytotoxic activity was evaluated using human cancer cell lines: MDA-MB-231 cells, HeLa, endometrial cancer cells Ishikawa, colorectal cancer DLD-1 cells and normal skin fibroblasts CCD-25Sk. The examination of the phytochemical profile of the alcoholic extract revealed the presence of rare methoxy derivatives of flavones and triterpenes as major constituents. MTT colorimetric assay was conducted to determine cell viability. Data show that the extract exerts concentration-dependent inhibition of cell viability. The MDA-MB-231 and HeLa cell lines were the most sensitive to the extract at each concentration. MDA-MB-231 cells showed 67%, 52%, 23% cell viability, and HeLa cells 74%, 61%, 16% viability of control cells after incubation with extract at 30, 100 and 300 µg/mL, respectively. However, inhibition of cell viability in fibroblast was similar or higher compared to cancer cells. Further studies of the chemical composition and biological assays are in progress.

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P-367 Prenatally exposition to *Luffa operculata* aqueous extract may have provided augment in the percentage of females in the litters

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The direct exposition to a tea made with the fruits of *Luffa operculata*, a plant traditionally used against sinusitis and for abortion, due to their cucurbitacin content, caused impairment in the behavior in adult male Wistar rats [1]. The present study aimed the evaluation of the offspring sex ratio from mothers that received the treatment of 1,0 mg/kg/five days of the aqueous extract obtained from the fruits of *L. operculata*, during a late period of gestation, GD17 to GD21. Sexage was performed at prost-natal day 2 (PND2), and it was verified that the experimental group presented a significant higher number of female pups (t=2.163, df=31, p=0.0386) than that of the control group, while the number of males diminished as expected (t=2.247; df=31, p=0.0319). According to the Trivers-Willard hypothesis, female mammals are able to adjust offspring sex ratio in response to the maternal condition [2]. In the present study, mother's conditions were directly influenced by the oral administration of *L. operculata*, which may have caused physiological alterations in the pregnant body, leading the gestation to privilege female born. Females are more likely to be mated even standing in an adverse condition, while males in unfavorable conditions tend to lose their mating chance to a male under a better health condition. The prenatal exposition to *L. operculata* changed the homeostasis of pregnant rats which causes the improvement in the rate of female born offspring.

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P-368 Antiangiogenic activity of iridoids from Lamiaceae and Plantaginaceae species

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Iridoids are a group of natural compounds, occurring in a great number of plant families, usually as glycosides. The considerable interest in iridoids is due to their ecological role as plant protectant and to their wide spectrum of biological activities, including cardioprotection, neuroprotection, anti-inflammatory, and anticancer activities [1]. Interestingly, some iridoid glycosides were found to have a potent antiangiogenic activity [2-3]. Angiogenesis process may be involved in tumour development, thus its inhibition appears to be a promising approach in anti-inflammatory and anticancer therapies [4]. Within this context, the aim of the present study was the isolation and characterization of iridoid derivatives from two Lamiaceae species, *Stachys ocymastrum* (L.) Briq and *Premna resinosa* (Hochst.) Schauer leaves, and from *Anarrhinum pedatum* Desf. aerial parts, belonging to Plantaginaceae family, together with the evaluation of their antiangiogenic potential. The chemical study of investigated plants afforded to the isolation of one new and four known iridoid glycosides from *S. ocymastrum*, nine known iridoid diglycosides from *P. resinosa,* and ten new and five known iridoid glycosides from *A. pedatum*, identified by NMR and MS analyses. The antiangiogenic effects of the isolates were reported on new blood vessels formation using two in vivo models: zebrafish embryos and chick embryo chorioallantoic membrane [5]. Among the tested iridoids, β -hydroxyipolamiide, ipolamiide, buddlejoside A5, and 6′-*O*-menthiafoloylmussaenosidic acid-11-(5-*O*-β-D-fructopyranosyl) ester showed a significant antiangiogenic activity in both assays, reducing the growth of blood vessels. Weaker antiangiogenic effects were also observed for some other iridoids, thus suggesting this class of compounds as promising antiangiogenic agents.

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P-370 Recovery effect of a monogalactosy imonoacylglycerol from the brown alga *Agarum clathratum* subsp. *yakishiriense* on neomycin-induced hair cell damage in zebrafish

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Marine plants are popular and abundant food ingredients mainly in Asian countries, and also known to have many beneficial biological activities. Therefore, the development of useful materials from marine plants is one of important contributions to human health. For this purpose, we previously investigated numerous marine plants on various biological activities. As a result, *Agarum clathratum* subsp. *yakishiriense* showed amelorative effect in the zebrafish model for hearing loss. *A. clathratum* subsp. *yakishiriense* Yamada ex G.H. Boo & P. C. Silva is a perennial brown alga in the family Costariaceae and is widely distributed in the coastal area of the East Sea in Korea [1]. The EtOH extract of *A. clathratum* subsp. *yakishiriense* was suspended in water and then partitioned consecutively with CH_2Cl_2, EtOAc, and *n*-BuOH. Among these fractions, the EtOAc fraction was subjected to column chromatographic separation. Two new and a known monogalactosylmonoacylglycerols were isolated from the EtOAc fraction. The structures of two new monogalactosylacyl glycerols were determined as (2*S*)-1-*O*-(6*Z*,9*Z*,12*Z*,15*Z*-hexadeca- tetraenoyl)-3-*O*-β-D-galactopyranosyl glycerol (**1**) and (2*S*)-1-*O*-(6*Z*,9*Z*,12*Z*-hexadecatrienoyl)-3-*O*-β-D-galactopyranosyl glycerol (**2**) from spectral data and chemical evidence. A known compound, (2*S*)-1-*O*-(6*Z*,9*Z*,12*Z*-hexadecatetraenoyl)-3-*O*-β-D-galactopyranosylglycerol (**3**), was identified by comparing its spectral data with literature values. The isolated compounds were investigated the amelorative effect on neomycin-induced hair cell damage in zebrafish. Compound **3** showed significant recovery on otic hair cell damage at concentration of 0.1 μM. In conclusion, (2*S*)-1-*O*-(6*Z*,9*Z*,12*Z*-hexadecatetraenoyl)-3-*O*-β-D-galactopyranosylglycerol (**3**) revealed potent recovery effect for the hearing loss.

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P-371 Red Wine and *Ginkgo Biloba* extracts induce vasorelaxant and antioxidant effects on the digital veins of healthy horses

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We evaluated the vasorelaxant and antioxidant properties of Red Wine (RW) and *Ginkgo biloba* (GB), two plant extracts available in standardized preparation, on isolated equine digital veins (EDVs).

EDVs were removed from limbs of healthy horses in a slaughterhouse. Then, concentration-response curves (CCRCs) to RW and GB (10-10 g/L - 10-3 g/L) were determined using the organ bath technique. The role of the endothelium and the nitric oxide (NO) pathway was evaluated by performing CCRCs on either des-endothelialized veins or veins incubated with NO pathway inhibitors (L-NAME or ODQ). The antioxidant effect of both RW and GB was estimated by an acetylcholine test. A reference CCRC was first performed to validate the oxidative effect of homocysteine (a superoxide anion generator) and the antioxidant effect of tempol (100 μM). Then, tempol was replaced by RW (30 μg/L) or GB (100 μg/L).

RW produced a relaxation which was inhibited on both des-endothelialized rings and on rings incubated with L-NAME or ODQ. Homocysteine-induced impairment of acetylcholine relaxation was partially restored by RW and GB. These results showed that RW and GB exert veins vasorelaxation but also antioxidant effects. The vasorelaxant effect of RW mainly involved a role of the endothelium and NO pathway, while GB induced endothelium-independent vasorelaxation.

We showed that RW and GB extracts possess vasorelaxant and antioxidant properties on EDVs. Beneficial effects of RW and GB reported in equine clinical practice could be linked at least to their vasculo-protective action that requires further investigation in laminitic horses.

P-372 Regulation of TRAF3-IKK interaction by an 8-hydroxydaidzein contributed to the inhibitory effect on the IRF-3 signaling pathway

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Cytokines and chemokines are transcriptionally regulated by inflammatory transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and interferon regulatory factor (IRF)-3. A daidzein derivative compound, 8-hydroxydaidzein (8-HD), isolated from fermented soy products, has recently gained attention due to various pharmacological benefits, including anti-inflammatory activities. However, regulation of the inflammatory signaling mechanism for 8-HD is still poorly understood, particularly with respect to the IRF-3 signaling pathway. In this study, we explored the molecular mechanism of 8-HD in regulating inflammatory processes, with a focus on the IRF-3 signaling pathway using a lipopolysaccharide (LPS)-stimulated murine macrophage cell line (RAW264.7). The 8-HD downregulated the mRNA expression level of IRF-3-dependent genes by inhibiting phosphorylation of IRF-3 transcription factor. The inhibitory mechanism of 8-HD in the IRF-3 signaling pathway partly relies on the ability of 8-HD to inhibit TRAF3 and IKK interaction, which subsequently downregulates phosphorylation of AKT and reduces activation of IRF-3, thereby mediating inhibition of IRF-3-dependent gene transcription such as that of *IFN-β*, C-X-C motif chemokine 10 (*CXCL10*), and interferon-induced protein with tetratricopeptide repeats 1 (IFIT1).

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P-373 Rose essential oils stimulate neural differentiation and autophagy in stem cells

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Lysosomes of stem cells are essential for maintaining cellular homeostasis, and dysfunction of the organelles has been observed in multiple cranial nerve diseases. Stem cells of olfactory bulb are highly dynamic and undergo fission and differentiation to maintain a functional nerve network. Here we have identified the formation and regulation of glycosylation of protein using zymogram, filter retardation assay and PA-staining. Glycosylation of protein formed in aromas untreated cells and were distinct from damaged protein that were targeted into lysosomes for degradation. Glycosylation was promoted in active 100kD receptor, and glycosylation untethering was mediated by recruitment of β-glucosidase to extracellular by geraniol to drive sugar chain hydrolysis and thereby inactivates the receptor. Functionally, glycosylation marks sites proliferation and differentiation of stem cells, allowing regulation of neural networks by stem cells, whereas conversely, glycosylation regulates receptor activity via β-glucosidase. Glycosylation thus allows directional regulation of receptor dynamics, and may explain the dysfunction observed in lysosome in various human diseases.

P-374 Safety profile and estrogenic activity of extracts and phytoestrogen rich fractions of *Vitex doniana* and *Millettia aboensis*

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Hormonal therapy has been used to treat menopause associated disease conditions. However, the risks have led to increased interest in the search for effective and safe alternatives to ensure improved quality of life for menopausal women. This study investigated the safety and estrogenic potential of methanol fruit and root extracts and phytoestrogen rich fractions of *V. doniana* (VD) and *M. aboensis* (MA), respectively, in ovariectomized rats. The LD_{50} of VD and MA extracts were 4472 and >5000 mg/kg and their ED_{50} 128.05 and 321.58 mg/kg, respectively. Their ethyl acetate fractions (EAF) showed the highest phytoestrogen contents of 90 and 115 mg genistein Eq/g respectively. Serum inflammatory marker - NF-kb was significantly ($P<0.05$) reduced by 200 mg/kg EAF of MA (2.82 ± 0.27 ng/ml) compared to vehicle treated control group (5.21 ±1.13 ng/ml) while bone resorption marker, ALP, was also significantly ($P<0.05$) reduced by both MA (60.79 ±0.78 IU/L) and VD (72.59 ± 1.32 IU/L) compared to vehicle control (94.63 ± 0.91 IU/L). The fractions significantly ($P<0.05$) increased serum catalase and superoxide dismutase enzyme activities. Ovariectomization-induced elevations in serum liver and kidney enzymes were dose dependently reduced by the extract and fractions. HPLC-DAD-MS analysis of the active fractions reveled the presence of these phytoestrogens: Daidzein, tectorigenin, 5-OMe tectorigenin, 7-OMe tectotigenin, genistein, 5-O-methyl genistein in VD and derrisoflavone derivatives in MA. MA and VD showed good estrogenic activities and a wide margin of effective and toxic doses that support their use for chronic management of menopause associated disease conditions.

P-375 Safflower (*Carthamus tinctorius* L.) Seed improves scopolamine-induced cognitive dysfunction in mice

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Alzheimer's disease (AD) is the most common type of aging-related neurodegenerative disease featuring gradually progressive cognitive and functional deficits. Neurodegenerative diseases are characterized by the presence of a state of oxidative stress and dysfunction of the cholinergic system. Safflower seed is mainly contained various anti-oxidant and cholinergic improvement compounds, such as serotonin and its derivatives, while the effect of Safflower seed in improving cognitive dysfunction has not been reported. In this study, we employed a scopolamine-treated learning and memory deficit mice to explore whether Safflower seed could alleviate cognitive dysfunction. Safflower seed water extract (SWE) was orally administered at dose of 100 mg/kg/day, and then behavior tests such as T-maze and novel object recognition test were conducted. Acetylcholinesterase (AChE) activity, reactive oxygen species (ROS) production, and antioxidant enzymes in the brain were measured. In behavior tests, novel route and object recognitions were improved by administration of SWE, indicating that SWE improved memory function in the scopolamine-treated mice. Also, SWE-administered group showed inhibition of the AChE activity. In addition, administration of SWE showed the lower ROS production and higher antioxidant enzymes levels compared with the scopolamine-treated vehicle group. The present results suggest that SWE improves scopolamine-induced memory deficits *via* inhibition of cholinergic dysfunction and oxidative stress. As a result of HPLC analysis, SWE contained serotonin and its derivatives such as *N*-(*p*-coumaroyl) serotonin and *N*-(feruloyl) serotonin. Therefore, safflower seed might be a promising candidate for the treatment of cognitive dysfunction.

P-376 *Sauromatum venosum* – an anti-proliferative plant from the Himalayas

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Cancer is an important cause of morbidity and mortality worldwide with 18.1 million new cases and 9.1 million deaths in 2018 [1]. Natural products are the leading source of anticancer drugs as 93 drugs from natural sources are among the 174 drugs approved for the treatment of cancer since 1980 [2]. At the foothills of the Himalayas, the local people living in Pakistan part of the Jummu and Kashmir use *Sauromatum venosum* (Dryand. ex Aiton) Kunth (family Araceae, for the treatment of cancer [3].

The aim of this study is to elucidate the active constituents of *Sauromatum venosum* possessing anticancer activity. For this, the plant extracts (*n*-hexane, dichloromethane and methanol) and isolated fractions from active extracts were tested against different cancer cell lines using a cell metabolic rate estimation assay. Dichloromethane and *n*-hexane extracts of tubers of the plant showed marked activity against both adherent and non-adherent cancer cell lines (MDA-MB-231 and CCRF-CEM). To identify the constituents responsible for this activity, column chromatographic fractions were subjected to LC-DAD-MS and LC-HRMS analyses for dereplication. The analyses revealed that tubers contain lignans, predominantly lariciresinol and pinoresinol. These lignans showed moderate activity against the cancer cell lines compared to the crude extracts. Therefore, isolation and structure elucidation of further active compounds is in progress.

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P-377 Screening for active substances with TLC bioautography: challenges with artefacts

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TLC, and its modern form HPTLC, is a standard method for identification and quality control of herbals [1]. Its combination with bioassays offers simultaneous separation and screening of plant extracts and other multi-compound mixtures for active substances. The intriguing simplicity and rapidness of the method might lead to underestimation of potential artefacts, first reported by Taibon et al. for HPTLC-Tyrosinase Inhibition [2]. In assays based on cells or

enzymes in hydrophilic media, high lipophilic substances prevent contact with the potentially active substance and might lead to false-positive or false-negative results. Additionally, diffusion problems from the layer to the biological assay agents can also lead to wrong interpretation of results, due to difference in the physico-chemical properties of the molecules in the sample mixture.

The planar Yeast Estrogen Screen Assay (pYES) is used for detection of human estrogen receptor alpha (hERα) agonists in mixtures [3]. The hERα is expressing β-galactosidase in presence of estrogenic substances. Substances with a strong blue autofluorescence at 366 nm detection and low estrogenic activity are difficult to differentiate from real estrogenic activity, when using the blue fluorescent dye 4-methylumbelliferyl-β-D-galactopyranosid (MUG) as substrate for β-galactosidase. Results of blue fluorescing phenolic acid rich plant extracts with known estrogen activity are demonstrated and a control assay using resorufin β-D-galactopyranoside (RGP) as substrate is proposed.

The poster summarizes potential artefacts from pYES and other antimicrobial assays and reviews the theoretical considerations and suggests further investigation to overcome the challenges and improve bioautographic methods.

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P-379 Secondary metabolites from marine sources as inhibitors of advanced glycation end products (AGEs) and collagenase

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Advanced glycation end products (AGEs) have recently received particular attention in aging research, and especially the extracellular matrix (ECM) proteins have been regarded as one of the major target structures for glycation. Collagen, the major component of ECM, which is important for mechanical stability and cell-interaction, loses its function during glycation leading to stiffness, decreased flexibility and impairment in tissue turnover [1]. Some natural products have already been identified as AGEs inhibitors, however connected to diabetes and other degenerative diseases but still poorly studied in skin aging. Marine species have received great attention as sources for natural photoprotective agents and unique biomolecules, which are often a result of adaptation strategies to the extreme marine environment [2]. Therefore, our study focused on secondary metabolites from various marine sources including mycosporine-like amino acids (MAAs) from Rhodophyta, coumarins from Chlorophyta, and sulfated flavones from seagrasses that were isolated by using various chromatographic techniques. Their structures were determined by nuclear magnetic resonance and mass spectrometry, and the compounds tested in two different fluorescence based *in vitro* assays to investigate their collagenase and pentosidine-like AGEs inhibitory effects. In total, 24 substances were investigated mostly showing comparable IC_{50} values in both assays. For example, IC_{50} values around 75-150 µM were determined for the MAAs. Interestingly, the sulfated flavones from *Zostera marina* exhibited higher activities than their non-sulfated equivalent, e.g luteolin-7,3′-disulfate showing an IC_{50} of of 60 µM compared to luteolin with 0.4 mM. Some substances showed even higher activities than the standard inhibitor rutin.

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P-380 Selected extracts from *Potentilla alba* modulate the viability of normal and cancerous cells of the human colon epithelium

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Potentilla alba (Rosaceae) has been used in the traditional folk medicine for several hundred years. However, data on the cytotoxicity effects of this plant are limited [1,2]. In the study the following extracts from aerial parts of *P. alba* were used: MeOH (PAL1), 50% MEOH (PAL2), water (PAL3), Et_2O (PAL4), EtOAc (PAL5), n-BuOH (PAL6) and 70% acetone (PAL7). MTT and NR assays for assessing cells viability were used. The tests were carried out on human colon tumour (HT29) and normal epithelial (CCD 841 CoTr) cell cultures. In NR uptake method, PAL7 extract (25 µg/mL) had the strongest cytotoxic (by 18% compared to the control) potential against cancer cells. Extracts PAL1, PAL4, PAL5 and PAL6 significantly decreased the viability of cells (to 83% compared to control) at 225 µg/mL concentration. PAL4 extract (125 µg/mL), was the most active against normal cells decreasing their viability to 77% comparing to the control. In MTT method, PAL2, PAL4, PAL7 extracts at concentrations lower than 125 µg/mL increased up to 60% the succinate dehydrogenase activity. When normal cells were analysed, the enzyme activity was not affected by the PAL2 and PAL3 extracts. The remaining extracts in a concentration dependent manner stimulated the enzyme activity in normal cells. *P. alba* extracts depending on the concentration used, type of solvent and type of cells exerted differential effects in the context of cell viability and the action of selected Krebs cycle enzymes. An analytical approach based on LC-ESI-MS was also applied to obtain phytochemical profile of all extracts.

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P-381 Semi-synthetic studies on astragaloside VII and immunomodulatory activities of the derivatives

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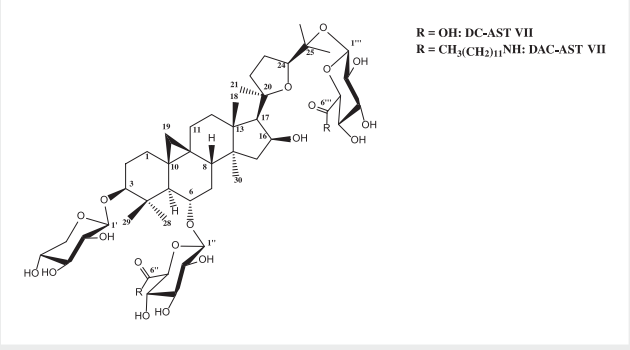
Adjuvants have been used in vaccine sector since 1920s to increase the immunogenicity of antigens, reduce the dosage and minimize frequency of immunizations [1]. The use of saponins as adjuvant in the prophylactic/therapeutic human and veterinary vaccines, and investigation of their immunomodulatory activities have gained importance in recent years [2,3]. Astragaloside VII (AST VII), a triterpenoid saponin isolated from *Astragalus* species, stimulates Th1 mediated immune response, antigen-specific antibody response and splenocyte proliferation [4,5].

The main goals of this study were the synthesis of immunologically active analogs of AST VII and investigating immunomodulatory properties of these compounds in human whole blood. The analogs of AST VII were prepared by selective oxidation of the primary alcohols to carboxylic acids in glucose residues to afford DC-AST VII, and followed by amidation reaction to give DAC-AST VII. AST VII and its analogs were evaluated for their immunomodulatory properties based on their effects on cytokine level alterations (IL-2, IFN-γ, IL-17A, IL-1β, IL-4, TNF-α) using ELISA method.

As a result, between 2 to 32 µg/mL concentrations, the compounds significantly reduced Th1 mediated cytokines such as IL-2, IFN-γ, and TNF-α ranging from 1.10 to 8.46-fold compared to the control (PMA-ionomycin), while inducing IL-1β and IL-17A. The most potent compounds were: DAC-AST VII (3.32-fold) for production of IL-1β, and AST VII (5.05-fold) for production of IL-17A. According to the cytokine release profiles, these compounds as single entities or in combination might have chance to be developed as vaccine adjuvants, and further studies are in progress to elucidate the mechanism of action.



▶**Fig. 1** Chemical structure of AST VII analogs

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P-382 Sexual behavior of ovariectomized female rats after chronic genistein administration

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Genistein is an isoflavonoid extracted from soy with phytoestrogenic effects. It is a β2-estrogen receptors agonist, having a similar molecular structure with endogenous estrogens [1]. Hormonal decline occurring in postmenopausal women causes decreased libido, besides other unpleasant symptoms like flushes, dryness of the vaginal mucosa etc. [2]. The purpose of this study was to highlight estrogen-like effects of genistein in female rats castrated prior to the onset of puberty. Four groups of female rats were divided as follows: lot GEN (10 castrated rats treated with genistein i.p. 10mg/kg/day for 8 weeks), lot ESTR (10 castrated rats treated with 10 µg/kg bw/day 17β-estradiol for 8 weeks), lot OVX (10 castrated and untreated rats) and lot NORM (10 rats in control group). The rats were left in the presence of the males for 2 hours a day and were monitored for one week. The prospective behavior (male interest) and the copulative behavior (lordosis) were evaluated. Results: There was a significant difference in rats behavior towards male, in the OVX lot compared to GEN, ESTR and NORM, as they became aggressive in the presence of the male. There was no difference between the behavior of the rats in the NORM and ESTR group, which shows that the hormone replacement therapy with estradiol was effective. In the GEN lot, the presence of prospective behavior was observed, but the number of lordosis positions adopted by animals was lower than the NORM and ESTR lot. The study demonstrates the influence of genistein on sexual behavior, but the agonist-antagonist effect depending on the endogenous level of estradiol should be also considered.

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P-383 Sidoamidines – 8-(pyrrolidine-1-carboximidamide-2-yl)-(epi)gallocatechinsNew components in the *Pelargonium sidoides* extract EPs® 7630

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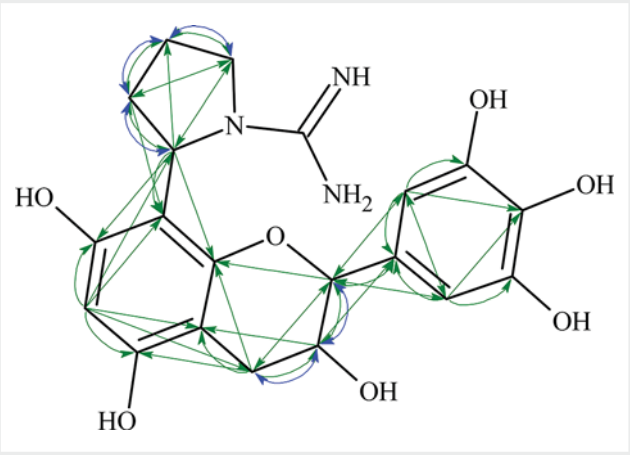
EPs® 7630, a 15% hydroalcoholic *Pelargonium sidoides* extract, is used for the treatment of common cold, especially bronchitis. Minor ingredients are the plant-characteristic highly oxygenated benzopyranons, like umckalin in free and sulfated form, in addition to the major monomeric and oligomeric (epi) gallocatechins, the prodelphinidins (OPDs).

Detailed analysis of purified OPDs with 1D-and 2D-NMR (HSQC, HMPC) revealed a predominant 4->8 B-type connectivity of the monomeric units gallocatechin and epigallocatechin in about equal quantities. In addition to the plain OPD, another oligomer with an unusual additional substitution was detected, which was characterized by an added mass of 111 Da,

leading to signals in the positive mass spectrum of (PES $[M+H]^+$: 418, 722, 1026 Da; plain OPD: 307, 611, 915 Da). Isolation of one low content monomeric entity in combination with 2D-NMR spectroscopy revealed a pyrrolidine-1-carboximidamide-2-yl conjugation at the 8-position of (epi) gallocatechins. These substances were not reported yet, and are thus named as sidoamidines. Biomimetic syntheses of four pure isomers starting with (-)-gallocatechin and (-)-epigallocatechin, respectively, made it possible to establish their exact chemical structure by HRESIMS, 2D-NMR, and CD-spectroscopy (▶**Fig. 1**). The chirality of the building blocks was unequivocally demonstrated by chiral chromatography and optical rotation of selected reference material.

Separation of the oligomeric sidoamidine fraction from plain OPD by cation exchange chromatography demonstrated quite similar structural characteristics (HSQC spectra) beside the extra substitution.

Evaluation of the new components in an animal disease model, the LPS induced sickness behavior, may argue for positive involvement in the (malady) treatment with EPs® 7630.



▶**Fig. 1** Constitution of sidoamidines with HMBC (green) and COSY (blue) correlations.

P-384 Study of neurodifferentiation potential of different extracts of *Bacopa monnieri*

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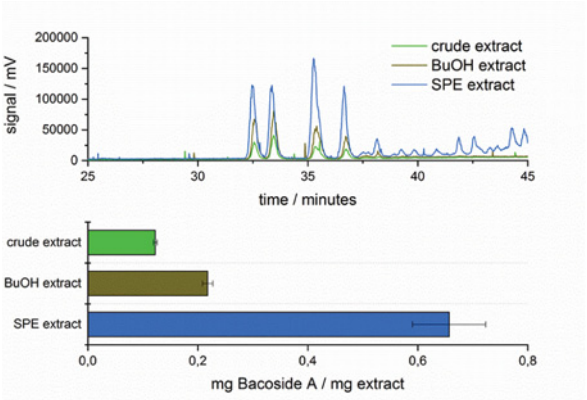
Bacopa monnieri (BM) belongs to the family of *Scrophulariaceae* and is a creeping herb, growing in shady marshlands [1-3]. It is found in Asia, Australia and the United States [1, 2]. BM contains different substance classes like alkaloids, flavonoids, glycosides, sterols and saponins [1, 3]. Commonly it is used in Ayurveda as nootropic for the treatment of memory loss, anxiety, poor cognition and lack of concentration [1, 2]. According to literature, the active compounds in BM seem to be triterpenoid saponins, which are named bacosides and are present as a mixture [1, 4]. This contribution describes the extraction of plant material, the enrichment of bacosides via liquid-liquid-extraction (LLE) and solid-phase-extraction (SPE), and the assessment of the biologic activity of the obtained extracts. The extracts were tested regarding their differentiation inducing activity of neuronal adult stem cells by an *in vitro* dual luciferase assay. To transfect the cells, a specific promoter of the doublecortin (DCX) gene was used [5]. Expression of DCX indicates the neuronal differentiation and the current neurogenesis [5]. For this test, primary mouse embryonic forebrain cells were transfected using firefly luciferase driven by the DCX promoter and *Renilla* luciferase as a control [5]. After LLE and SPE an enrichment factor of five was determined using Bacoside A as reference substance. Some extracts containing flavonoids show slight differentiation inducing activity, while others are not active. For further biologic investigations the neuroprotective potential of these extracts will be tested.



▶**Fig. 1** Top: Chromatograms of different extracts (1 mg/mL), Bottom: Concentration of Bacoside A in different extracts (mg Bacoside A/mg extract)

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P-385 Synergistic anti-hyperuricemic effect of combined medication of *Alpinia oxyphylla* and Allopurinol

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Alpinia oxyphylla Miquel, belonging to the Zingiberaceae family, is a well-known traditional medicine used to treat urosis, diuresis, and chronic glomerulonephritis in China and Korea. Allopurinol is a commonly used medication to treat hyperuricemia, however, the drug exhibits many adverse effects. Thus, it is desirable to combine a compound

with allopurinol to reduce the high doses of the drug for more safety treatment. In this study, we investigated the possible synergistic effects of *Alpinia oxyphylla* fruit extract (AE) and allopurinol in decreasing serum uric acid level in rats with potassium oxonate-induced hyperuricemia. We examined the effects of allopurinol combination with *Alpinia oxyphylla* on the serum uric acid, creatinine and blood urea nitrogen (BUN) levels in hyperuricemic rat model. The effects of allopurinol plus AE on the xanthine oxidase (XOD) activities were measured. The combination of allopurinol and AE significantly decreased the serum uric acid level, leading to the normalized serum uric acid concentrations. Furthermore, the combination of allopurinol and AE decreased the serum creatinine and BUN levels. The attenuation of hyperuricemia-induced renal dysfunction was related to the inhibition of both serum and hepatic XOD activity. The anti-hyperuricemia effects of allopurinol are improved by combination administration of *Alpinia oxyphylla*. As an edible fruit, *Alpinia oxyphylla* has a good safety record in humans. Our results suggest that the combined use of allopurinol and AE may have a clinically potential value in the treatment of hyperuricemia.

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P-387 Tannin basic building blocks as potential scavengers of chemical carcinogens: a computational study

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Tannins are natural compounds that were historically used in the tanning of leather. This structurally rather diverse group is divided into hydrolysable (gallotannins and ellagitannins), complex and condensed tannins.[1] Since they are omnipresent in various plant tissues, they also represent a common component of food (e.g. ellagitannins in berries, epigallocatechin in green tea). Tannins are believed to exert several health enhancing effects,[2] although those effect are rather difficult to study.[3] Therefore computational studies can provide us with some guidance of possible molecular mechanisms of tannin actions.

In this study we examined chemical scavenging capacity of three tannin basic building blocks (i.e. gallic acid, ellagic acid and (-)-epicatechin) against nine ultimate carcinogens of the epoxy type at the Hartree-Fock level of theory in conjunction with flexible basis sets and implicit solvation models. The reactivity of tannin basic building blocks was then compared to the reactivity of identical ultimate carcinogens against guanine, which represents the most reactive nucleobase of DNA.[4] The tannin basic building blocks with reactivity towards ultimate carcinogens that exceeds the reactivity of guanine were presumed as good scavengers of ultimate carcinogens. The studied monomeric tannin building blocks exhibit a significant scavenging potential, with (-)-epicatechin representing the best scavenger.

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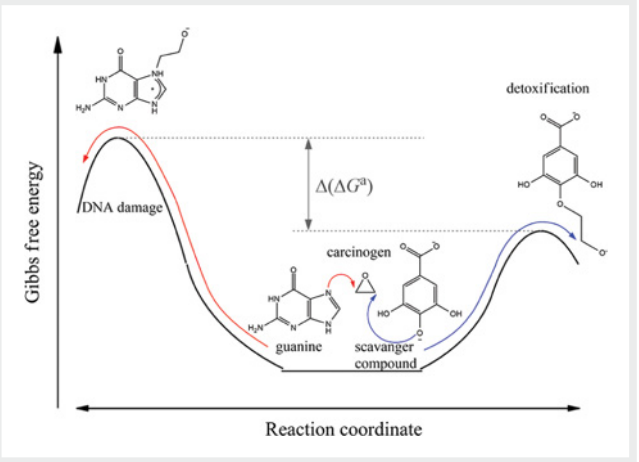

▶ **Fig. 1**

P-388 Tannins from Chestnut (*Castanea sativa* Mill.) leaves and fruits show promising in vitro antiinflammatory properties in gastric epithelial cells

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IL-8 plays a central role in the immune-pathogenesis of H. *pylori*-induced tissue injury. H. *pylori cag*$^+$ strains are clinically related to more severe outcomes of gastritis, like ulcers and cancer. The present study investigated the biological effect of tannins-containing extracts from *Castanea sativa* Mill., using *in vitro* models of gastric inflammation. Gastric epithelial cells (AGS and GES-1) were stimulated with TNFα (10 ng/mL) or co-cultured with different bacterial strains from clinical samples. Interestingly, IL-8 and IL-6 release after 6 h was found *cag*A independent. Basing on our previous work on TNFα-challenged cells and chestnut fruits, we tested the effect of the extracts in AGS co-cultured with H. *pylori cag*$^+$ (ATCC strain). Extracts from fruit episperm and pericarp, both rich in condensed tannins, inhibited H. *pylori*-induced IL-8 release at 25 µg/mL, compared to 5 µg/mL during previous TNFα treatments. Moreover, we investigated the effect of an extract from chestnut leaves, rich in tannins, which was never previously evaluated against gastric inflammation, in addition to castalagin and vescalagin, ellagitannins which occur in bark and leaves. Leaves extract inhibited TNFα and H. *pylori*-induced IL-8 secretion at 10 µg/mL and 50 µg/mL, respectively. Both ellagitannins strongly inhibited TNFα-induced IL-8 with the same IC50 (0.04 µM), whereas H. *pylori*-induced IL-8 was impaired at 50 µM. Although the extracts exhibited a lower inhibitory potency in co-culture model with respect to TNFα induced gastric epithelial cells, the higher concentrations of extracts tested (25-50 µg/mL) may be easily achieved *in vivo* after oral consumption.

P-389 Targeting mechanisms of the DNA damage response (DDR) by natural products

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Natural products (NP) are important lead substances for the design of new and potent anticancer therapeutics. The common target of approved conventional anticancer drugs is the genomic DNA of tumor cells. If the DNA is damaged, a complex stress response, called DNA damage response (DDR), is activated. The kinases ATM and ATR are the key regulators ensuring the coordinated regulation of DNA repair, activation of cell cycle checkpoints and apoptosis. Therefore, mechanisms of the DDR are attractive target structures for the development of new therapeutic approaches. The aim of the study is to identify NP derived from endophytic fungi, plants, lichens or marine sponges that influence the DDR.

For this purpose, the cytotoxic and DDR modulating potency of 296 natural compounds, used alone or in combination with the cAT cisplatin or doxorubicin was investigated by fluorescence-based analysis of the ATM/ATR-catalyzed S139 phosphorylation of histone 2AX (γH2AX), a surrogate marker of DNA damage. Upon, analyzing DNA double-strand breaks (DSBs), other types of DNA damage and the influence of the NP on the DDR on protein level, a total number of 10 natural products were identified that interfere with the DDR in monotherapy and/or modulate the cAT-stimulated DDR. No interactions with drug transport mechanisms were ascertained.

Based on these results and published data we suggest the NP 5-epi-nakijiquinone Q, 5-epi-ilimaquinone and secalonic acid F as most promising NP-based lead structures for future testing. Mode of action and their anticancer activity in tumor cells of different origin will be determined in forthcoming studies.

P-390 The benign prostate hyperplasia medication WS®1541 positively influences sexual behavior in a stress-induced model in rats

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WS®1541, a phytopharmaceutical combination of extracts from *Sabal serrulata* fruits (WS®1473) and *Urtica dioica* roots (WS®1031), used for treatment of benign prostate hyperplasia (BPH) in men. This process is frequently accompanied by a disturbance of the sexual function, which can also be caused by stress from the clinical picture and the associated fear of sexual failure.To investigate if WS®1541 has a positive effect on the sexual behavior in rats, we used a stress-induced sexual behavior model, which leads to a reduction of libido. In this experiment male rats were exposed once daily for 3 days to a 30 minutes interruption of the light/dark cycle, 60 minutes after treatment. The animals were treated daily with WS®1541 (100, 300, 900 mg/kg) or vehicle over three consecutive days one hour before stress. For the measurement of sexual behavior males were placed for 12 minutes together with estrus synchronized ovarectomized females. The total number of mounts and of licking the penis was counted. In the stressed group treated with vehicle a significant decrease of the total number of mounts and of licking the penis in comparison with the vehicle treated unstressed group was observed. The treatment with WS®1541 significantly antagonized the sexual dysfunction in a dose dependent manner in comparison with the vehicle treated stressed group.

These results demonstrate that WS®1541 used for the treatment of BPH also reduces sexual dysfunction in a stress-induced rodent model. These results should now also be investigated in a BPH model together with a stress induction.

	Treatment	Dose (mg/kg)	Number of mountings with licking penis mean ± SEM
1	Vehicle	--	14.00 ±0.89
2	Stress + Vehicle	--	2.88 ± 0.64 *
3	Stress + WS® 1541	100	5.57 ± 1.02 *
4	Stress + WS® 1541	300	10.00 ± 0.87 *
5	Stress + WS® 1541	900	14.50 ± 1.41 *

*p<0,05

▶ **Tab. 1**

P-392 The effects of *Sida rhombifoia* L. on benign prostatic hyperplasia in testosterone propionate-induced animal model and its active constituents

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Benign prostatic hyperplasia (BPH) is a pathologic process that is more likely to develop after middle age [1]. More than 50% of men aged over 60s have BPH and 15 to 30% of those affected suffer lower urinary tract symptoms, such as, urinary retention, bladder infection, bladder calculi, or renal failure [2,3]. BPH is known to depend on androgens [4], especially dihydrotestosterone (DHT), which is the predominant hormone in immature, mature, and hypertrophic prostate glands. DHT is synthesized from testosterone by two isoenzymes of 5α-reductase, that is, types 1 and 2 [5], and type 2 5α-reductase (5AR) is found predominantly in prostate. An excessive accumulation of DHT leads to hyperproliferation of stromal and epithelial cells of the prostate gland which results in prostate size increase. Therefore, the inhibition of the enzyme, 5AR and thus normal masculinization appears to be one of the sensitive targets for the treatment of benign prostatic hyperplasia. During the search for active natural resources to treat or improve the symptoms of BPH, it was found that the extract of *Sida rhombifolia* L. showed the potent inhibitory effects on 5AR2 enzyme activity. This study aimed to investigate the beneficial effects of *S. rhombifolia* extract on testosterone propionate-induced benign prostatic hyperplasia (BPH) in rats. The administration of ethanolic extract of *S. rhombifolia* (100 mg/kg body weight, *p.o.*) for 6 weeks markedly attenuated prostate enlargement and reduced serum and prostate levels of DHT and 5AR in testosterone propionate-treated rats. The expressions of androgen receptor, prostate specific antigen (PSA), and proliferating cell nuclear antigen (PCNA) in the prostate tissues were remarkably suppressed in the extract-treated rats. Through activity-guided isolation, the compounds belong to terpenoid have been successfully isolated from the extract of *S. rhombifolia*. The structures of the isolated compounds were elucidated by extensive 1D and 2D spectroscopic methods including 1H NMR, 13C NMR, 1H-1H COSY, HMQC, HMBC and NOESY. These findings regarding the effects of *S. rhombifolia* extract on of rats with testosterone-induced prostate hyperplasia suggest that the extract of *S. rhombifolia* and its active constituents may be useful for the treatment of prostate hyperplasia.

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P-393 The natural product Oleuropein shows enhanced anticancer and cardioprotective activity when co-administered *in vivo* with Doxorubicin

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Oleuropein (OLEU) is a phenolic compound widely consumed as part of the Mediterranean diet and found in large amounts in olive fruit and leaves of *Olea europaea L*. OLEU has pleiotropic pharmacological properties, such as antioxidant, anti-inflammatory, anti-atherogenic and anti-cancer. Doxorubicin (DXR) is an anthracycline broadly used as chemotherapeutic for the treatment of solid tumors, although it presents significant side effects. We investigated the *in vivo* anticancer and cardioprotective effect of OLEU when given together with DXR in mice with melanoma and colon cancer. We designed chronic cardiotoxicity models [1] in C57/BL6 and Balb/c mice that were injected (day-0) subcutaneously with syngeneic B16.F1 (10^5/mouse) and CT-26 (10^6/mouse) cells, respectively. When tumors were palpable (day-11 post-inoculation), we administered intraperitoneally OLEU and/or DXR (total 2100 mg/Kg and 18 mg/Kg, respectively; divided in 6 doses given every other day) and monitored tumor growth (tumor dimensions measured by a caliper) and cardiotoxicity (with echosonography performed before cancer cell inoculation and prior sacrifice) for 23 days. The concomitant administration of OLEU and DXR significantly retarded tumor growth, particularly of melanoma. Additionally, the DXR-induced chronic cardiotoxicity was reduced (ΔFS ~4%) in mice treated with the OLEU/DXR combination compared to animals treated only with DXR (ΔFS ~10%). Further experiments concerning the impact of this co-treatment protocol are needed to acquire more holistic and solid conclusions.

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P-394 The polyketide soraphen A exerts beneficial effects on cholesterol homeostasis in macrophages

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Atherosclerosis is a chronic disease of the arterial wall, characterized by the development of plaques that contain cholesterol crystals and lipid-laden foam cells. HDL-mediated cholesterol efflux from macrophages is an important mechanism to prevent foam cell formation, and an early step in the reverse cholesterol transport pathway, for subsequent excretion of cholesterol in bile. Major regulators of cholesterol homeostasis are the nuclear receptors LXR (liver X receptor) α and β that promote the expression of cholesterol efflux transporters, like ABCA1 (ATP-binding cassette transporter A1) [1]. Soraphen A is a macrolide produced by a myxobacterium and is characterized as an inhibitor of acetyl-CoA carboxylase activity [2, 3].

The aim of this study was to assess the influence of soraphen A on cholesterol homeostasis in THP-1-derived macrophages.

Soraphen A reduced intracellular lipid contents and increased cholesterol efflux from THP-1-derived macrophages (EC_{50}: 0.014 μM). In HEK293 cells transfected with a luciferase reporter plasmid, it increased the transactivation activity of LXRα and LXRβ. Soraphen A upregulated ABCA1 in THP-1-derived macrophages at the mRNA and protein level. The ABCA1 inhibitor probucol reversed the soraphen A-mediated increase in cholesterol efflux. In addition, the ratio of free cholesterol to total cholesterol was increased upon treatment with soraphen A, which led us to hypothesize that an increased level of cholesterol metabolites may act as ligands for LXRs.

In conclusion, soraphen A enhances cholesterol efflux from THP-1-derived macrophages *via* ABCA1. If cholesterol metabolites are implicated in these effects still remains to be elucidated.

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P-395 The relevance of alkaloid proportions in *Chelidonium maius* extracts

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DOI 10.1055/s-0039-3400100

Pharmacologically important compounds of *Chelidonium majus* show broad spectrum of bioactivities, for example antimicrobial, antiprotozoal, pro-apoptotic [1]. The complex alkaloid composition of the species is still under exploring, and so is the bioactive potential of the plant. The phytochemical profile that determines the biological activity is difficult to characterize due to large number of compounds produced in the plant and the multitude of factors determining their formation. The aim of our research was to evaluate the antimicrobial potential of *C. majus* extracts obtained from intact plants and *in vitro* cultures. Phytochemical analysis showed that roots contained higher number and amounts of alkaloids in comparison to aerial parts. All tested plant extracts manifested antimicrobial activity, related to different chemical structures of the alkaloids. Root extract used at 31.25 - 62.5mg/L strongly reduced bacterial biomass. From the seven individually tested alkaloids, chelerythrine was the most effective against *P. aeruginosa* (MIC at 1.9 mg/L), and sanguinarine against *S. aureus* (MIC at 1.9 mg/L). Strong antifungal activity was observed against *C.albicans* when chelerythrine, chelidonine and aerial parts extract was used. The experiments with plant extracts, individually tested alkaloids and their mixtures allowed for a deeper insight into the potential mechanisms affecting the activity of this group of compounds.

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P-396 The rule of natural products in belowground interactions between plant species

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Plants are consistently releasing root exudates into the rhizosphere which have the potential to influence the growth and development of neighboring plant species. Root exudates contain low and high molecular weight compounds such as sugars, amino acids, secondary metabolites, phytohormones, proteins, enzymes, and polysaccharides. Although aboveground plant interactions through volatile compounds has been extensively studied, only few studies investigated role of the belowground chemical interactions between plant species. The aim of this study is to elucidate the belowground chemical interactions between rye and hairy vetch grown together as cover crop mixture. Rye and hairy vetch were cultivated alone and together in pots filled with micro glasssbeads. Plant were grown for three weeks in climate chamber and their root exudates were collected for the chemical analysis. Targeted analysis with LC-MS/MS was done to identify the changes occurring in secondary metabolite profile of the rye and hairy vetch's root exudates as result of co-cultivation. Quantification results displayed that both hairy vetch and rye are altering their root exudation in response to rye-hairy vetch co cultivation. Hairy vetch significantly increased concentration of root exuded flavonoids such as kaempferol and pratensein in response to presence of rye. Concentrations of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and **BOA** (1,3-benzoxazol-2-one) in rye's root exudate were increased significantly as result of co cultivation with hairy vetch. The results from this study will increase our understanding about root exuded natural products with growth suppressive effects, which finally leads us to more sustainable plant protection system.

P-397 The vitamin E derivative α-amplexichromanol as anti-inflammatory lead inspired from traditional African medicine

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The nut seeds of *Garcinia kola* are traditionally used in African medicine and known for their anti-microbial, anti-oxidative and anti-inflammatory activities. Structural optimization of the anti-inflammatory ingredient and potent 5-lipoxygenase inhibitor garcinoic acid [1] yielded α-amplexichromanol (α-AC) which is a semi-synthetic analog of endogenous vitamin E metabolites that mediate immune functions of vitamin E [2]. α-AC possesses superior 5-lipoxygenase-inhibitory activity as compared to garcinoic acid and the bioactive vitamin E metabolite α-T-13′-COOH. Here we show that α-AC limits inflammation in murine peritonitis and experimental asthma *in vivo* and address the compound's expected higher metabolic stability using a liver-on-chip model. α-AC significantly reduced 5-lipoxygenase-derived leukotriene (LT)C_4 levels along with inflammatory cell infiltration and bronchial hyperreactivity. Lipid mediator profiles in plasma, lung and bronchia are comprehensively altered and α-AC was detected in lung up to six days after the last i.p. administration in contrast to α-T-13′-COOH. While α-T-13′-COOH is efficiently degraded by β-oxidation, α-AC is predominantly sulfated but not truncated. Our results indicate α-AC as a promising natural product-inspired lead that is orally active and seemingly metabolically more stable as compared to garcinoic acid and endogenous vitamin E metabolites.

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P-398 Toxicological effect of Brazilian bamboo extracts in zebrafish larvae

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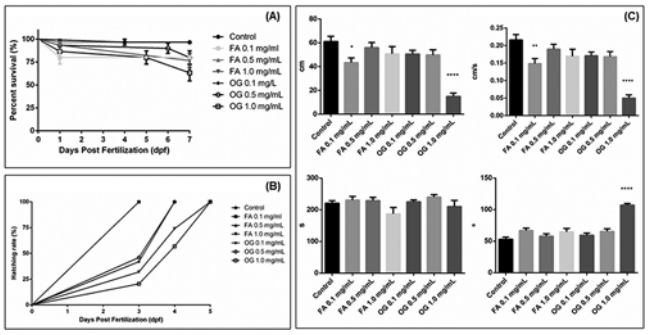
Zebrafish (Danio rerio) has been considered as a promising model for in vivo screening of bioactive molecules of plant extracts [1]. Bamboos possess a diversity of medicinal properties [2]. This study aimed to analyze the toxicological effect of the aqueous extracts from Olyra glaberrima (OG) and Filgueirasia arenicola (FA) on the locomotion of zebrafish larvae. Dried leaves were powdered and submitted to infusion. Embryos were exposure to freeze-dried extracts (0.1, 0.5 and 1.0 mg/mL) immediately after fertilization for seven days. Daily the survival rate was recorded. Animals were individually submitted for a session of exploratory behavior analyses. Total distance traveled (cm), mean

speed (cm/s), time mobile (s), and absolute turn angle (°) were considered the main parameters of exploration of a new environment and for the evaluation of movements. After that, larvae were submitted to avoidance-escape behavior from a visual stimulus. The number of larvae in the non-stimulus area during the session was considered indicative of deficits in the avoidance response [3]. There was a delayed larvae hatching rate for the treated animals (▶**Fig. 1**). The lowest survival rate was observed after exposure to 1.0 mg/mL OG. At the exploratory analysis, 1.0 mg/mL OG and 0.1 mg/mL FA induced a significant decrease in total distance traveled and mean speed. Only 1.0 mg/mL OG increased erratic movements. There were no changes in the larvae avoidance response after bamboo extracts treatment. It was possible to conclude that extracts of both species in their highest concentration showed a toxicological effect in zebrafish larvae.



▶**Fig. 1 (A)** Larvae percent survival: FA 0.1, FA 1.0, OG 0.5 and OG 1.0 mg/mL differed from the control group ($p \leq 0.05$). **(B)** Percentage of hatching rate: FA 0.5, FA 1.0, OG 0.1, 0.5 and 1.0 differed from the control group ($p \leq 0.0001$). **(C)** Exploratory parameters: FA 0.1 and OG 1.0 showed significant differences from the control group. For all analyses, One-way ANOVA was used, followed by a post-hoc Tukey's test. * represents significant difference $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ and **** $p \leq 0.0001$ in relation to control.

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P-399 Triterpenes present in *Eucalyptus tereticornis* inhibit adipocyte lipid accumulation and reduce their toxicity when they are in a plant extract

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Obesity is a complex condition with an enlargement of adipose tissue to store excess energy intake. We have identified Ursolic acid (UA), Oleanolic acid (OA) and Ursolic acid lactone (UAL) as the main molecules (78%) in an *Eucalyptus tereticornis* (Eu) extract (OBE100) and shown OBE100 has an important effect of reducing lipogenesis in adipocyte cell lines [1]. Our objective is to compare the effects of the triterpenes mixture present in the natural extract with the same amount of the molecules outside the extract. Murine 3T3-L1 adipocyte cell line was used to evaluate OBE100, M1 (mix of triterpenes present in OBE100), UA, OA and UAL biological activity. We determined treatments concentrations after the analysis of cytotoxicity levels of the different compounds and mixes. We found that cell lipid storage and leptin protein levels were reduced with each treatment but principally with OBE100, M1 and UA. However, M1 and UA outside of the plant extract present a significant higher cytotoxicity and have no effect on cellular oxidative stress while OBE100 no-triterpene fraction reduces the oxidative burst. OBE100 treatment also inhibits the expression of adipogenic and lipogenic genes (PPAR-γ, C/EBPα, SREBP-1c, ACC) and increases the expression of lipolytic genes (PPAR-α) in a higher level than the triterpenes outside the extract. These results suggest triterpenes present in Eu have anti-obesity properties but the combination of these triterpenes with other minor molecules present in the vegetal extract may have a synergistic or additive effect that improves them, reducing their toxicity and improving their biological properties.

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P-400 Ursolic acid lactone from leaves of *Eucalyptus tereticornis* regulates lipid accumulation and glucose uptake in cellular models

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Obesity is a risk factor associated with insulin resistance and it is linked with cardiovascular disease and type 2 diabetes mellitus. The potential of natural products for treating obesity is under exploration and interest has increased in the development of treatments from natural sources with fewer adverse effects. Ursolic acid lactone (UAL) is a triterpene found in leaves of *Eucalyptus tereticornis* (Eu)[1][2]. Triterpenes constitute a large family of plant natural molecules with diverse structures and functions and have been found to manifest hypolipidemic, anti-obesity and antidiabetic activity[3]. The purpose of our study is to analyze if UAL have similar properties. A crude methanolic extract (OBE100) from leaves of Eu was partitioned with ethyl acetate, concentrated and purified by Sephadex LH-20. UAL was purified by Sephadex column and preparative chromatography from OBE100. Murine 3T3-L1 adipocyte and C2C12 myocyte cell lines were used to evaluate UAL effects. We determined treatments concentrations after the analysis of cytotoxicity levels of the molecule. We found UAL reduced 32% adipocyte lipid content after 7 days of treatment compared to 18% reduction by metformin treatment. This effect was accompanied by inhibition of leptin production. We also found that this triterpene stimulated up to 24% glucose uptake in muscle cells. To the best of our knowledge, it is the first time that UAL has been proven to exhibit anti-lipogenesis effect in adipocyte cells and stimulation glucose uptake in myocyte cells suggesting its potential future application as antiobesity and antidiabetic agent.

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P-401 Using animal model systems to evaluate the impact of botanicals on the gut microbiome

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We have reported that *Rhodiola rosea* increased lifespan and improved healthspan of fruit flies [1]. The fruit fly, *Drosophila melanogaster,* is emerging as an important model system in biomedical research to evaluate the impact of various interventions on a number of phenotypes including changes in the microbiome. The main hypothesis of this project was that "*Rhodiola rosea* improves healthspan by modulating the microbiome". We tested our hypothesis in flies and then in mice with two main aims: 1) to examine whether *Rhodiola rosea* improved the healthspan of fruit flies by changing their microbial composition and 2) to evaluate the impact of *Rhodiola rosea* on the microbiome of leptin deficient mice. Flies and were fed a normal diet and were randomized to either receive *Rhodiola rosea* or control diet. *Rhodiola rosea* significantly altered the microbiome of *Drosophila* and increased the ratio of the genus *Acetobacter* and decrease the order *Lactobacillales* throughout the lifespan and increased the total bacterial load throughout the fly lifespan [2]. We then evaluated the impact of this plant extract on the gut microbiome of a severe diabetic and obese phenotype in mice; leptin deficient mice. Our preliminary data showed that *Rhodiola rosea* improved glucose levels and changed the microbiome of leptin deficient mice. We are now in the process of a more detailed data analysis. Animal model systems, both insects and mammals, can be used in the pre-clinical stage to evaluate the impact of botanical extracts on the gut microbiome as it relates to their healthspan.

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P-402 Variation of bioactive principles in different varieties of *Perilla frutescens* var. *crispa*

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Perilla (*Perilla frutescens*) is an economically and medicinally important annual crop in the Labiatae family, native to East Asia. Depending on morphological characteristics and availability, this species is divided into two varieties such as *P. frutescens* var. frutescens, the oilseed crop for source of perilla oil, and *P. frutescens* var. crispa for the aromatic leafy herb [1]. In addition, *P. frutescens* var. crispa (Jasoyeop in Korean) can be further categorized based on various morphological forms in leaf shapes and colors and flower colors. In this study, to investigate the variation in bioactivities among Jasoyeop varieties, we selected varieties based on their characteristic leaf colors like green-leaved forms, purple-leaved forms, mixed color-leaved forms, and leaf forms that are purple only on the abaxial side. Selected Jasoyeop varieties have been classified into three chemo-types, according to the main components of their essential oils. Comparative analysis on anti-melanogenic, anti-inflammatory and anti-cancer activities was preformed using 70% EtOH extracts obtained from leaves of each varieties. Although no significant difference in anti-inflammatory activity between varieties were detected, the extracts of Pfc 51 exhibited the highest anti-melanogenic activities and Pfc 13 and Pfc 22 extracts strongly inhibited the proliferation of human lung cancer A549 cells. The difference in activities seems to be related to the variations in phytochemical content and composition between varieties. The variations observed here should be useful for selection of a beneficial material for the food and pharmaceutical industries.

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P-403 Xanthine oxidase inhibitory activities of some Vietnamese medicinal plants

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In the search for novel xanthine oxidase (XO) inhibitors to treat gout and other diseases associated with the XO activity, 165 chloroform, ethanolic and aqueous extracts from 55 parts of 21 plants, belonging to 7 families, selected from Vietnamese medicinal plants, were screened for their XO inhibitory [1] potential. 21 extracts of 7 plants showed the XO inhibitory activity higher than 50% at concentration of 1mg/ml. Of these, 8 extracts belonging to 3 plants still inhibited XO activity higher than 25% at concentration of 0.125mg/ml.

The 2 highest active plants were further investigat, guided by XO assay. From trunk of *Flacourtia rukam* Zoll. et Mor. (Flacourtiaceae), vanillic acid and poliothrysosid were isolated. Their IC_{50} (μM) of XO inhibitory activity compared to that of allopurinol were 61, 18 and 22, respectively. From stem woods of *Gnetum latifolium* Blume (Gnetaceae), 7 pure compounds, *i.e.* gnetol, *trans*-resveratrol, gnetifolin A, chrysoeriol, isorhapontigenin, 2,4-dihydroxy-3-methoxystilbene and 4′,5,7-trihydroxy-3′,8-dimethoxyflavone were isolated. IC_{50} (μM) of gnetol and *trans*-resveratrol, compared that of allopurinol on XO inhibitory activity were 242, 164 and 23, respectively.

The chloroform extract of *Gnetum latifolium* was also studied on hyperuricemia pretreated with oxonate. At dose of 150mg/kg, after 7 and 14 day of experiment, the extract revealed the effect of reducing serum uric acid level comparable with that of allopurinol (10mg/kg). The study showed the potential of Vietnamese medicinal plants for XO inhibitors that can be developed, upon further investigation, for treatment of gout and other XO-related disorders.

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P-404 Young adult male mice weight gain after being late prenatally exposed to *buchinha-do-norte* aqueous extract

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Tea made with the fruits of *Buchinha-do-norte* (BDN), or *Luffa operculata*, is popularly used in sinusitis, as a direct instillation in the nostrils, or as abortifacient, once is drunk. Previous work has shown that the administration of the BDN tea impaired behavior in adult male rats [1]. The aim of the present study was to verify the alterations caused by the prenatal exposition to 1,0 mg/kg/five days, during a late period of gestation, GD17 to GD21, that are related to weight gain

of the offspring. Female and male pups were weighed at post-natal day 2 (PND2) and at PND60, and the pup weight and pup weigh gain were obtained. It was observed that weight at PND2 showed no alterations in the female or male pups ($p<0.05$). Weight gain was not observed in female pups ($p<0.05$), while the male pups showed weight gain ($t=2.206$, $df=33$; $p=0.0344$). Previous work [1] stated that BDN oral administration to adult male rats did not cause weight gain, while the young male pups which received a prenatal exposition to BDN showed weight gain. Present findings may suggest that BDN compounds, such as cucurbitacins, may have a pivotal role in the pup uterine late development, causing a predisposition to weight gain. The consumption of BDN tea during pregnancy affects the offspring, in terms of weigh gain predisposition, although the causes are not established up to today.

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P-405 Zebrafish-based evaluation of antiepileptic activity of *Pimpinella major* (L.) Huds. and its main constituent pimpinellin

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Epilepsy represents the most common chronic neurological disorder with diverse etiology, affecting at least 70 million people worldwide. Taking into consideration that the antiepileptic drugs (AEDs) are effective in only 70% of the patients, the discovery of new AEDs with novel mechanisms of action is a very important task. There is a large body of literature reporting research on the antiepileptic effects of plants, with several plant-derived compounds, such as cannabidiol, cannabidivarin and huperzine currently under development as AEDs [1,2].

The aim of this study was to perform an initial zebrafish-based evaluation of the antiepileptic activity of *Pimpinella major* (L.) Huds. (Apiaceae), plant used in traditional medicine for treating mental disorders, and isolate its main active constituents. The methanolic root extract of *P. major* was screened in a zebrafish epilepsy model based on the $GABA_A$ antagonist pentylenetetrazol (PTZ), which induces an increased locomotor activity, seizure-like behavior and epileptiform electrographic activity in zebrafish larvae [3].

At 10 µg/mL, the extract decreased PTZ-induced locomotor activity by 45.87%. As a purification tool that enables fast and effective separation of compounds, high-performance countercurrent chromatography (HPCCC) led to the isolation of pimpinellin (16 mg, purity > 98%) from 738 mg of crude extract in 30 minutes. Additionally, three other coumarin derivatives were isolated and tested. Of these, pimpinellin (60 µM) was able to potently decrease PTZ-induced locomotor activity by 63.79%. Thus, the HPCCC and zebrafish epilepsy model could be used as efficient platform for the fast identification and isolation of compounds with promising antiepileptic activity.

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P-406 A multicenter, prospective, pharmacy-based cohort study with a combination of essential oils from peppermint and caraway in functional gastrointestinal disorders

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Functional gastrointestinal disorders (FGID) are defined by lack of organic cause with interindividual differences in symptom emergence and severity. Functional dyspepsia (FD) and irritable bowel syndrome (IBS) belong to the most prevalent FGIDs.

The present pharmacy-based cohort study aimed at gaining insights into effects and tolerability of Menthacarin[a] - a proprietary specified combination of essential oils from *Mentha x piperita* L. (90 mg WS® 1340) and *Carum carvi* L. (50 mg WS® 1520) - applied in subjects suffering from FGID, particularly with mild cramps, bloating and/or fullness. Occurrence and severity of 13 dyspeptic symptoms were assessed at the beginning and during 3 weeks of Menthacarin intake using a modified Gastrointestinal Symptoms Rating Scale (GSRS) [1]. Also, patient satisfaction and tolerability were evaluated by pharmacists and pharmacy customers.

50 participants (mean age 54 years) were recruited. After 3 weeks, the GSRS total score was reduced from 48.6±17.1 to 22.8±12.3 points ($p<0.001$). 44.9% of the patients rated the perceived effects as “very good” or “good” and 30.6% as “satisfactory”, which was comparable to the pharmacists’ rating. 83.3% of the pharmacy customers´ and 87.7% of the pharmacists´ ratings for tolerability of Menthacarin were “very good” or “good”.

These findings clearly indicate the safety and the good applicability of Menthacarin in pharmacy-supported self-medication of patients suffering from FGID.

[a]Menthacarin® is the active agent of the product Carmenthin® bei Verdauungsstörungen (Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe).

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P-407 *Althaea officinalis* root extract in the light of the PhytoVIS study, a NIS in 20,870 users of herbal medicinal products

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STW 42, a marshmallow (*Althaea officinalis* L.) based cough medicine, is used in the therapy of mucosal irritation with dry cough, a condition frequently occurring in the cold season. It acts locally, in contrast to a number of other therapeutic options with systemic effects and, accordingly, a higher potential of side effects.

The PhytoVIS study, a pharmacoepidemiological data base on the use of herbal medicinal products [1], containing data from 20,870 users of herbal medicinal products, which have been captured in doctors' practices and pharmacies in compliance to the ENCePP Code of Conduct [2], was screened for users of STW 42. Patients included all age groups and most described their symptoms as cough resp. dry cough. 126 patients used STW 42 syrup and 15 STW 42 lozenges. Nearly 90% of the users rated the efficacy of the product as moderate to very good, only 3.5% reported their condition to be unchanged or worsened. The effect tended to be rated better in moderately severe as compared to mild symptoms. Overall tolerability was very good.

Together with the information on more than a thousand patients documented in earlier surveys [3, 4], these data underline the efficacy of STW 42 in relieving cough symptoms and its good tolerability. They further support its use not only in adults, but also in pediatric patients, where it presents itself to be a safe and highly used option, so giving a useful insight into epidemiology as well as data regarding efficacy and safety of STW 42.

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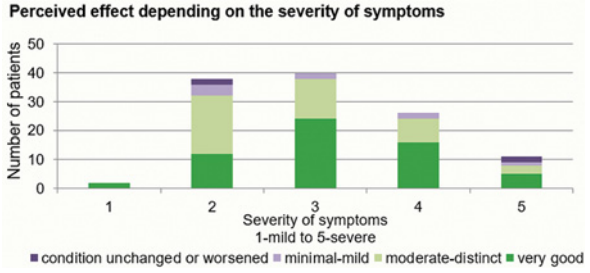

▶ **Fig. 1**

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P-408 An open-labeled randomized controlled trial on the efficacy of Thai traditional medicine for pressure ulcer

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The traditional medicine with robust researches was paid more attention for alternative pressure ulcer (PU) treatments. There is a promising Thai Traditional Medicine (TTM) practice at Kabchoeng Hospital, Thailand. Honey and a Thai Herbal Oil preparation (THO) was used specific to TTM wound diagnosis. This study aimed to compare an efficacy of the TTM practice with a standard practice in PU treatment. To evaluate an efficacy of the practice, we incorporated TTM wound diagnosis into an open-labeled randomized controlled trial design. A TTM practice group received honey or THO depended on TTM wound diagnosis, using the Thai Traditional Medicine Pressure Ulcer Assessment Tool (TTM-PUAT) [1]. A standard practice group received advance dressings, e.g. hydrogel, foam, and alginate. Study setting was home-based care in 7 hospitals in Thailand. Sixty-six patients with at least a PU, in stage 2-4 or unstageable [2], were allocated to two groups equally by minimization method. The Pressure Ulcer Scale for Healing (PUSH) scores was used to monitor 6-week PU healing [3]. PUSH scores of both groups reduced significantly comparing to the scores at baseline. However, PUSH score reduction was not difference significantly between the groups (p=0.284). The mean of PUSH scores reduction after 6 weeks was 2.58 ± 3.38 in the TTM practice group and 3.24 ± 3.49 in the standard practice group. In summary, TTM practice was effective similar to the standard practice for PU, in home-based care setting within 6-week period. This study supported the TTM practice to be an alternative treatment for PU.

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P-409 Assessment of the effectiveness of Ivy leaf (*Hedera helix*) Syrup compared to acetylcystein in adults and children with acute bronchitis

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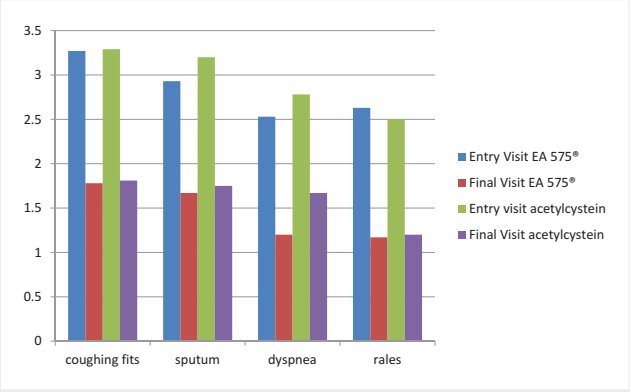
Acute bronchitis is a clinical term implying a self-limited inflammation of the upper airways that is characterized by cough. It is the most common disease in industrialized countries [1, 2] and thus, herbal expectorants enjoy high popularity in many European countries due to their favorable risk-benefit ratio [3, 4].

The present non-interventional study was intended to gain further data on the application of Ivy leaf Cough Syrup containing extract EA 575® by evaluating its effectiveness and safety in children and adults with symptoms of an acute bronchitis. Acetylcystein was chosen as comparator drug (common mono preparations).

The study was conducted on 25 sites throughout Switzerland as prospective, open, observational non-interventional study by physicians and medical practitioners.

At entry visit all clinical assessments including coughing fits, sputum, dyspnea, rales, severity of the disease and coughing quality were rated with a moderate intensity in both groups. At the final visit after seven days of treatment there was a comparable improvement in both groups for all assessments except dyspnea and number of cough attacks which showed a higher improvement in the extract EA 575® group (▶ **Fig. 1**). Cough-associated sleeping disorders improved also more in this group. Both, physicians and patients described the effectiveness of extract EA 575® as slightly better than acetylcystein. Observations of the tolerability were comparable for both products.

The study results indicate that ivy leaf extract might be an effective alternative to acetylcystein with respect to the improvement of respiratory function in children and adults at a slightly better evaluation of safety.



▶**Fig. 1** Average reduction of assessment score by physicians at final visit compared to entry visit

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P-410 CU03-1001, 50% ethanol-extract of Moutan radices cortex and *Cinnamomi ramulus* (1:1) inhibited laser-induced choroidal neovascularization (CNV) in Brown Norway rats

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Age-related macular degeneration (AMD) is the cause of blindness in people over 60 years of age. More than 30 million individuals worldwide suffer from visual impairment due to AMD [1]. Anti-VEGF therapies require repetitive intravitreal injections and have been successful in improving central vision about 30% [2]. long-term treatment associated with geographic atrophy, cardiovascular event and loss of the retinal neurotrophic activity [3, 4, 5]. Our goal is to develop an oral herbal drug to prevent AMD exacerbation, and reduce repetitive intravitreal injections and avert side effects of anti-VEGF therapies.

CU03-1001 was standardized with 12 compounds by HPLC. 30 compounds were identified using UPLC/Q-Orbitrap. After CNV, 60 and 90 mg/kg CU03-1001 were administrated orally BID for 15 days. CNV lesion areas and fluorescence leakage were significantly reduced. CU03-1001 constituents significantly inhibited reduction in the thickness of subretinal outer nuclear layer in N-methyl-N-nitrosourea-treated zebrafish or rats. CU03-1001 significantly decreased permeability in ARPE-19 cells, and migration and tube formation in HRMECs. Based on the GLP repeated toxicity test for 13-weeks in rats, NOAEL was determined as 1,250 mg/kg. The maximum tolerable dosage in beagle dogs after 28-day repeated oral administration was considered over 1,500 mg/kg. No evidence of GLP genotoxicity and safety pharmacology were found. Studies on CYP450 and UGT revealed CU03-1001 as a poor interactor. Finally, a combination therapy of current anti-VEGF agent and CU03-1001 might be possible to prevent against deterioration of AMD and to decrease side effects and the injection frequency of anti-VEGF therapies. [KIOM grants: K18270, KSN1911711]

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P-411 Echinacea reduces antibiotics through prevention of respiratory tract infections in children: a randomized, blinded, controlled clinical trial

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In children, up to 30% of viral respiratory tract infections (RTIs) develop into bacterial complications associated with *pneumonia, sinusitis* or *otitis media* to trigger a tremendous need for antibiotics.

Echinaforce® Junior tablets [400 mg freshly-harvested *Echinacea purpurea* alcoholic extract] or vitamin C [50mg] were applied tree times daily for the prevention of RTIs and cold days in children 4 – 12 years. 2 x 2 months of prevention were separated by a 1-week treatment break. Parents assessed respiratory symptoms in children via *e*-diary and collected nasal secretions for screening of respiratory pathogens (Allplex® RT-PCR).

Overall, 429 cold days occurred with *Echinacea* (N_{ITT}=103) in comparison to 602 days with vitamin C (N_{ITT}=98; p<0.001, Chi-Square test). *Echinacea* prevented 32.5% of RTI episodes resulting in an odds ratio of OR=0.52 [95% CI, 0.30-0.91, p=0.021]. Six children (5.8%) with *Echinacea* required antibiotic treatment on 45 days in comparison to 15 children (15.3%) with antibiotics on 216 days in the vitamin C group, indicating a strong reduction by 76.3% (p<0.001).

Eleven (11) and 30 events of bacterial superinfections and RTI complications occurred with *Echinacea* and vitamin C, respectively (p<0.05). *Echinacea* significantly prevented influenza (3 vs. 20 detections, p<0.05) and membranous virus infections (28 vs 47 detections, p<0.05). Finally, 76 adverse events occurred with *Echinacea* and 105 events with vitamin C, only 3 events were related with the study medication.

Our results strongly support Echinaforce® Junior tablets for the prevention of RTIs in children for a reduced need of antibiotics in this population.

P-412 Effect of administration of hydroxytyrosol on weight and fat loss: preliminary data from a randomised double blind prospective study

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DOI 10.1055/s-0039-3400123

Hydroxytyrosol (HXT) is a phenolic compound of extra virgin olive oil which is derived from oleuropein and has antioxidant and anti-inflammatory properties. In the current study a randomized double blind prospective study was performed examining the effect of HTX administration for 6 months on weight and fat loss in overweight and obese women. Thirty women with a BMI 27-35 kg/m^2 and stable body weight during the past 3 months, without serious health problems were included and randomly assigned to 3 groups: group A received 15mg of HXT daily, group B received 5mg of HXT daily and group C received placebo for 6 months. HXT or placebo was administered in the form of capsules 3 times daily before meals. All visited a dietitian on a monthly basis for 6 months and all investigators were blinded as to group assignment. Body weight, fat mass and visceral fat were measured at baseline and after 1, 3 and 6 months. The findings suggest that mean weight loss and visceral fat mass loss were statistically significant at months 1, 3 and 6 months for women receiving 15mg of HXT daily. Similarly, mean fat mass loss was statistically significant at 3 and 6 months of the study. No significant differences were found between group B and C. Based on these results, consumption of 15 mg HXT daily was effective in reduction of body weight, fat mass and visceral fat in overweight and obese women. The intervention was

P-414 Effects of lavender oil on insomnia in patients with anxiety disorders – a meta-analysis

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The efficacy of lavender oil in patients with anxiety disorders has been shown in several clinical trials [1]. Anxious patients often suffer from short sleep duration and poor sleep quality as a consequence of their anxiety disorder.

The effects of lavender oil on the Hamilton-Anxiety-Scale (HAMA) item 4 'insomnia' were investigated systematically in a pooled dataset.

A meta-analysis of five clinical trials was performed to describe subsequent effects of lavender oil on sleep in patients with anxiety.

We examined the accompanying symptoms regarding sleep based on item 4 'insomnia' of the HAMA after ten weeks of treatment. The HAMA total score was the main outcome variable of all trials. Within the single trials the change between baseline and the last value under treatment was analyzed using a model of covariance analysis with treatment as a factor and the baseline value of the outcome variable as covariate. A random effects meta-analysis was performed to combine the results of the single trials.

The ratings of 1172 patients were included in the analysis. In the pooled dataset a mean baseline value of 2.7 points (moderate to severe) for insomnia was rated in the lavender oil group and in the placebo group. A statistically meaningful difference of 0.32 points in favor of lavender oil was calculated in the meta-analysis (p = 0.0038).

Treatment with lavender oil resulted in a relevant improvement of sleep symptoms in the context of anxiety disorders in addition to the proven efficacy on the core symptoms of anxiety.

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P-415 Herbal medicinal products are an important option for the treatment of functional GI diseases in children - a systematic review

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Herbal medicinal products play an important role in the treatment dyspepsia (FD) and irritable bowel syndrome (IBS) [1]. Given the high relevance of these indications in children (2), there is the need for treatment options applicable in this age group.

A systematic review was conducted focusing on FD and IBS in children, searching for clinical studies and reviews via PubMed-Medline complemented by hand searching and cross-referencing.

The search gained 79 hits for FD with 13 of which 2 were herbals. For IBS it gained 321 hits, 5 on herbal treatments. Herbal options mentioned for IBS were Psyllium, Peppermint oil and STW 5 (Iberogast) as well as Turmeric, Cannabis, Aloe vera and Ginger, with clinical studies in children mentioned only for Psyllium, STW 5 and Peppermint oil [1, 2, 3, 4, 5]. For FD Hippophae rhamnoides and STW 5 were identified with clinical evidence on both [1, 6]. Only Iberogast was mentioned for both diseases. A number of studies supported the therapeutic usefulness in children of all ages, retrospectively [7, 8] as well as prospectively [9–14]. Data from 44.315 children showed an excellent safety profile with only few mild side effects and a convincing rating of the therapeutic usefulness.

Herbal preparations provide effective treatment options in children with FD and IBSwith a low risk profile and therefore play a prominent role in their management. While Psyllium and Peppermint oil as well as Hippophae rhamnoides show evidence only for IBS or FD respectively, grades of evidence are most convincing for Iberogast likewise in FD and IBS.

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P-416 Herbal medicinal products in the public pharmacy: a surveillance study with STW 5 in patients with functional gastrointestinal diseases

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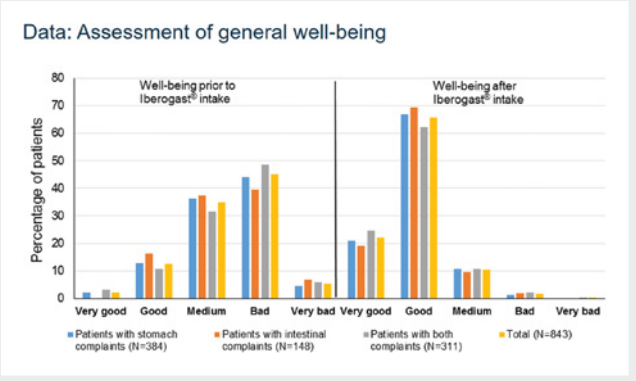
Pharmacy-based surveys on everyday life therapeutic use and patient satisfaction are tools to gain real life evidence on patient needs [1]. The use of STW 5 (Iberogast®) for treatment of functional gastrointestinal diseases is supported by a multitude of clinical, pharmacological and toxicological studies [3].

Pharmacy customers with product desire or recommendation for STW 5 received a questionnaire with the product to be answered within a week.

Data from 843 patients were evaluated. The majority was 30-49 years old, 70.6% were female. In 384 patients, complaints were related to the upper, in 139 to the lower abdomen, in 311 patients to both regions. In 7.3% of patients a functional dyspepsia had been diagnosed, in 16.9% of the 139 patients an irritable bowel syndrome. Up to 64% of the patients specified a good or very good improvement of the predominant symptom after treatment. Symptom relief was perceived as fast and covered the comprehensive spectrum of complaints. Tolerability was rated good to very good in 97.4% of all patients. Correspondingly 93.2% of the users were „very satisfied“ or „satisfied“. 91% of the customers would recommend STW 5 to others.

It can be concluded that this pharmacy-based survey gives a reliable picture of the view of patients on STW 5, with high satisfaction values as well in irritable stomach as in irritable bowel syndrome. The ratings are well in accordance to the favorable ratings of tolerability and patient perceived satisfaction from the clinical studies on the product.



▶ **Fig. 1**

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P-418 Mallow extract-containing hyaluronic (HA) acid eye drops for the treatment of dry eyes is perceived superior over an HA-only product

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Dry eye syndrome is caused by insufficient lubrication of the cornea. Extensive screen time, wearing contact lenses or staying in an air-conditioned environment are frequent triggers for dry eye symptoms

In an observational clinical phase IV study the comparative efficacy of Visiodoron Malva® (0.15% hyaluronic acid (HA) *plus* 0.5% mallow extract, HA+M) or a competitive HA-only product (0.15% HA, HA) was investigated. The following parameters were recorded: subjective efficacy, individual patients` preference, tear film breakup times (TBUT), lissamine green staining of the cornea, symptoms grade, Schirmer test, ocular surface disease index (OSDI). Furthermore, physical properties (viscosity/surface tension) of the eye drops with and w/o mallow extract were analyzed.

With HA+M in 90% of the patients OSDI improved from ‘moderate’ to ‘mild’/‘normal’, whereas with the competitor w/o mallow extract, this rate was only 60%. No difference was shown for TBUT, lissamine green staining, symptoms or Schirmer test. However, subjective efficacy perceived by patients and the patients’ preference was superior for HA+M.

Rheology of eye drops is negatively influenced by HA concentration as evidenced by concentration-dependent increase of viscosity. Notably, in HA-containing eye drops *plus* mallow extract surface tension was significantly reduced. Reduction of surface tension and thereby prevention of tear film break-ups represents an important physiological mechanism provided by surfactant proteins in tears. Although there was no difference in lubrication between the two treatments, HA *plus* mallow extract is perceived superior by patients over an HA-only product. This superiority might be mediated by the reduced surface tension caused by mallow extract.

P-419 No clinically relevant interactions of St. John’s wort extract Ze 117 low in hyperforin with cytochrome P450 enzymes and P-glycoprotein

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Background *Hypericum perforatum L.* (St. John’s wort) is used to treat mild-to-moderate depression. Its potential safety risks are pharmacokinetic drug interactions *via* cytochrome P450 enzymes and P-glycoprotein, presumably caused by hyperforin.

Aims: In a phase I, open-label, non-randomized, single-sequence study, the low-hyperforin *Hypericum* extract Ze 117 was investigated using a drugs cocktail in 20 healthy volunteers.

Results No pharmacokinetic interactions of Ze 117 were observed for CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4 and P-glycoprotein. AUC and Cmax of the used probe drugs showed 90%-confidence intervals of the geometric mean ratios of the drugs taken together with Ze 117 *vs*. probe drug alone well within the predefined bioequivalence range of 80% to 125%.

Though Ze 117 did not induce dextromethorphan metabolism by CYP2D6, it weakly increased dextromethorphan AUC ratio (mean 147.99, 95% CI 126.32-173.39) but not the corresponding metabolic ratio.

Conclusion Ze 117 does not show clinically relevant pharmacokinetic interactions with important CYPs and P-glycoprotein.

P-420 Salvia extract for the treatment of menopausal symptoms: a randomized, controlled, blind clinical trial

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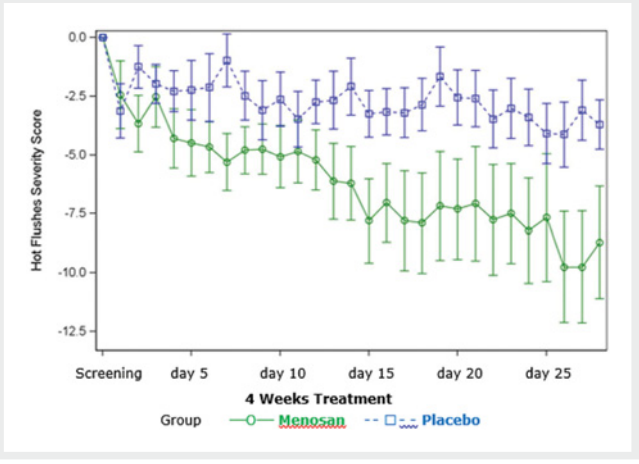
DOI 10.1055/s-0039-3400129

Menopausal transition is typically accompanied not only by hot flashes but also by mood alterations and sleep disorders. *Salvia officinalis* has been traditionally used to treat those symptoms.

Eighty (80) menopausal women between 48 – 65 years of age received Menosan® tablets [3′400 mg ethanolic extract of *Salvia officinalis*] or placebo under randomized double-blind conditions for 4 weeks. The primary clinical endpoints menopausal rating scale [MRS] and hot flush severity and incidence [HFS] were monitored throughout therapy and were finally correlated with quantitative electroencephalographic [qEEG] measurements in a per protocol analysis.

Salvia off. potently reduced MRS by 39.2% from 15.3 ± 6.87 to 9.3 ± 5.75 and significantly in comparison to placebo (p=0.002). The somato-vegetative subscale improved by 40.8% from 7.1 ± 2.67 to 4.2 ± 1.84 (p<0.001). The HFS score was reduced by 55.3% from 15.9 ± 13.77 to 7.1 ± 7.41, reaching significance compared to placebo on week 3 onwards (▶**Fig. 1**, p=0.0284). Sleep quality as per qestionnaire [SF-B/R] and fatigue in profile of mood state [POMS] significantly improved with *Salvia*, whilst the HAMA showed a non-significant trend only. Clinical effects of *Salvia off.* found a pharmacological correlation in changes of alpha1/2, delta, theta and beta1/2 qEEG values.

Menosan® Salvia showed a significant and clinically relevant amelioration of the broad complex of menopausal symptoms with a high specificity on hot flushes. It induced a higher mental capacity and a more relaxed state of mind compared to placebo. EEG data documented an impact on central nervous transmitter systems involved in neuroadaptive processes as required by the physiological estrogen decline in menopause.



▶**Fig. 1** Change of hot flush severity score during 4 weeks treatment with *Salvia off.*

P-421 St. John´s wort in a large epidemiological study on the use of herbal medicinal products

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DOI 10.1055/s-0039-3400130

The PhytoVIS study, presumably the largest study worldwide on herbal medicines, is a non-interventional study (NIS) conducted in pharmacies and physician´s offices in Germany with 20,870 patients. It aims to describe the epidemiology, medical needs and perceived effectiveness of the therapy with these products [1]. In this setting, it is of special interest to evaluate the respective data on the St. John´s wort (*Hypericum perforatum* L.) preparations used in psychic depression to identify potential specific patterns of its therapeutic use.

The total database of 24,056 patient questionnaires was screened for the use of a leading product in this field, Laif 900. Epidemiological data on gender, age and disease state as well as on the use an perception of the herbal product were evaluated.

80 patients taking Laif 900 were identified. The majority of patients was female. The general tolerability of the product was good. In 41% of all patients the perceived effect was very good, and another 43% gave it to be moderate to distinct. Only 4% of patients perceived no effect.

Due to the provenience of the present data from a large non product-specific survey, they augment, despite of the rather small sample size, the available evidence on *Hypericum* products in moderate depression. The majority of patients gave the effect to be moderate or better with more than a third stating it to be very good. This confirmed the positive rating of its use already described in previous non-interventional and interventional studies [2].

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P-422 STW 5 in 1515 patients with functional gastrointestinal diseases

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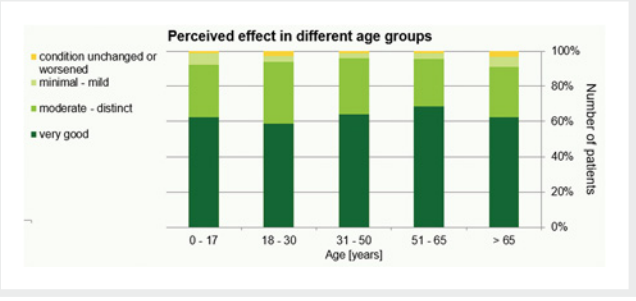
Herbal medicinal products are an important treatment option for patients with functional gastrointestinal diseases (FGID). Data on the use of STW 5 [1] in FGID were selected from the PhytoVIS study, a non-interventional study in 20,870 patients who had used a herbal product within the last 8 weeks before the survey [2]. The aim was to describe the epidemiology, medical needs and the perceived effect of the therapy in these patients.

Questionnaires related to STW 5 were evaluated regarding epidemiological data on gender, age and disease state as well as on the use of the medicine and its perception.

From the PhytoVIS data 1515 patients taking STW 5 could be identified. About two thirds of the patients had symptoms associated with FD, ca. one third associated with FD or IBS. 94% of patients reported the therapeutic experience to be moderate or better, 63% stated it to be very good, with 30% of all patients experiencing an effect after a few minutes, 80% after some hours. Side effect profile and tolerability of STW 5 were convincing.

PhytoVIS turned out to be a suitable tool to study the epidemiology of patients with functional GI diseases treated with STW 5. STW 5 proved to be a fast-acting therapeutic option rated positive not only in a vast variety of gastrointestinal complaints, but also regardless of factors like the age of the patients, thus making it an interesting option for vulnerable patient groups and patients suffering from often multi-causal gastrointestinal diseases with different symptoms.



▶ **Fig. 1**

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P-423 Treatment of restless legs syndrome with *Bryophyllum pinnatum*: a case series

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Restless legs syndrome (RLS) may seriously affect patients' sleep and quality-of-life, but established pharmacological therapy often have severe side effects. Therefore, new therapeutic approaches are needed. Preparations of leaves from *Bryophyllum pinnatum* (Lamarck) Oken [syn.: *Kalanchoe pinnata* (Lamarck) Persoon] have been proposed to possess both sedative and muscle relaxant properties [1]. Moreover, prospective observational trials revealed good effectiveness in the treatment of sleep disorders with *Bryophyllum pinnatum* 50% chewable tablets [2,3].

We set out to experimentally treat RLS patients with preparations of *Bryophyllum pinnatum* [4].

The pre/post comparison of polysomnography tests revealed a frequency reduction of periodic legs movements during sleep (PLMS) in four out of our five patients, most interestingly, also in the two patients with the highest PLMS indexes. Moreover, patients with low sleep-efficiency, moderate sleep-latency and high arousal index at treatment-begin could show improvement for these sleep parameters. The one patient who did not show improvements either in RLS symptoms or in sleep quality was able to abstain from benzodiazepines, which favourably affected her health status. Questionnaire results were in line with the polysomnography data.

This case series suggests that RLS treatment with *Bryophyllum pinnatum* preparations (mainly 50% chewable tablets, or the lactose-free alternative, 33% mother tincture) can reduce restless legs symptoms and improve sleep quality. Spasmolytic properties and the sedative effect of *Bryophyllum pinnatum* preparations seem to play a role. Randomised controlled trials with appropriate size and treatment duration are urgently needed.

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P-425 Antimicrobial activity and phytochemical analysis of *Combretum collinum* leaves extract

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Herbal medicinal products show significant contributions to mankind in the treatment of infections with pathogenic bacteria and fungi. The present study aimed at finding the antimicrobial potential of ethanolic leaf extracts from *C. collinum*. The phenolic profile of the ethanolic extract could already be characterized in previous studies [1]. However knowlegde about the lipophilic part of the extract is still scarce. In order to obtain further information on the lipholilic components of *C.collinum*, a GC-MS analysis of the hexane extract of the leaves was performed.

During the GC-MS-analysis 22 new substances including myristic acid, palmitic acid, stearic acid, oleic acid, lignoceric acid, oleic acid amide, campesterol, stigmasterol and beta-sitosterol could be identified by comparison with spectra from the NIST2 database.

The antibacterial activity of the ethanolic extract of *C. collinum* against eight bacterial strains was evaluated by comparing the diameters of the inhibition zone with standard antibiotics. Subsequently, the MICs of the extract were tested against selected bacterial strains that had been shown to be sensitive.

Inhibition zones were found for the ethanolic extract of *C.collinum* for the following microorganisms: *S.epidermidis, S.aureus, MRSA, K.pneumoniae, Enterococcus, P.aeruginosa.* The effect was strongest against *S.epidermidis, S.aureus* and *MRSA*. MIC values resulted in 275.0 µg/ml for *S. epidermidis* respectively 385.5 µg/ml for MRSA.

The tested ethanolic leaf extract showed a promising antibacterial effect. In further experiments it has to be investigated which volatile components of the hexane extract also occur in the ethanolic extract and thus can contribute to the antibacterial effect.

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P-426 Antimicrobial and cytotoxic properties of the *Copaifera reticulata* oleoresin and its major diterpene acids

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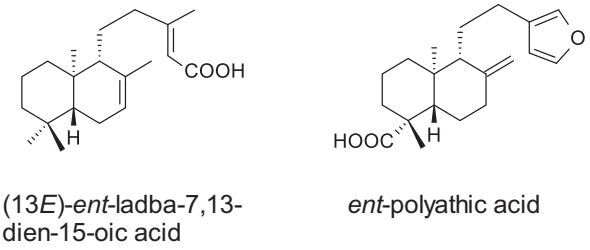
The oleoresin of Brazilian *Copaifera reticulata* (Fabaceae) is traditionally used for the treatment of skin and urinary tract infections, respiratory diseases, rheumatism, ulcer and tumors. Thus, playing an important role in the primary health care of the indigenous population [1,2].

As previous pharmacological tests used the crude oleoresin and only a few studies dealt with pure chemically defined compounds, the aim of this study was to systematically evaluate the antimicrobial and cytotoxic properties of the oleoresin and to assign traditional uses to specific secondary metabolites.

Whereas no cytotoxic activity was detected, the oleoresin showed activity against gram-positive bacteria *Enterococcus faecium* (IC_{50} value of 4.2 µg/mL) and methicillin-resistant *Staphylococcus aureus* (MRSA, IC_{50} value of 5.3 µg/mL). Fractionation of the oleoresin yielded two dicarboxylic diterpene acids and the four major diterpene acids, comprising three different diterpene scaffolds. Interestingly, the activity was not restricted to a certain diterpene-type but rather correlated with the compounds' lipophilicity, with the most active compound, (13*E*)-*ent*-labda-7,13-dien-15-oic acid, displaying IC_{50} values of 1.6 (*E. faecium*) and 2.5 µg/mL (MRSA), respectively. The major diterpenoid, *ent*-polyalthic acid, was significantly active against dermatophytes with IC_{50} values of 6.8 µg/mL (*Trichophyton rubrum*) and 4.3 µg/mL (*T. mentagrophytes*).

The present study proved the antimicrobial effects of the *C. reticulata* oleoresin and its diterpenoid constituents, supporting its wide use in folk medicine for the treatment of skin and urinary tract infections and provides potential lead structures for the treatment of two clinically relevant bacterial strains.



(13*E*)-*ent*-ladba-7,13-dien-15-oic acid

ent-polyathic acid

▶**Fig. 1**

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P-427 Can one monograph cover all traditions? The *Cyperus rotundus* L., case

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The drive to incorporate Traditional Chinese Medicine (TCM) and Ayurvedic herbal drugs in Western Pharmacopoeias is undoubtedly a positive action towards safeguarding public health.

C. rotundus rhizome is an herbal medicine within at least two traditional systems from outside of the EU and is currently available on the EU market. In this study, a diverse collection of samples from the EU market are analyzed using a three-tier testing approach (Fig. 1) to investigate whether the ethnobotanical origin of raw materials has a significant impact on characteristics.

DNA barcoding analysis successfully confirmed the identity of most unprocessed market samples as *C. rotundus*, and samples fell into two subgroups within the species. Two main processing types were detected within the *C. rotundus* sample set of TCM origin, dried with the fibrous roots removed and sliced/dry-fried. The Ayurvedic *C. rotundus* rhizome market samples were of high macroscopical conformity, and adhered to the standards set by the Quality Standards of Indian Medicinal Plants[1] rather than the Ayurvedic Pharmacopoeia of India[2]. The generated HPTLC profiles correlated with the traditional origin of the material and were unaffected by the processing type.

By providing an amalgamated investigation on the *C. rotundus* rhizome material available to EU consumers, this study highlights the significance of the traditional background of materials. In a global commerce environment, the standardised labelling of goods based on Latin binomials does not give a sufficient level of detail. The quality standards required for each tradition are separate, and often not interchangeable.

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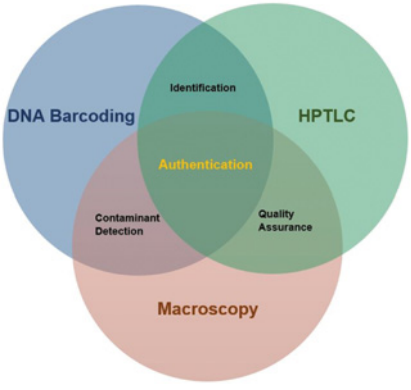

▶**Fig. 1** The three-tier approach followed in the study.

P-428 Coumarins and anthelmintic A-type procyanidins from the roots of *Paullinia pinnata* L.

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Extracts from the roots of *Paullinia pinnata* L. are used in West Africa as traditional remedies for a variety of diseases including infestations with soil-transmitted helminths [1]. Based on the results of an ethnopharmacological survey in Ghana [2] an aqueous acetone (70%) extract was investigated in this study for its anthelmintic and phytochemical properties. The crude extract showed lethal effects against the free-living nematode *Caenorhabditis elegans* (LC_{50} 2.5 mg/mL after 72h of incubation; positive control: Levamisole-HCl 40 mM, negative control: DMSO 1%) and bioassay-guided fractionation using Sephadex® LH-20 followed by preparative HPLC lead to oligomeric procyanidins (OPC) as the predominant class of active compounds. Structure elucidation by LC-MS and NMR revealed cinnamtannin B1 (epicatechin-(2β→O→7,4β→8)-epicatechin-(4β→8)-epicatechin), parameritannin A1 (epicatechin-(2β→O→7,4β→8)-[epicatechin-(4β→8)]-epicatechin-(4β→8)-epicatechin), epicatechin-(4β→8)-epicatechin-(2β→O→7,4β→8)-epicatechin-(4β→8)-epicatechin and epicatechin-(2β→O→7,4β→8)-*ent*-catechin-(4β→8)-epicatechin as the major OPCs in the extract. The anthelmintic activity of OPCs is well known [3], however suprisingly, the A-type trimer cinnamtannin B1 caused a significantly higher mortality in *C. elegans* than the structurally related B-type trimer procyanidin C1 (86 % vs. 47 % at 1 mM respectively).

In addition to the OPCs, a glucosyloxy-4-methyl-2(5H)-furanone along with several coumarins, including cleomiscosins A-D and the novel isofraxidin-7-O-β-D-glucopyranosyl-(1′′→6′)-α-L-rhamnopyranoside, were isolated. None of these compounds contributed to the anthelmintic activity, however, the presence of coumarins has not been described previously for *Paullinia* species.

In summary, this study provides an insight into the composition of phenolic compounds in the root extract from *P. pinnata* and is one of the first investigations of A-type proanthocyanidins against nematodes, supporting the traditional use as an anthelmintic remedy.

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P-429 CYP450 activity inhibition by *Guazuma ulmifolia* and major phytochemical constituent, procyanidin β2 *in vitro*: assessing the potential for drug interactions

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Research in Jamaica reports prevalence rates for medicinal plant use of 73%, with 27% concomitant use with pharmaceutical drugs, highlighting the importance of assessing potential plant-drug interactions in a region where little such work has been undertaken to date. This *in vitro* study aimed to undertake an initial assessment of the potential inhibitory impact of a standardized *Guazuma ulmifolia* aqueous bark extract (GUABE), against the activity of key drug-metabolizing cytochrome P450 enzymes (CYPs). GUABE is used across the Caribbean for a number of health conditions and, in Jamaica, often used in root tonics, a commonly consumed health beverage. Results indicated potent inhibitory activity against CYPs 1A2 and 3A4 (IC_{50} = 5.2 and 6.7 µg/ml respectively) and moderate activity against CYPs 2C9, 2C19 and 2D6 (IC_{50} = 10.8, 79.9 and 28.5 µg/ml respectively). HPLC analysis of GUABE identified procyanidin β2 (Pβ2) as the most abundant phytochemical present. Assessment of Pβ2 against the activity of CYPs 1A2 and 3A4 demonstrated moderate (IC_{50} = 43.7 µM) and weak activity (IC_{50} > 145.5 µM), respectively. Synergistic analysis, using isobologram and combination index analysis, indicated slight synergistic interaction between GUABE and Pβ2 for the inhibition of CYP1A2. Pβ2, whilst not solely responsible for the observed potency of GUABE against CYP1A2, appears to play a part through synergistic interaction with other phytochemicals present in the extract. The potent inhibition of CYPs 1A2 and 3A4 *in vitro*, provides a useful preliminary indication of the potential for adverse herb-drug interactions and warrants further *in vivo* and clinical studies.

P-430 Cytotoxic effect of white forsythia (*Abeliophyllum distichum* Nakai) extracts on human melanoma SK-MEL-2 cells

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Abeliophyllum distichum Nakai, a monotypic genus with a single species of deciduous shrub in the olive family, has been used as an ornamental plant due to its horticultural value. Current pharmaceutical studies have revealed the pharmaceutical application of *A. distichum* based on its anti-diabetic activity [1] and anti-inflammatory effect [2]. Although these indicate the considerable potential of *A. distichum* as a crude drug and a dietary health supplement, studies on the biological activity of *A. distichum* have been limited. In this study, the methanolic extracts of of *A. distichum* leaves, stems and fruits were evaluated for cytotoxicity to human melanoma cell (SK-MEL-2). MTT assay revealed that leaf extract at 50 µg/ml, 100 µg/ml and 200 µg/ml reduced cell viability to 48.02%, 21.88% and 13.69% respectively. Annexin V+ cell populations, increasing activity of caspase 3 further demonstrated that apoptosis induced by leaf extract. To investigate the molecular mechanisms underlying apoptosis induction, cells were treated with leaf extract for 48 h, and western blot analysis was performed using specific antibodies. Western blot analysis suggested that anti-proliferation and apoptosis induction by leaf extract should be associated with MEK–ERK-dependent caspase activation. Taken together, these results suggest that *A. distichum* leaves have compounds with cytotoxicity against SK-MEL-2 cells, and could be developed as a potential source for improving human health.

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P-432 Harnessing the wound healing potential of a conifer balm: re-epithelialization enhancing effects of Norway spruce balm (*Picea abies*)

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Wound healing and anti-infective preparations from natural sources were of fundamental importance in the pre-antibiotic era. Nowadays wound therapy faces resistances against multiple microorganisms and new agents derived from nature are in demand. One of those traditional herbal medicines is the balm of Norway spruce (Picea abies (L.) H. Karst., Pinaceae) which has been shown to be effective in subjects with chronic wounds by clinical trials. [1] [2] However, the active constituents, their mode of action and the quantitative composition remain unknown.

For this reason the isolation and identification of constituents with the subsequent examination of their effect in an in vitro re-epithelialization model was targeted. Flash chromatography, SFC, HPLC-UV-DAD as well as LC-MS, 1D- and 2D-NMR were used for isolation and structure elucidation of pure compounds. A potential effect on proliferation or migration (re-epithelialization) in a HaCaT keratinocyte based model was examined by monitoring the closure of a gap in the cell monolayer.

Four extracts of Norway spruce balm and nine pure compounds (resin acids, their hydroxylated derivatives, the labdane diterpene abienol and the lignan pinoresinol) were tested in the cell model. Among them three compounds were detected to induce a faster closure of the cell free area compared to the vehicle control.

First insights into the complex composition of Norway spruce balm were gained. Additionally the effect on re-epithelialization could be – at least in part – traced back to some of the isolated pure compounds.

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P-434 *In vivo* examination of anti-hyperlipidaemic effect of *Stellaria media* in rats

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Stellaria media has traditionally been applied as medicinal plant in the treatment of hypercholesterolemia. Since there are no firm experimental proof to support the rationale of this practice, we aimed to assess the *in vivo* effect and safety of *Stellaria media* tea in hypercholesterolemic rats.

Adult male Wistar rats were divided into 3 groups. Beside control group, who received standard laboratory chow, the hypercholesterolemic group received cholesterol-enriched diet, and the chickweed-treated hypercholesterolemic group received cholesterol-enriched diet and 100 mg/kg *Stellaria media* tea lyophilizate for 8 weeks. Blood samples were collected to determine liver and kidney function and serum lipid profile, and echocardiography was performed to assess cardiac morphology and function.

The serum total cholesterol, LDL- and HDL-cholesterol levels were significantly elevated, but no change were observed in triacylglycerol concentrations in group receiving cholesterol-enriched diet. The treatment with chickweed did not cause any significant change in serum lipid profile or in body weight increase. Moreover, liver and kidney functions were unaltered, cardiac morphology and function were not changed due to *Stellaria media* tea lyophilizate.

Although chickweed does not seem to be toxic when consumed as tea, our results do not support the rationale for its use in the treatment of hypercholesterolemia.

P-436 Inventory of medicinal plants in the sacred forests of the Manon Community of Guinea

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DOI 10.1055/s-0039-3400141

Background. Today, many animal and plant species are threatened with extinction, even in the sacred forests which are usually rigorously protected. Within the Guinean Manon community, the protection of biodiversity has always obeyed their beliefs and cultural values through their sacred forests[1-2]. Today, these forests are shrinking alarmingly. Aim. To evaluate the vegetation in sacred forests in Guinean Manon area. Results. An interview of 45 indigenous traditional healers and patriarchs led to the collection of 128 plant species from which 110 were identified in the sacred forests. These plants are mainly used in the management of prevalent infectious diseases. Botanical investigations have highlighted the probable extinction of important medicinal plant species such as *Pavetta owariensis* (Rubiaceae), *Cola simiarum* (Sterculiaceae), *Afrosersalisia afzelii*, (Sapotaceae), *Calamus deerratus* (Arecaceae) along with 37 endangered species including *Thaumathococus danielli* (Zingiberaceae), *Bussea occidentalis* (Caesalpiniaceae) and *Hannoa klaineana* (Simaroubaceae). Many other plant species are in danger of extinction due to deforestation linked to the industrial palm oil, rubber, intensive urbanization and decadence of cultural values. Conclusion. The gradual loss of biodiversity is likely to endanger the foundations of the traditional medicine which was developed and passed on from generation to generation, Considerable efforts are needed to safeguard the little remaining of these Manon sacred forests, sanctuaries of cultures, traditions, and medications

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P-437 Investigations on Ayurvedic medicinal plants towards Inhibition of quorum sensing and biofilm produced by periodontal bacteria isolated from diabetic patients

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Periodontitis includes diverse inflammatory circumstances that are common in diabetics and arise due to bacterial biofilms produced by quorum sensing (QS). The current project aims to evaluate the efficacy of certain herbs and formualtions to eradicate biofilms and inhibit quorum sensing of oral pathogens. Plants were extracted with 90% methanol using cold maceration. A primary analysis of extracts was performed using UHPLC and ATR-FTIR. Clinical strains from female diabetic patients were isolated and identified using 16S rRNA. A total of 35 medicinal plants were analyzed and a significant inhibition of *Pseudomonas aeruginosa* by *Syzygium aromaticum* oil (IC_{50} 0.024 mg/ml), *Myristica fragrans* (seed) (IC_{50} 0.024 mg/ml) and *Juglans regia* (root bark) (IC_{50} 0.048 mg/ml) was recorded. Against *Staphylococos aureus* a higher inhibition was seen i.e *Juglans regia*">*Juglans regia* (IC_{50} 0.097 mg/ml), *Syzygium aromaticum* (IC_{50} 0.024 mg/ml) and *Myristica fragrans* (IC_{50} 0.097 mg/ml). *Syzygium aromaticum, Myristica fragrans* and *Juglans regia* were active against most tested clinical strains. The clinical strains were found resistant to various antibiotics including ceftriaxone, cephradine, imipenem, meropenem, ofloxacin, amoxicillin, and amoxicillin-clavulanic acid. During antiquorum sensing experiments, the order of QS inhibition was *Syzygium aromaticum* > *Juglans regia* ≥ *Myristica fragrans*. During antibiofilm assays, excellent inhibition was observed for *Syzygium aromaticum* (72%) *and Juglans regia* (65%). We are further exploring the plant extracts for isolation of active compounds.

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P-441 Phytochemical and pharmacological investigations of *Albizia julibrissin* DURAZZ. bark extract with respect to anti-inflammatory effects

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Chronic inflammation is involved in a number of diseases such as rheumatoid arthritis, psoriasis or ulcerative colitis. The most common treatment for chronic inflammatory diseases are nonsteroidal anti-inflammatory drugs (NSAIDs). Their long-term use, however, may cause a series of adverse effects. The goal of this research is to find plant based alternatives and novel compounds with the ability to improve classical treatment of chronical inflammatory diseases. [1]

The dichloromethane, methanol and water extracts of *Albizia julibrissin DURAZZ.* bark have been found active in an anti-inflammatory assay at 50µg/ml in LPS and IFNγ induced murine RAW 264.7 macrophages and microglial BV-2 cells. [2] After liquid-liquid extraction for fractionation of the active compounds, all 6 fractions showed inhibitory activity on NO production. However only the lipophilic fractions 1-3 did not interfere with cell viability, compared to cells treated with solvent control. Therefore, the reduced production of NO in cells treated with the polar fractions 4-6 is most likely due to cytotoxic effects.

Active components of the extracts and the active compounds of Albiziae cortex are investigated by LC-MS dereplication and isolation. Since the genus *Albizia* is known for triterpenoid saponins, saponins are expected among the active constituents. [3, 4]

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P-442 Phytochemical characterization and *in vitro* assessment of oral-health related pharmacological activities of *Salvadora persica* leaves

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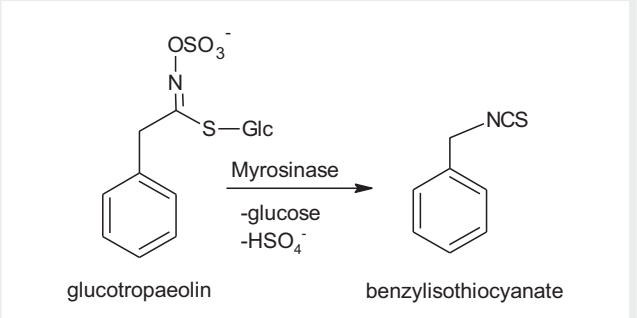
The stems and roots of the ever-green shrub *Salvadora persica* L. (Salvadoraceae) are important sources of plant-based tooth-cleaning sticks (miswak) commonly used for oral hygiene throughout the Arabian peninsula and the wider Muslim world [1][2]. Also the leaves of the plant are traditionally eaten as salad and used for medicinal purposes [3], but studied in less detail so far. Therefore, the aim of this study was the phytochemical and pharmacological investigation of *S. persica* leaves.

The phytochemical composition of a methanolic *S. persica* leaf extract was assessed by LC-HRMS analysis. Some of the major compounds were assigned as the glucosinolate glucotropeolin and as a series of kaempferol and isorhamnetin glycosides.

Furthermore, the oral health promoting-effects of the methanolic leaf extract (SPML), of glucotropaeolin (GT) and of benzylisothiocyanate (BT), a glucotropeolin degradation product formed by myrosinase-catalyzed cleavage (▶**Fig. 1**), were assessed in cell-based assays on anti-inflammatory and anticancer activity.

Only BT was able to inhibit the growth of HNO97 human tongue carcinoma cells. Also LPS/IFN-γ-induced NO production in RAW264.7 cells was only inhibited by BT. However, SPML as well as GT and BT potently inhibited IL-1β, TNF-α and IL-8 production in neutrophils stimulated with LPS. Moreover, SPML and BT strongly inhibited ROS production in this model.

Since primed and hyperactivated neutrophils are known to be involved in inflammatory oral diseases like periodontitis [4], the effects observed for SPML, GT and BT in stimulated neutrophils may substantiate the traditional use of *S. persica* leaf preparations for oral health purposes.



▶**Fig. 1** Myrosinase- catalyzed degradation of glucotropaeolin to benzylisothiocyanate.

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P-443 Plaunol A isolated from *Croton stellatopilosus* suppress inducible nitric oxide synthase (*iNOS*) and cyclooxygenase-2 (*COX-2*)

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A cyclic diterpene, namely plaunotol from *Croton stellatopilosus* Ohba or plaunoi (Thai name) has long been used in the treatment of gastric ulcer and it has been manufactured in a gelatin capsule under the trade name as Kelnac® (Sankyo Daiichi, Japan) [1]. Other cyclic diterpenes have also isolated from plaunoi including labdane-, kaurene-, clerodane-type diterpenes[2, 3]. Previously, plaunotol, plaunol E and plaunol F been reportedanti-inflammatory activity in lipopolysaccharide (LPS)-induced murine macrophage RAW264.7 cells [4]. In the present study, plaunol A, isolated from the stem, was assessed for an anti-inflammatory activity in the LPS-induced RAW264.7 cells model. The result showed that plaunol A exhibited an inhibitory effect on nitric oxide (NO) production with an IC_{50} of 11.69 μM and cytotoxicity at the concentration more than30 μM. In addition, the transcription profile analysis of inducible nitric oxide synthase (*iNOS*) and cyclooxygenase-2 (*COX-2*) genes in the RAW264.7 cells using qRT-PCR technique that plaunol A inhibited the NO production by suppressing the *iNOS* and *COX-2* mRNAs. In conclusion, plaunol A possessed an anti-inflammatory effect by suppressing the iNOS and COX-2 in the macrophage RAW cells.

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P-444 Variation in bioactive compounds and bioactive principles of rosehip (*Rosa rugosa* Thunb.) during repening

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The fruit ripening is a genetically programmed process that involves a number of biochemical and physiological processes assisted by variations in gene expression and enzyme activities. This process generally affects the phytochemical profile and the bioactive principles in fruit and vegetables. To appraise the variation in bioactive principles of rosehip from *Rosa rugosa Thunb.* during its ripening process, we analyzed the changes in antioxidant and anti-elastase activities and polyphenolic compounds during the four ripening stages of rosehips. Overall, an extract of unripe rosehip (stage 1) contained the highest levels of total phenolic and flavonoid contents, radical scavenging activity, reducing power, oxygen radical antioxidant capacity and elastase inhibitory activity, compared with the extracts of rosehip at other stages of ripening. In addition, we found that the reduction of flavonoid content occurs because of decreased transcriptional levels of genes involved in flavonoid biosynthesis pathway during ripning process. Based on HPLC analysis, we found that the extract of unripe fruit contained highest amount of myricetin(0.00±0.00 to1.51±0.76 μg/10 mg of extract), caffeic acid(7.18±0.20 to 39.69±2.76 μg/10 mg of extract), chlorogenic acid(6.08±0.54 to 39.69±2.76 μg/10 mg of extract), syringic acid(2.62±1.31 to 69.56±19.66 76 μg/10 mg of extract) and p-coumaric acid(0.00±0.00 to 15.68±1.69 μg/10 mg of extract), and suggested that the antioxidant and anti-elastase activities of the extract obtained from stage 1 should be mediated by the presence of these compounds. In addition, we analyzed the interaction sites and patterns between these compunds and elastase using the structure-based molecular docking approach, and suggested that Chlorogenic acid strongly interacted with elastase. Taken togather, these findings suggest that the maturity of rosehip has profound effects on the pharmaceutical value of *Rosa rugosa Thunb.*

P-445 Proper planting density for direct seedling of 5 years old ginseng in rain shelter house

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Ginseng *(Panax ginseng C.A. Meyer)* is widely cultivated in Korea as a medicinal herb. Gangwon-do(Province), especially in the northern part state of South Korea, has a favorable environment for ginseng cultivation due to its cool climate. So, the area of ginseng cultivation in Gangwon-do is Steadily increasing. Conventional ginseng cultivation uses an inclined shading facility. In order to improve productivity and reduce workforce, it is necessary to cultivate using rain shelter house and direct seedling. It is possible to reduce the number of pesticide spraying (12~15 times → 2~4 times). Therefore, ginseng production also increases more than twice as much as conventional cultivation. Direct seedling in ginseng cultivation using the rain shelter house is expected to reduce the labor, improve growth and promote eco-friendly cultivation. In this study, we conducted to determine the proper planting density of 5-year-old ginseng in the rain shelter house. We conducted the study through four treatments; 63, 72, 108, 180 root/m^2. 1~2 lips of dehisced ginseng seed had a hill dropping, covered with it 2~3 times thicker than the seeds and then covered with it and fully watered.
We concluded that the 72root/m^2 could be a proper planting density of the rain shelter house in northern region of Korea.

▶**Tab. 1** Growth characteristics and yield of 5-year-old ginseng according to the planting density.

Trt. (root/m^2)	Root length (cm)	Tap root diameter (mm)	Fresh weight (g/root)	Red coloration (%)	Postharvest root rots of ginseng (%)	Yield (kg/1.6m^2)
63	28.6 ±3.6	23.8±0.1	82.1 ±9.0	10.1	0.4	3.8
72	28.9 ±2.4	22.5±1.3	98.5 ±6.1	5.6	0.2	4.2
108	28.2 ±1.0	23.9±0.8	65.6 ±2.2	8.7	1.3	3.2
180	28.0 ±2.6	21.9±1.8	83.6 ±1.5	2.3	0.1	3.1

P-446 Fuctuation in active component biosynthesis in ginseng sprouts according to cultivated period

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Background: Major active components of ginseng are ginsenosides which pharmacological effects include anticancer, anti-stress, anti-fatigue, antioxidant and anti-aging effects. Ginsenosides levels are higher in leaves than in roots. Therefore, consumers are increasingly interested in using ginseng sprouts.

Methods and Results: Ginseng sprouts were cultivated from June to late July in greenhouse. Ginseng sprouts cultivated during 20, 30, 40, 50 and 60 days was harvested, washed and used in the experiment. Two indicators, namely, 30 kinds of ginsenosides and 8 kinds of phenolic acids were monitored by HPLC(detection). Total ginsenoside content increased 1.27 times in leaves and decreased 0.67 times in roots after 60days.The contents of ginsenoside Rg1, Re, Rb1, Rc, F3 and F4 of leaves were increased and ginsenoside Rg1, Re, Rb1, Rb2 and Rf of roots were decreased after 60 days, especially. The total phenolic acid contents of leaves decreased slightly until 40 days and then increased. The major components of ginsenoside and phenolic acid in leaves were Re and sinapic acid, respectively.

Conclusion: From the above results, ginseng sprouts cultivated for 50 days is more effective ingredient than roots, so it can be said that it is good for consumers to use.

P-447 Characterization of glycosylated flavonoids obtained from enzymatic reaction by matrix free laser desorption ionization mass spectrometry (LDI-MS)

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Plant polyphenols such as stilbenoids and flavonoids exhibit a wide range of biological effects such as antioxidant activities and therefore represent an interesting field of research [1]. However, due to their poor solubility in water, glycosylation is generally employed to improve their bioavailability as well as biochemical and pharmaceutical properties [2]. Among different possible pathways, enzymatic glycosylation using sucrose phosphorylase from *Bifidobacterium adolescentis* (BaSP) as enzyme, sucrose as donnor and stilbenoids or flavonoids as polyphenolic acceptors allows the formation of α-glucosylated compounds with high yields [3].

The present study therefore focuses on two main aspects, first the formation of glycosylated flavonoids by enzymatic reactions and secondly their rapid and direct detection by matrix free laser desorption ionization mass spectrometry (LDI-MS).

The polyphenol glucosylation was carried out using engineered variants of BaSP as catalyst. For example, the resveratrol-α-glucoside was obtained with high yields using variant BaSP Q345F. Following a SPE pre-purification for eliminating sugars from the reaction medium, all target compounds were directly detected from the methanolic wash solution by LDI-MS. Next to the quasi-molecular ions $[M-H]^-$ of glycosides also those of their aglycons were systematically observed in the spectra.

Overall present results show that LDI-MS, preceding SPE separation, represents a direct and efficient technique for the rapid identification of glucosylated stilbenoids and flavonoids originating from enzymatic reactions.

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P-448 Chemical composition and antioxidant compounds of *Rehmannia glutinosa* during steaming process

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Rehmannia glutinosa(RG) can be used in food or medicine as 3 types; fresh rehmannia root, dried rehmannia root and steamed rehmannia root. This study was performed to compare chemical compositions and antioxidant compounds depending steaming process.

For the study, raw roots of RG were dried, soaked in traditional Korean wine and steamed for 9 times. As a result, the catalpol content of raw root was 26.32mg/g but dramatically decreased after 1st steaming. It is not detected after 4th steaming. The content of 5-HMF was not detected at raw roots but started increased from 2nd steaming. The value exceed 0.1% from 5th steaming. The contents of two antioxidant compounds including total flavonoids, total polyphenols increased gradually during steaming. Each value was from 0.13mg/g to 0.31mg/g and 0.34mg/g to 0.81mg/g. From the results, we could find that the composition of RG was significantly affected by steaming process. Therefore, suitable process would be helpful for utilizing RG substances in industry.

P-449 Comparison of growth of Ginseng by eco-friendly soil disinfecting method in rain-sheltered shade house

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Ginseng (*Panax ginseng* C.A. Meyer) is a perennial crop, and it can be seen much damage by pests during the growing season. The Ginseng Rain-sheltered Shade House is one of the efficient cultivation techniques as reducing the damages caused by natural disaster. To grow ginseng once in ginseng house and grow again, soil must be changed or disinfected.

This study was conducted to shorten the period of re-cultivation in the ginseng rain-sheltered house. Each section was treated with cultivation of sudan grass+flooding, rice straw+flooding and flooding was covered with vinyl, all areas were solarized and steam sterilization. Ginseng growth characteristics such as plant length, stem diameter, root weight and root diameter were investigated at 4-years-old ginseng. Aerial part growth was generally good in disinfection treatment compared to conventional treatments. The evaluations of root weight by measurements were heavy in the following order: steam sterilization, sudan grass+flooding, flooding, rice straw+flooding and

conventional treatments. The incidence of root rot was less in the order of steam sterilization, sudan grass+flooding, flooding.

The purpose of this study was to reduce the period of ginseng re-cultivation in a ginseng house. Growth of 4-years-old ginseng showed the best in steam sterilization method and the lowest incidence of root rot disease. The most optimal disinfection method to shorten the re-cultivation period is to look at the growth until the 6-years-old ginseng.

▶**Tab. 1** Growth characteristics of 4-years-old ginseng according to soil disinfecting method

Treatments	Root diameter (mm)	Root length (cm)	Retest-Reliabilität (Zeit-Intervall)
sudan grass +flooding	16.5±2.27	24.2±3.00	20.5±6.93
rice straw +flooding	13.2±1.64	22.6±2.73	13.0±3.71
flooding	16.7±1.86	21.2±3.31	19.4±4.65
steam sterilization	18.6±2.18	22.0±3.02	22.3±4.57
conventional cultivation	14.0±1.50	20.7±2.63	10.8±1.99

P-450 Correlation analysis between free sugar and organic acid contents during growing period of sprout Ginseng

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Background: Sprout ginseng is used not only for herbs but also for fresh vegetables and salads. Sprout ginseng is characterized by its short growth period of about 30~40 days. Even if sprout ginseng is used as a fresh vegetable, research on active ingredients should support the development of sprout ginseng. In this study, the contents of free sugar and organic acid were investigated for the growth period of sprout ginseng.

Methods and Results: On the cultivation of the plastic house, the box was filled with the substrates, and the seedling ginseng was planted. Free sugars and organic acid were measured for 20, 30, 40, 50 and 60 days of growth of sprout ginseng. The leaves and roots of the sprout ginseng were dried and extracted, and the free sugars and organic acids were analyzed by HPLC. Total sugar content of sprout ginseng was 15.82~16.58% on dry weight. The contents of sucrose and glucose were high. Total organic acids content of ginseng were 7.06~9.94% on dry weight. The contents of galacturonic acid and succinic acid were high.

Conclusion: The total free sugar and organic acid of spout ginseng showed the tendency to decrease with the growing period. Sucrose, which was high in free sugars, showed a negative correlation with growth period and glucose showed a positive correlation. Galacturonic acid, which was high in organic acid, showed a negative correlation with growth period and succinic acid showed a positive correlation.

P-451 Cultivation environment under rain shelter and growth characteristics of 5-year old ginseng

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This study is designed to develop the high temperature reduction technology in ginseng's facility cultivation responding to climate change. Accordingly, the research was focused on the effect of the covering materials of ginseng's facility house and the high-temperature reduction treatment on the growth of 5-year old ginseng. For the blocking of light, the scattering film, blue-white double-sided film, and PE film were installed while for the reduction of high temperature, aluminum screen 40%, isoprene and non-treatment were used. The aluminum screen 40% installed inside the facility had the lowest high temperature compared to other methods during the period of hot temperature. The photosynthesis speed in the scattering film + aluminum screen 40% showed the highest. As for the high temperature's damage to the ginseng, it was the lowest in case of aluminum screen 40%, followed by the isoprene and non-treatment. As for the growth of the underground part of ginseng, the weight of root of ginseng cultivated under the scattering film + aluminum screen 40% and isoprene was the highest. In conclusion, it was found that when the ginseng is cultivated in the facility house during the period of high temperature, the scattering film + aluminum screen 40% showed reduced damages to the ginseng but enhances the quantity of ginseng.

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P-452 Effects of silicate or germanium foliar spray on Korean Ginseng growth in plastic house

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Three species of genus *Panax*, *P. ginseng* C. A. Meyer, *P. quinquefolius* and *P. notoginseng* have been recognized and traded in markets around the world. *P. ginseng* (korean ginseng) is distributed in Asia's Far East including Korea and China. The main medicinal ingredient of ginseng is ginseng saponin (ginsenoside). Korean ginseng has 23-34 kinds, North American ginseng (*P. quinquefolius*) 13 kinds and *P. notoginseng* has 15 kinds of ginsinoside. Ginseng is effective in improving mental disorders, learning, and sensory functions. This study was conducted to investigate the effects on ginseng growth and ginsenoside content at the plastic house facilities using foliar spray of water-soluble silicate or germanium in 5-year-old Korean ginseng. The bacterial, fungus and actinomytes density of the soil foliar sprayed with diluted soluble silicate was 75 times higher than that without spraying(control). The photosynthesis rate of ginseng foliar sprayed with soluble silicate or germanium solution diluted by 1,000 times were 3.2 μmol $CO_2/m^2/s$ and 3.1 μmol $CO_2/m^2/s$, respectively. The photosynthesis rate of non-spray was 2.2 μmol $CO_2/$

m^2/s. Stomatal conductivity and transpiration rate were also higher in foliar spray treatment than in control. The growth status of aerial parts (plant height, etc.) and underground parts (taproot length, etc.) in ginseng were excellent in sprayed with silicate or germanium solution diluted 1,000 times. Fresh root weight was 65.0 to 68.8g per plant, up 34.7% to 43.9% from control. Ginsenoside content was increased 1.6-1.8 times when a 500 times dilution solution of soluble silicate or germanium was foliar sprayed.

P-453 Evaluation of different inoculation methods for screening of Sclerotinia rot and phytophthora blight in Korean medicinal Perilla germplasm

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Perilla_(*Perilla frutescens* L.) has been used as an important traditional herbal medicine and functional food for a long time in Asia. Sclerotinia rot (caused by *Sclerotinia sclerotiorum*) and Phytophthora blight (caused by *Phytophthora nicotianae*) which were first seen in 2006, have been serious diseases causing damage in perilla cultivation in Korea. The use of resistant cultivars is considered the most cost-effective and technically feasible control method. The exploration of genetic resources with disease-resistance is very important to breed resistant cultivars. This study was conducted to establish a simple and rapid disease inoculation method for screening of Sclerotinia rot & Phytophthora blight in Perilla germplasm.

The effectiveness of three different inoculation methods, detached leaf (agar plug placed on start point of vein, 1/3 point of leaf vein & front side, back side), stem tip and soil drenching with three-growth stages (two, four & six-leaf stage) at two different temperatures (20 & 25 °C) were assayed using two accessions of perilla germplasm. Based on disease lesion, 20 °C in two-leaf stage with detached leaf method (start point of vein) was found to be an efficient method for screening of Sclerotinia rot, whereas 25 °C in two-leaf stage with stem tip and detached leaf method were effective method for screening of Phytophthora blight in perilla germplasm.

For screening of Sclerotinia rot and Phytophthora blight, the detached leaf method of agar plug placed on start point of vein and back side, respectively, at two- leaf stage is a preferable inoculation method in perilla germplasm.

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P-454 Gamification in teaching plant systematics

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Teaching plant systematics can be tough because taxonomic terms seem unspeakable to the students and the sheer number of plant examples with their specific morphologic characteristics quickly overloads them. Their motivation to learn usually lingers at low levels.

Why not make the whole thing a little more interesting?

Gamification is the use of typical elements of games in a context that is usually not related to games. For instance high scores, rankings, progress bars, virtual awards, competition, small surprises and a joyful atmosphere [1]. This tool can increase the motivation during the learning process [2].

To give the gamification aspect a sense of a treasure hunt, first plant specimens have to be located via (previously determined) GPS-coordinates. Using a free app running on Android and iOS devices we start a quiz game then. Suitably the app is called "Quizizz". With Quizizz we ask small groups of students several multiple-choice questions about the nature of inflorescence, leaf position, types of fruits and herbal ingredients. All questions refer to a certain plant family or plant specimen.

When the treasure hunt is over, a winner team will be awarded. During the whole game process the teacher has a full overview of the answers given to the questions. To optimize the learning process, after the game some poorly answered questions can be reviewed with the students and additional explanations and facts can be given. We hope to catch the attention of the students and motivate them to learn more independently.

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P-455 Historical pharmacognostic collection and library at the University of Zagreb

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The study of pharmacy was introduced at the University of Zagreb (Croatia) in 1882 following the Decision of the Emperor Francis Joseph I. Since then, pharmacognosy has been an important part of the pharmacy education at the University of Zagreb. Today, pharmacognosy is taught and researched at the Department of Pharmacognosy of the Faculty of Pharmacy and Biochemistry, which was established in 1896 as the first independent institute of pharmacognosy in the world [1]. At this unique Department formerly called Institute of Pharmacognosy, its founder Professor Julije Domac and his successors preserved an exceptional collection of medicinal drugs as well as a collection of very old and rare pharmaceutical and medical books and manuscripts dating from 17th to 19th centuries. The Pharmacognostic collection was renovated in 2016 on the occasion of the 120th anniversary of the Institute. Today, the collection counts more than 1200 specimens of medicinal drugs of plant and animal origin that found their use in traditional medicine and contributed to the development of modern-day pharmaceuticals. Most of the specimens are very rare and old and come from all corners of the world, such as the aloe in monkey skin from Africa or curare from South America. Beside its scientific and educational significance for new generations of pharmacists, the Pharmacognostic collection and library have an important historical and cultural value.

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P-456 Abstract see SL YRW-12

Abstract see on page 1399

P-457 Impact of herbal substances on efflux pumps in bacterial and human cells

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The rapid global expansion of multidrug (MDR) and extensively drug-resistant (XDR) bacteria reflects the urgent need of a novel course in antibiotic therapy to tackle infectious diseases such as tuberculosis [1]. Considering the rising numbers of multidrug resistance especially in *mycobacteria* including the *Mycobacterium tuberculosis* complex, they are one of the critical global health concerns [2]. Efflux pumps (EP) present one of the key strategies of bacteria to protect themselves against antimicrobials [3]. We investigated specific isolated flavonoids from *Scutellaria* species for their antimicrobial activity against the non-pathogenic surrogate models for *Mycobacterium tuberculosis,* i. e. *Mycobacterium smegmatis* mc^2 155, *Mycobacterium aurum* ATCC 23366 and *Mycobacterium bovis* BCG ATCC 35734. The MICs of the plant compounds against the studied strains were determined using microbroth dilution and SPOTi- assays [5].

Prior to efflux assays, all compounds tested at sub inhibitory concentrations, were evaluated for their synergistic effects with ethidium bromide (EtBr) and rifampicin against *M. smegmatis* strain. *M. smegmatis* and *M. aurum* were further assessed for accumulation of EtBr in the presence and absence of the plant compounds as putative efflux pump inhibitors (EPIs) including the reference inhibitors verapamil (VP) and chlorpromazine (CPZ) by detecting efflux activity through a fluorometric method. Based on the results obtained from our experiments, skullcapflavone II exerts potent antimycobacterial activity against *M. aurum* (MIC = 7.8 mg / L) and *M. bovis* BCG (MIC = 31.25 mg / L) and considerably increases the susceptibility of *M. smegmatis* to ethidium bromide (MF = 128) and rifampicin (MF = 4).

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P-458 *In vitro* permeation, skin-layers distribution and environmental emission of bioactive Tea Tree essential oil components from topic formulations

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Melaleuca alternifolia essential oil (Tea Tree Oil, TTO) is well-known for its several biological activities [1,2] and it is largely used as ingredient in skin care products.

The aim of this study is the quantitative evaluation on the permeation, skin-layers distribution (*stratum corneum*, epidermis and dermis) and release in the surrounding environment of TTO markers (i. e. -pinene, β-pinene, α-terpinene, 1,8-cineole, γ-terpinene, 4-terpineol, α-terpineol) when applying a 5 % TTO cream at a finite dosing regimen. Permeation kinetics were studied *in vitro* on pig ear skin using conventional and *ad hoc* (for evaluation of released compound in the surrounding environment) static glass Franz diffusion cells. Formulation, receptor phases and skin-layers were analyzed by adopting a fully-automatized and solvent-free analytical method using Headspace Solid Phase Microextraction (HS-SPME) combined with Gas Chromatography-Mass Spectrometry (GC–MS).

Skin-layers overall contained less than 1 % of each TTO marker. Only oxygenated terpenes (i. e. 4-terpineol, α-terpineol and 1,8 cineole) significantly permeated through the skin layers, while hydrocarbons were found at trace level in the receptor phase. As expected, due to the volatility of TTO markers, a substantial fraction of each TTO component applied on the skin was released in the surrounding environment.

To the best of the authors' knowledge, in this work for the first time 1) a completely solvent-free approach is used to quantify the TTO markers from skin layers, and 2) an *ad hoc* modified static Franz cells is used to quantify the release of TTO components in the surrounding environment.

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P-459 Isolation of Lyngbyatoxin and other Teleocidin species from, *Streptomyces Blastmyceticum"* culture

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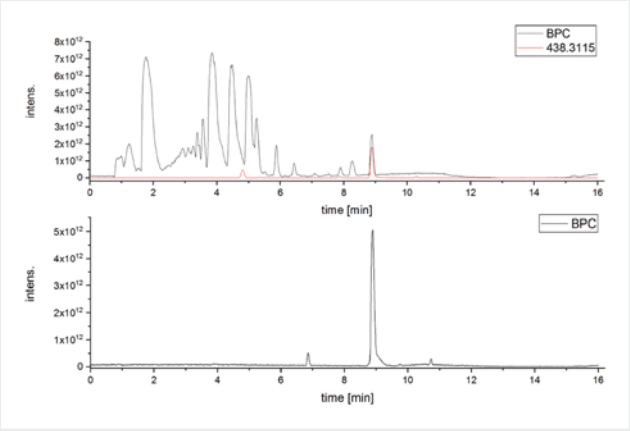
Teleocidines originally found in japanese soil bacteria e. g. "*Streptomyces mediocidicus*" or "*Streptomyces blastmyceticum*" have aroused interest due to their ability of promoting tumor growth[1]. The structural feature which combines all these Teleocidin type substances is a 9-membered lactam ring linked to an indole nucleus[2]. It was reported that also Lyngbyatoxin, a secondary metabolite of the Hawaiian blue-green algae "*Lyngbya Majuscula*", shows this structural feature[3]. Lyngbyatoxin is well-known for causing seaweed dermatitis to swimmers in Hawaii or Okinawa. Due to the structural similarity Lyngbyatoxin apparently was also proved to be a Tumor-promoter[1,4]. We hereby report that "*Streptomyces Blastmyceticum"* can be induced to produce a slightly different spectrum of metabolites, by an altered cultivation scheme. Bacteria growth was performed in a very peptone rich medium for 4-5 days. LC / MS analysis of the culture solution showed presence of Lyngbyatoxin and other Teleocidin species. After centrifugation of the biomass, the supernatant solution was extracted with Amberlite XAD 1180N for 1 day. The crude extract was further purified by open normal phase and HPLC reversed phase chromatography. The LC / MS diagram of the purified compound can be seen in the figure below. The exact mass of Lyngbyatoxin was detected with less than 1 ppm error. Other Teleocidin compounds such as Olivoretin and Teleocidin B have been isolated in a similar manner. For further experiments other cultivation conditions will be tested on different strains of "*Streptomyces*" and the occurrence of Teleocidin compounds will be investigated.



▶**Fig. 1** Upper picture: Chromatogram of crude Amberlite extract, lower picture: Enrichment of purified compound Lyngbyatoxin

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P-460 MediHealth – an interdisciplinary and international research and innovation staff exchange project to identify novel natural products for healthy ageing

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The percentage of elderly people in Europe is rapidly increasing [1]. As a result, also the prevalence of age-related diseases will significantly increase. The major aim of the MediHealth project is to introduce a novel approach for the discovery of novel bioactive natural products of food plants from the Mediterranean diet and other global sources to promote healthy ageing. To achieve this aim, a series of plants from the Mediterranean diet and food plants from Africa, Asia, and South America has been carefully selected and subjected to *in silico, in vitro* (advanced cell-based assays), *in vivo* (flies and mice models) and metabolism analysis. Advanced analytical techniques complement the pharmacological evaluation process for the efficient isolation and identification of bioactive plant constituents. Furthermore, pharmacological profiling of bioactive natural products as well as identification and synthesis of their metabolites are carried out. Finally, process optimization studies are performed to proceed to the development of innovative nutraceuticals, dietary supplements or herbal medicinal products [2].
The project is based on a well-balanced exchange of researchers between 5 universities and 4 companies from European countries as well as 4 universities from non-European countries. It is developed on the needs and interests of both sectors exploiting the existing complementary expertise. Moreover, knowledge is transferred by training of seconded researchers in environments with different research orientation where complimentary skills are required. MediHealth aspires to comprise a successful model promoting researchers' competences and long-lasting collaborations between Industry and Academia generating innovation potential at international levels.

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P-461 New advances for an improved content of Sweroside and Swertiamarin in cultivated *Gentiana lutea* L. var. *aurantiaca* roots

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Gentian lutea L. var. *aurantiaca* is an endemic plant growing in the northwestern mountains of the Iberian Peninsula. Its roots are mainly used in the elaboration of liquors and medicinal products due to the high concentration in bitter compounds. Novel medicinal properties described for the sweroside [1] and swertiamarine [2] and the increasing demand, encourage for a stable source of high quality roots.

The evolution patterns on the content of sweroside and swertiamarine in the roots of cultivated *G. lutea* var. *aurantiaca* have been studied. Methanolic extracts of complete root systems collected in spring and autumn during three consecutive years were analyzed by HPLC, evaluating the effect of the age and development (weight) of the roots in the content of the cited compounds.

The evolution of the content for both compounds was widely different. Analyzing the data by a linear regression model, the obtained R-squared (R^2) for the swertiamarin was 0.9549 and 0.6223 for the sweroside. These results show how the evolution in the content of swertiamarin is not dependent on the age and weight of the roots. On the other hand, sweroside content is random, but not dependent on the age or the weight.

These results suggest that the architecture of the root systems play an important role in the content of sweroside, since a higher concentration of this compound is in the cortex tissues [3]. Further research must be conducted in order to improve the quality of gentian roots.

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P-462 Photosynthesis according to chlorophyll contents of ginseng

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Ginseng is cultivated mostly in Korea and China. As a medicinal plant, it has traditionally been used for treating such diseases as hypertension, diabetes mellitus, liver and kidney dysfunction, mental disorders, and skin inflammation since long ago. Ginseng can be harvested three years after its cultivation. As a half-shade plant, ginseng is cultivated in a sun-screening facility. If the sun-shading material of ginseng has a high light transmission rate, chlorophyll contents reduce and ginseng withers away; if a low light transmission rate, chlorophyll contents increase but a growth amount decreases. Therefore, this study analyzed photosynthesis of 6-year ginseng according to chlorophyll contents. At 20℃, when the value of chlorophyll contents was 35, the photosynthesis was the highest, or 3.98 μmol / m^2s. At 25℃, when the value of chlorophyll contents was 30, the photosynthesis was high, or 4.38 μmol / m^2s. At 30℃, when the value of chlorophyll contents was 25, the photosynthesis was the highest, or 2.89 μmol / m^2s. In terms of quantity, a typical sun-shading method had 762 kg / 10area; when the shading method was adjusted in order to make chlorophyll contents suitable to photosynthesis, it had 1,174 kg / 10area. In terms of the useful element ' total ginsenoside ', a typical sun-shading method had 5.7 mg / g; when the shading method was adjusted in order to make chlorophyll contents suitable to photosynthesis, it had 27.51 mg / g. In conclusion, to increase a quantity of ginseng and total ginsenoside, it is necessary to adjust a sun-shading facility on the basis of chlorophyll contents.

P-464 Practical advice to ensure legal certainty when accessing your genetic resource samples

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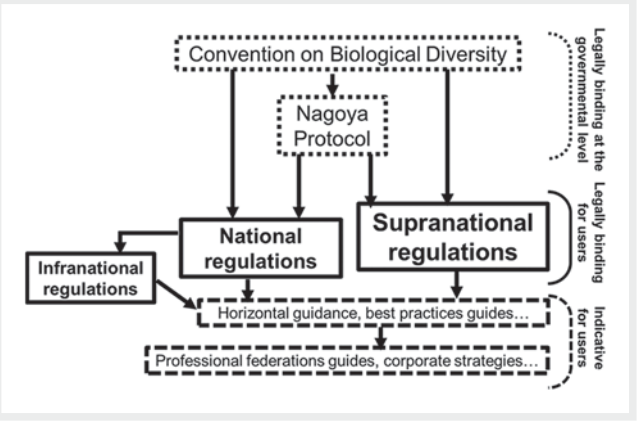
For every natural product researcher in either academic or industrial sectors, legal access to biodiversity has become nowadays a strategic and very sensitive issue. The need to share the fair and equitable benefits arising from the utilization of genetic / biological resources established by the Rio Convention (1992) was reaffirmed by the Nagoya Protocol (2010) [1] which entered into force internationally on 12th October 2014. In this framework national access laws and the European regulation EU N°511 / 20142 have been implemented and enforced[2]. They must be clearly understood by researchers and fully integrated into working practices.

The practical processes for legally accessing our biodiversity samples from source countries[3] are crucial points. The temporal and geographical scopes and the nature of utilization (R&D, commercial use ...) must be clearly defined as they determine the legal framework. Prior Informed Consent (PIC), Mutually Agreed Term (MAT) contracts, administrative formalities and obligations must be undertaken. The "ABCD" main issues of the Nagoya Protocol implementation are Access, Benefit sharing, Compliance and Due Diligence Declarations.

Clear understanding and respect of the regulations should be achieved in the interest of all stakeholders: source countries, local populations, academic researchers, industries, patients and customers of biodiversity derived products.



▶ **Fig. 1**

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P-466 Schiff base ligands derived from phenylenediamine and its metal complexes as enhancer against two mechanisms of antibiotic resistance

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The increasing mortality rate caused by serious infection due to widespread emergence of multidrug resistance microorganisms that are highly resistance to current available antibiotic can make the treatment of infectious diseases become difficult and ineffective. Therefore, the development of new antibacterial agents with potent and broad antibacterial activity with minimum toxicity is urgently needed. Schiff base are formed from the condensation reaction between primary amine reacts with aldehyde or ketone-like compounds under specific conditions and exhibit wide range of biological activities. Antimicrobial activity of 41 Schiff base compounds was analyzed against three strains of Gram-positive bacteria, *Staphylococcus aureus* (ATCC 25923), *Staphylococcus haemolyticus* (ATCC 29970), and *Staphylococcus cohnii subsp.urealyticum* (clinical strain); and four strains of Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), *Klebsiella pneumonia* (ATCC 700603), and *Shigella flexneri* (ATCC 12022) using resazurin microtiter based assay [1]. Schiff base compound Ovan(me)MPD and its tetranuclear metal complexes showed the highest percentage inhibition at 51.3 %. The synergistic effects of Schiff base on the susceptibility of *K. pneumoniae* and *P. aeruginosa* to antimicrobial agents with or without PAβN (Phe-Arg-β-naphtylamide) were measured. The MICs of chloramphenicol, nalidixic acid, gentamicin, tetracycline and streptomycin in the presence of PAβN, were significantly reduced, decreasing to 30–16 μg/mL. The effects of PAβN on the susceptibility of bacteria to Schiff base indicate that some that some Schiff base compounds are substrates for efflux pumps in Gram-negative bacteria and the presence of more metal centers in structure of Schiff base could increase the susceptibility of antimicrobial agent activity.

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P-467 Selection of seed disinfectant of *Angelica acutiloba* Kitagawa for reducing seed fungi

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This study was conducted to select seed disinfectants to improve the germination rate and the cultivation environment because the fungi on the seed surface can disturb germination and should be controlled for introduction into the plant factory for mass production. The seed material was harvested in the field of Department of Herbal Crop Research, Rural Development Administration, Korea in 2017. 2 pieces of filter paper were placed in 9 cm Petri dish with 5 mL of sterilized water, and the seeds were put on the filter papers and irradiated at 375 nm for 2 days in a 25℃ incubator. The fungi formed on the seeds were isolated and grown on PDA medium, and the most vigor area of mycelial growth was cut and stored at -70℃. 10 kinds of seed disinfectants such as tebuconazole, which is registered for domestic cultivation disinfectant, and NaOCl 1 %, 4 % (v/v) solution were used. The control value (%) was calculated as efficacy effect whilst germination rate (%) was calculated as harm effect. As a result, the treatments recognized as excellent in efficacy was tebuconazole, prochloraz copper chloride complex tebuconazole, prochlorza, hymexazol+metalaxyl-M and NaOCl 4 % (v/v) treatment. And thiophanate-methyl+triflumizole, prochloraz, hymexazol+metalaxyl-M and ipconazole was recognized that the harm effect is low so that germination rate is statistically higher or equal to that of the non-treated. Thus, prochloraz and hymexazol+metalaxyl-M, which have passed both the efficacy effect and harm effect, were selected as seed disinfectant of *Angelica acutiloba* Kitagawa and further *in-vivo* experiment will be conducted.

P-468 Abstract see SL YRW-11

Abstract see on page 1399

P-469 Simultaneous determination of malondialdehyde, glutathione, and glutathione disulfide with UPLC-Q-TOF/PDA in human lung cancer cells (A549)

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To evaluate the antioxidative effects of medicinal plants *in vitro*, intracellular glutathione (GSH), oxidized glutathione (GSSG), and malondialdehyde (MDA) levels after treatment with an oxidizing agent and a medicinal plant extract are determined. Quantification is generally carried out with bio-assay kits and high-performance liquid chromatography (HPLC). As the compounds are measured individually, the procedure becomes time-consuming and expensive, which pose a disadvantage. Here, our aim was to establish a method to simultaneously determine GSH, GSSG, and MDA levels in cultured cells with ultra-performance liquid chromatography quadrupole time-of-flight/photodiode diode array (UPLC-Q-TOF/PDA).

All cellular analytes were purified using self-manufactured solid-phase extraction (SPE) cartridges, and SPE reproducibility was noted to be less than 7.26 %. Instrumental analysis methods verified by ICH guidelines demonstrated excellent linearity and sensitive detection limits. tert-Butyl hydroperoxide treatment was applied to induce oxidative stress because an increase in MDA and GSSG levels and decrease in GSH levels were assumed to be due to oxidative stress. GSH and GSSG exhibited significant changes with respect to the oxidative stress levels; however, MDA levels remained unchanged.

We simultaneously analyzed *in cellular* MDA, GSH, and GSSG levels in A549 cells. This study is the first attempt of this fast, time-saving, and economical method. In future, to evaluate medicinal plant antioxidant effect(s), it would be used *in vitro*.

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P-470 Studies on the physicochemical properties on 3 types of *Rehmannia glutinosa*

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Rehmannia glutinosa (RG) is a widely cultivated medicinal crop in Asia. Its rhizomes are widely used for food or traditional medicine in raw, dried or steamed state depending on their purpose. This study was performed to compare physicochemical properties of 3 types of RG. For this study, the korean domestic cultivar 'Dagang' was used. To prepare the 3 types of RG, drying and steaming process were conducted. For dried root, raw root was dried at 60℃ in hot air dryer for 48hours. For steamed root, dried root was steamed and dried repeatedly for 9 times. As a result, the color value of lightness was highest in raw root and lowest in steamed root. Each value was 86.83 and 38.49. The value of

redness and yellowness was lowest in steamed root. Dried root has the highest value in hardness but lowest in adhesiveness. The content of catalpol was highest in dried root and the next was in raw root. Each value was 29.47 mg / g and 27.57 mg / g. It didn't detect in steamed root. The aucubin content was highest in steamed root. The value was 0.95 mg / g. A value of 0.55 mg / g of aucubin was in dried root and 0.46 mg / g in raw root. The results show that physicochemical properties of RG was significantly different depending on the types of RG. It may work differently in drug and using proper types of RG would be important factor. Therefore, further study on pharmacological activities related these properties is needed.

P-471 The stability of medicinal plant microRNAs in herb preparation process

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Herbal medicine is now globally accepted as a valid alternative system of pharmaceutical therapies. Various studies around the world have been initiated to develop scientific evidence-based herbal therapies. Recently, microRNAs (miRNAs) have been indicated as new bioactive ingredients in medicinal plants [1]. However, the stability of miRNAs during herbal preparation process remains unclear. This study evaluated the stability of mistletoe miRNAs in different mistletoe preparations. Fresh mistletoe leaves and prepacked dried leaves were powdered, extracted at room temperature, 80 °C or 100 °C with or without mechanical treatment, to mimic the clinical medicinal use as well as folk medicinal use of mistletoe. The total RNA was isolated from these extracts and the miRNAs including miR166a-3 p, miR159a, miR831-5 p, val-miR218 and val-miR11 were quantified by stem-loop qRT-PCR. As a result, the miRNAs showed the highest level in fresh plants. A significant decrease of miRNAs was observed in the extracts made from fresh plants, and mechanical treatment increased miRNA damage. Unexpectedly, a relatively high number of tested miRNAs were found in prepackaged dried mistletoe plants, indicating that these miRNAs could survive air-drying and storage process. Only miR166a-3 p and miR159a with relatively low levels were determined in the mimicked mistletoe tea preparation (soak dried mistletoe in hot water for 10 min). The Ct values of tested miRNAs in mistletoe decoction (boiled in water for 30 min) were below the limit of detection. These data suggested that the miRNA could survive some preparation process, and indicated our consumption that medicinal plants preparations might introduce miRNAs into mammals [2].

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P-472 The Tramadol origin: the end of a story or an endless controversy?

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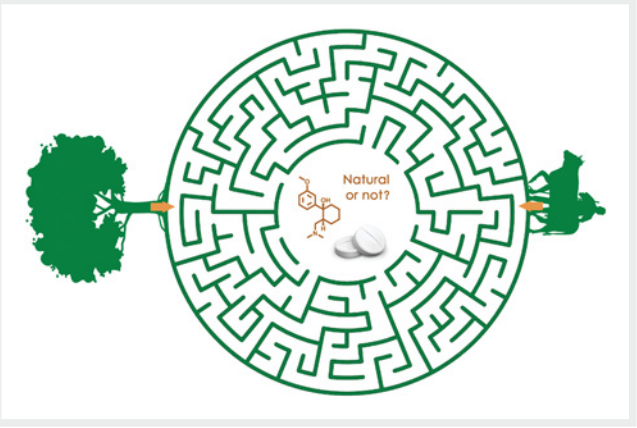
In 2013, Tramadol, a fully synthetic drug was extracted from the roots of an African shrub named *Nauclea latifolia* [1]. As it could be expected, this major discovery has stimulated both considerable excitement and controversy about its natural origin. In 2014, the presence of tramadol in the roots of the plant was suggested to be an anthropogenic contamination due to ethnoveterinary practices [2]. Clearly, further investigations are required in order to decipher the Tramadol origin. Hence, we have investigated 13 *Nauclea latifolia* roots collected at different areas of the national Bénoué park in Cameroon (designated by the UNESCO as a Biosphere reserve). HRMS analyses showed the occurrence of tramadol in all methanolic extracts of *Nauclea latifolia*. The new tramadol-targeted isolation procedure was carried out by semi-preparative HPLC with dry-load injection of the sample and the structure was confirmed by 1 D and 2 D NMR spectroscopic methods. Highly pure tramadol was isolated and is being analyzed by the ^{14}C accelerator mass spectrometry in order to determine the radiocarbon content in the tramadol isolated from *Nauclea latifolia versus* synthetic Tramadol. Indeed, natural products, recently biosynthesized should contain radiocarbon and synthetic compounds (petroleum derived molecules) should not contain ^{14}C as the latter is decayed.



▶ **Fig. 1**

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P-474 Variability of total phenolic content and main phenolic compounds in the germplasm collections of *Perilla frutescens* leaves

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Perilla plant is an important source of bioactive compounds with potential pharmacological properties. In this study, we evaluated the total phenolic content (TPC) and profiles of major phenolic contents of perilla leaves in 762 germplasm collections. TPC was estimated using Folin–Ciocalteu method [1]. Rosmarinic acid (RA), Caffeic acid (CA), pigenin-7-O-diglucoronide (ADG), scutellarein-7-O-glucoronide (SG), and apigenin-7-O-glucoronide (AG) were analyzed by using Ultra Performance Liquid Chromatography (UPLC) equipped with PDA detector. The TPC and individual phenolic compounds showed a wide variation across the

entire germplasm collections. TPC was ranged from 9.87 to 133.69 mg GAE / g dried extract (DE). RA, CA, ADG, SG, and AG were ranged from 0.27 to 21.05 mg / g, 0.08 to 1.16 mg / g, 0.00 to 2.18 mg LUE / g, 0.20 to 5.25 mg LUE / g, and 0.00 to 2.81 mg LUE / g of DE, respectively. Based on the surface and back color of the leaves, samples were divided into seven groups (A, B, C, D, E, F and G). Group C showed higher AG than other groups. While samples in group E were characterized by higher values of TPC, RA, and SG compared to other groups. Group G contained samples with higher value of CA and ADG relative to the other groups (p>0.05). Accessions IT226732, IT226741, IT274300 and IT274302 could be utilized in research and food processing industries for developing new functional materials with high content of phenolic compounds.

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P-475 *In vitro* antiviral effect of Cortex Mori Radicis water extracts against influenza viruses

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Cortex Mori Radicis (CMR) is root peel of Morus alba L, which is widely distributed throughout Asia including South Korea, China, and Japan. CMR has been used to treat diseases such as cough, headache, edema and diabetes. The antiviral effect of CMR on influenza virus had not been reported until now.

In this study, we demonstrate the anti-influenza viral activity of CMR water extract (WCMR) in murine Raw 264.7 macrophage cells. Pretreatment of WCMR reduced viral replication in a dose-dependent manner, as evaluated using a green fluorescent protein (GFP)-tagged influenza virus in the cells. Consistently, we found that the expressions of viral proteins were decreased in the presence of WCMR, compared with no treatment, through immunoblot analysis and immunofluorescence. To investigate the underlying mechanism on antiviral efficacy of WCMR, we checked whether WCMR could affect the type I IFN production and proinflammatory cytokine activation. WCMR remarkably activated STAT-1 and TBK at both 4 h and 24 h post-treatment, dose-dependently. Furthermore, the pretreatment of WCMR increased the phosphorylation levels of ERK and JNK in the cells. These result implies WCMR induced the production of type I IFN including pro-inflammatory cytokines and subsequent stimulation of antiviral state in the cells.

In conclusion, the pretreatment of WCMR suppresses viral replication and viral gene expression via type I IFN activation and proinflammatory cytokine such as STAT-1, TBK, ERK, and JNK, followed by induction of antiviral state, in murine Raw 264.7 macrophage cells.

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P-476 Acute oral dose toxicity studies of fermented *Platycodon grandiflorus* powder in Sprague-Dawley rats and Beagle dogs

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Platycodon grandiflorus, called balloon flower, is a species of herbaceous flowering perennial plant of the family *Campanulaceae* and inhabits East Asia (Korea, China, Japan and the Russian Far East). The traditional use of balloon flower root is to promote healthy lung function, to remove phlegm from the lungs, and to treat diarrhea and other inflammatory diseases in Korea [1]. We intend to develop a health functional food of fermented *Platycodon grandiflorus* powder, which helps improve the condition of the skin caused by immune overreaction. The fermentation-derived bioactive substance is expected to reduce itching by suppressing the secretion of inflammation mediators in mast cells [2] and to induce Th1 immunity by suppressing the Th2 immune overreaction [3]. However it's not known whether the excessive intake of the fermented powder causes toxic effects. The present study was carried out to evaluate the acute toxicity of the fermented *Platycodon grandiflorus* powder following single oral administration to Sprague-Dawley rats and beagle dogs. Rats and dogs were administered single dose at dose levels of 0, 750, 1500 and 3000 mg / kg by oral gavage or capsule. In the rat and dog studies, there were no abnormal changes in mortality, clinical sign, body weight, food consumption, hematology, clinical chemistry and macroscopic finding following administration. Based on these results, the maximum tolerated dose (MTD) was considered over 3000 mg / kg under this study condition. Also, we suggest a dose level of 3000 mg / kg be recommended as a high dose in repeat toxicity studies for the acceptable daily intake (ADI).

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P-477 Analysis of the volatile and non-volatile fraction of cocoa liquors and chocolates produced with cocoa beans from West-Africa and Ecuador

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Cocoa liquor is the product obtained from the beans of *Theobroma cacao* L. and together with cocoa butter, sugar and soy lecithin, it is the major ingredient of chocolate. There are four main varieties of *T. cacao*, including the Forastero variety, considered as "bulk", and the Nacional variety, considered a "fine-flavor" cocoa.

In this research, both cocoa liquor and chocolate, produced from a blend of West African ("bulk") cocoa beans and cocoa beans originating from Ecuador (Nacional variety, "fine-flavor"), were thoroughly investigated with regard to their composition. The nutritional composition was determined to obtain

information about the levels of the primary metabolites, while secondary metabolites were investigated by means of GC-MS (gas chromatography – mass spectrometry) and UPLC-HRMS (ultra performance liquid chromatography – high resolution MS), for volatile and non-volatile compounds, respectively.

A wide range of compounds were analyzed: almost 70 volatile compounds were identified and semi-quantitatively determined by GC-MS, while more than 40 compounds were (tentatively) identified by UPLC-HRMS, of which 15 were quantified. Interestingly, various volatiles, which contribute significantly to the final flavor, were more abundant in the West African cocoa liquor and chocolate, while with regard to the non-volatile secondary metabolites, the Ecuadorian samples were richer, when it comes to most of the quantified substances. Additionally, principal component analysis (PCA) of the datasets confirmed that the four samples can be clearly distinguished based on their chemical profile. Certain specific constituents, including alcohols, pyrazines, amino acids and biogenic amines were found to be highly influential in causing this clear differentiation between the samples.

P-478 Anti-allergenic effects of peanut sprouts extract in a systemic anaphylaxis food allergy mouse model

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An allergy to peanuts is a major cause of fatal food-induced anaphylaxis, with food allergies becoming an increasingly important health research issue. This study was undertaken to verify the effect of peanut sprout extract (PNS_E) on the inhibition of allergic and anaphylactic responses using a peanut (PN)-immunized food allergy mouse model. Fresh peanut sprout, which was germinated for 0, 3, 5, and 7 days with mature peanut. Mice were sensitized to cholera toxin plus PN or PNS_E by intragastric administration on days 0, 7, and then only PN or PNS_E challenged on day 21, 35. Five weeks later, we compared the mucosal mast cell degranulation, ear swelling and systemic anaphylaxis that were stimulated with PN extract, compared to PNS_E. Subsequently, Ara h1, a biomarker of PN allergy, levels in serum and Th1 / Th2 cytokine production in cultured supernatants of splenocytes were measured. PNS_E (germinated for 3, 5, 7 days) treatment significantly attenuated the secretion of the Ara h1 antibody, mucosal mast cell degranulation, the degree of systemic anaphylaxis and ear swelling, and a higher level of production of IFN-□ and IL-10 cytokines, and reduced secretion of IL-4 that are related to the Th2 immune response in the PN-immunized food allergy mouse model. The results of this study show that the allergenicity of PN extracts could be reduced by germinated for 3, 5, and 7 days which caused downregulation of Th2 lymphocyte activity, systemic anaphylaxic response, mast cell-mediated ear swelling in the PN-sensitized mice.

P-479 Characterization of a standardized extract of fresh *Curcuma Longa* rhizomes

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Part of our research and industrial expertise is focused on the production of plant extracts with a phytochemical profile similar to that of the plant *totum*, which is defined as all the active compounds present in the part of the plant used for extraction, and on which the fundamental principles of additivity and synergy are based. The objective of this study was to determine the phytochemical composition of a hydroethanolic extract of fresh *Curcuma Longa* rhizomes, made according to the patented process Phytostandard® (standardized extract marketed under the name EPS Curcuma, PiLeJe Laboratoire, France), per comparison with the native plant.

The phytochemical composition of the extract was determined in HPTLC, HPLC, LC / MS, MS / MS and NMR experiments. The phytochemical profile of the extract was similar to that of the native plant. We identified 16 compounds including phenolic compounds, curcuminoids, amino acids, polysaccharides and terpenoids. NMR experiments after purification of the extract allowed the identification of α-tumerone, β-tumerone and arturmerone, three compounds known to be present in the essential oil. By combining several analytical methods we identified precisely different compounds present in the extract and showed that the patented extraction process used allows to obtain an extract of fresh *Curcuma Longa* rhizomes in which the majority of metabolites found in the native plant are preserved and therefore with a composition close to the *totum*.

P-480 Genistein therapy in an animal model of estrogen deficiency

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Genistein is a partial β2-estrogen receptor agonist, having a similar endogenous affinity to 17β-estradiol [1]. Due to the hormonal decline in postmenopausal women, the incidence of bone mass loss increases, together with mammary gland atrophy, mouth and vaginal mucosa dryness [2].

To establish the effects of genistein in ovariectomized female rats. Forty female rats were divided into 4 groups (10 rats / group): group I: treated with 10 mg / kg/day genistein (intraperitoneally), for 8 weeks; group II: treated with 17β-estradiol for 8 weeks with 10 μg / kg/day; the group III: untreated rats and group IV: non-ovariectomized and untreated rats. Bone samples were taken from the femoral and lumbar head, mammary gland, vaginal and buccal mucosa and were histologically analyzed. The bone density evaluation was made by radiological method. All the experiments and research protocols were approved by the Research Ethics Comitee.

Results: In group III the imaging results show a slight bone demineralization at the level of the femoral head compared to the other groups. Bone histological analysis confirms bone loss in group III and in group I the bone density is lower than in groups II and IV. Regarding the mammary gland and vaginal mucosa, substitution treatment with estradiol produces hypertrophy, whereas genistein prevents oral mucosal atrophy at this level, but does not reduce vaginal and mammary gland atrophy.

The effects of genistein on bone density are beneficial and the reduced effects on the mammary gland and vaginal mucosa make it useful in long-term treatment in postmenopausal women.

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P-481 Iminosugars from *Baphia pubescens* Hook (Fabaceae) as a potential source of antidiabetic phytopharmaceuticals

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Baphia pubescens Hook (Fabaceae) is a shrub or small tree found in the tropical regions of Africa. Locally, the leaves and seedpods of *Baphia* species are used as a vegetable or spice and also for treating various ailments including inflammation, pain and diarrhoea [1], but ethnobotanical literature does not list these plants amongst those traditionally used for the treatment and management of diabetes.

Previous phytochemical investigation has shown that plants belonging to this genus of legumes are rich in iminosugars. These compounds closely resemble sugars structurally and mimic them along metabolic pathways. Iminosugars often show digestive enzyme inhibition and thus have a therapeutic potential in managing diabetes (by helping to stabilise blood glucose levels) [2].

This research focuses on the isolation and purification of iminosugars from *Baphia pubescens* by ion exchange chromatography and aims to establish whether HPCCC (high performance counter current chromatography) could offer an alternative method for isolation.

An ethanolic extract (50 % vol:vol) of whole seed pods, prepared and fractionated using ion exchange resins, yielded several iminosugar compounds and two related glycosides. A method of isolation by HPCCC is currently being developed.

As iminosugars previously isolated from *Baphia nitida* have demonstrated significant glycosidase inhibition (e. g. 1-deoxynojirimycin (DNJ)) [2], *Baphia pubescens* may serve well as a potential source of antidiabetic phytopharmaceuticals.

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P-483 *In vitro* antioxidant activity of Modern Botany™ products and selected natural product ingredients

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The project aim was to investigate antioxidant activity of selected Modern Botany™ products and their ingredients. Modern Botany™ oil from different manufacturing sources was tested for antioxidant activity and results showed, compared to the control Tocopherol (Vit E) a known antioxidant that the Irish manufactured Modern Botany Oil exhibited 80 % DPPH radical scavenging activity, compared to UK manufactured product which had a DPPH of 73 %. Selected ingredients such as *Borago officinalis* (Borage), *Calendula officinalis* (Marigold), and *Linum usitassimum* (Flaxseed) (table 1)[1] [2], also exhibited significant antioxidant activity and would warrant further analysis. These tests of Modern Botany™ products suggest that further testing is warranted for antimicrobial and antioxidant activity.

▶**Tab. 1** % DPPH scavenging and Trolox equivalence (mM) for Modern Botany Oil & Selected Ingredients

	Average ABS (Con)	Average Abs (Sam)	Std-Dev (Sample)	% DPPH (0.05-mM)	Trolox equivalent (mM)
Tocopherol (control)	0.544	0.086	0.020	84	1.61
MB(IE) Oil (new)	0.544	0.111	0.003	80	1.45
MB(IE) Oil (old)	0.544	0.118	0.004	78	1.40
MB UK Oil	0.544	0.147	0.025	73	1.22
Borage (IE)	0.544	0.186	0.016	66	0.96
Calendula	0.544	0.196	0.030	64	0.90
Flax (UK)	0.544	0.25	0.086	54	0.54
Flax(IE)	0.544	0.297	0.090	45	0.24
Borage (UK)	0.544	0.395	0.033	27	-0.40
Rosewood	0.544	0.453	0.065	17	-0.79
Chamomile	0.544	0.486	0.038	11	-1.0

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P-484 In vitro α-glucosidase and α-amylase enzyme inhibitory effects and free radical scavenging activity of *Garcinia schomburgkiana* extracts

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The genus Garcinia (Clusiaceae) is distributed in tropical and subtropical region of America, Africa and South East Asia. *Garcinia schomburgkiana* Pierre has been traditionally used for medicinal purposes as anti-inflammatory and anti- diabetic agent [1]. Thus, the aim of the present study was to evaluate the alpha α-amylase and alpha α-glucosidase inhibitory activities and *in vitro* antioxidant activities of the extracts. The plant extracts were examined using DPPH scavenging method, ABTS radical scavenging assay,

and lipiperoxidation assay for antioxidant activity. The 80 % ethanolic extracts of *G. schomburgkiana* bark, leaves and fruits were prepared by sonication technique. The study revealed that the different parts of the plant extracts possessed the potent activities. The strongest activity of the extract was bark extract that exhibited significant α-amylase and α-glucosidase inhibitory activities with IC_{50} value of 20.04±1.33 µg / mL, 2.81±0.43 µg / mL, respectively and well compared with standard acarbose drug. For *in vitro* antioxidant activities, the bark extract exhibited IC_{50} value of 28.96 ±1.62 µg / mL, 9.79±0.14 µg / mL and 574.89±14.68 µg / mL for DPPH scavenging method, ABTS radical scavenging assay, and lipiperoxidation assay, respectively. Thus, the bark of *G. schomburgkiana* should be recommended as the high quality material for pharmaceutical and nutraceutical development in treatment of hyperglycemia, diabetes and the related condition of oxidative stress. The active compounds should be elucidated for standardization in the future study.

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P-485 Investigation of ellagic acid sourcing as a dietary supplement by UFLC-PDA-ESI-MS/MS

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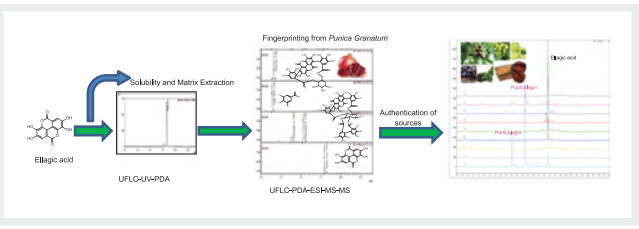
Ellagic acid (EA)is a very common plant phenolic with occurrence in bark, stem, fruit or wood part in many plants. It occurs in nature either in a free or complex form like ellagitannins which further gets hydrolyzed in the body into EA and metabolized in to urolithins and its glucuronide. In this way, EA entered dietary supplement (DS) industry worldwide from last decade with diverse claims like antioxidant, immunity booster and digestive health.

The regulations for the DS globally have common requirement "authentication of botanical source as per label claim". EA has been introduced in the DS industry from a potential source i. e. *Punica granatum* (PG). PG is a tropical fruit with biochemical markers as punicalagin A & B, punicalin A-B, gallic acid and EA. PG mainly contains gallotannins-ellagitannins class compounds which on hydrolysis convert to EA.

The current different methods have inability to confirm the sourcing of raw material from which EA have manufactured and varied solubility of EA in different solvents while analysis. Therefore, this research update solving this problem with optimization and validation of a simple, stable matrix-based sample preparation method created on UFLC-UV-PDA.

This method adapted for investigating the sourcing of botanical DS of ellagic acid from 10 potential sources other than PG with the help of ESI-MS/MS. Also, a fingerprinting of PG has been separately established for reconfirmation of its sourcing.

This study provides a valuable strategy for rapidly screening and identifying chemical components of PG and source identification of EA as DS.



▶ **Fig. 1**

P-486 New Zealand spinach (*Tetragonia tetragonoides*) as a functional food alternative for anti-obesity and anti-hyperuricemia

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Obesity is a serious public health problem, associated with the development of metabolic disorders. New Zealand spinach (NZS) growing around the Korean peninsula has been used, but no report is available on its antiobesity effects and mechanisms. Here, we examined the anti-obesity and anti-hyperuricemic effects of NZS and underlying mechanisms in high-fat diet (HFD) obese mice. NZS extract was prepared with 70 % ethanol under reflux in a condensation system at 85◦C for 3 h. Mice were fed the normal fat diet (NFD); high-fat diet (HFD); HFD with 75, 150, or 300 mg / kg NZS extract; or 245 mg / kg Garcinia cambogia (GC) as a reference. Compared to HFD, NZS extracts decreased body weight gain, total white adipose tissue (WAT), and liver weight by the max. 58 %, 64 %, 75 %, respectively, and improved hepatic and plasma lipid profiles, which appeared to be related to the reduced adipogenesis genes including aP2 / FABP4. With NZS, the plasma levels of the leptin and uric acid were significantly decreased while the adiponectin levels were increased. Furthermore, NZS decreased the expression levels of adipogenesis-related genes and xanthine oxidoreductase (XOR), which is involved in uric acid production, while increasing that of proteins associated with fatty acid oxidation. The dose treatment of 300 mg / kg used here would be comparable with about 1500 mg / day in a 60-kg human, which implies that the effect of NZS may be expected for antiobesity. Results indicated that NZS exerts anti-obesity, anti-hyperlipidemia, and anti-hyperuricemic effects by regulation of lipid metabolism-related genes and proteins, and decreased expression of XOR.

P-487 Perinatal taurine supplementation in maternal diabetes prevents insulin-glucose dysregulation in adult rat offspring

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This study evaluated the effect of perinatal taurine supplementation on insulin secretion in adult offspring of maternal diabetic rats. Female Wistar rats were fed normal rat chow and tap water with (Diabetes group) or without diabetes induction by intraperitonial streptozotocin injection (50 mg / kg of body weight) (Control group) before pregnancy. Then, they were supplemented with 3 % taurine in water (Control+T and Diabetes+T groups) or water alone from conception to weaning. After weaning, male offspring were fed normal rat chow and water throughout the study. At 16 weeks of age, fasting blood sugar (FBS), oral glucose tolerance test (OGTT), plasma insulin level, insulin resistance, and blood lipid profiles were measured. Body weight, total cholesterol, high-density lipoprotein, low-density lipoprotein and insulin resistance (estimated by HOMA-IR) were not significantly different among the four groups. Further, compared to Control, the Diabetes group displayed significant increases in fasting blood sugar, OGTT and plasma triglyceride, but a decrease in plasma insulin, pancreatic weight and pancreatic to

body weight ratio. Perinatal taurine supplementation in drinking water significantly improved, at least in part, these adverse effects of maternal diabetes mellitus in the adult offspring (the Diabetes+T compared to other groups). These data suggest that maternal diabetes mellitus affects pancreatic growth and insulin-glucose homeostasis in adult male offspring, and these adverse effects can be prevented, in least in part, by perinatal taurine supplementation

P-488 Quantitative control of dietary supplements containing caffeine

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European legislation is more permissive regarding authorizing a dietary supplement than a pharmaceutical. There are many caffeine-containing dietary supplements that are used as energizing or lipolytic (weight loss) supplements [1,2]. The term "dietary supplement" often misleads the consumer, assuming that the active principles are of vegetal origin.The purpose of the study was to compare the caffeine concentration declared by the manufacturer with that actually present in the dietary supplements containing green tea or guarana extract [3]. Therefore, 5 dietary supplements were analyzed by HPLC-UV and the amount of caffeine contained in each supplement was compared with the declared one. Results: The 5 dietary supplements had a reported caffeine content of 80 to 160 mg / tablet/capsule, but the actual caffeine content determined by HPLC exceeded the one declared by the manufacturer by 30-60 %. The highest differences were found in preparations also containing guarana or green tea extract with known caffeine content. If different plant extracts containing the same active substance are combined, the manufacturer should declare the total cumulative dose of the active principle in order to avoid side effects or even some pharmacodynamic or pharmacokinetic interactions between the dietary supplement and a possible chronic medication.

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P-489 Selenium-enriched ricegrass juice prevent cadmium-induced toxicity on HEK293 kidney cells

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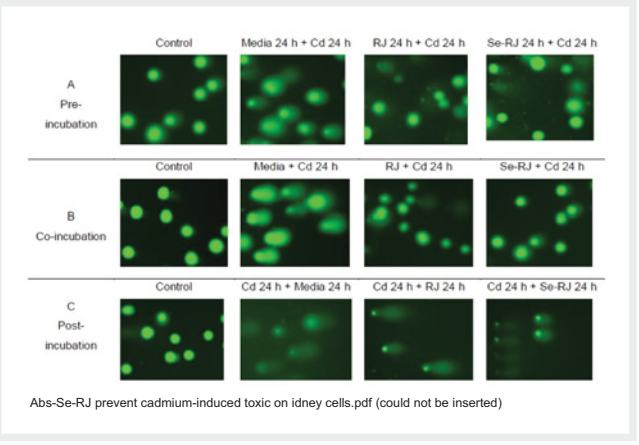
Selenium-enriched ricegrass juice (Se-RJ) has been claimed as a functional food by provide high nutritive value and various biological activities [1,2,3]. In this study, Selenium-enriched ricegrass juice (Se-RJ) and ricegrass juice (RJ) extracts were investigated for cell viability and their anti-cadmium (Cd) toxicity on kidney cells (HEK293). Pre-incubation, co-incubation and post incubation of Se-RJ and RJ on HEK293 towards the exposure to CdCl2 were analysed for DNA protective property by COMET assay. Results confirmed that Se-RJ and RJ had no toxicity to kidney cells. Se-RJ and RJ pre-incubation and co-incubation exhibited DNA protective effect against cadmiuminduced toxicity on HEK293 kidney cells by decreasing % DNA damage in tail and tail length of COMET over the CdCl2 treated cells (Fig. 1). Moreover, Se-RJ showed additional benefits over the RJ for DNA protective properties. In conclusion, this study provides a clear evidence that SeRJ can a candidate functional food for renal protection.



▶ **Fig. 1** Abs-Se-RJ prevent cadmium-induced toxic on idney cells. pdf (could not be inserted)

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P-490 Simultaneous HPLC quantitative analysis of major components of *Maclura cochinchinensis* stem extract and antityrosinase activity

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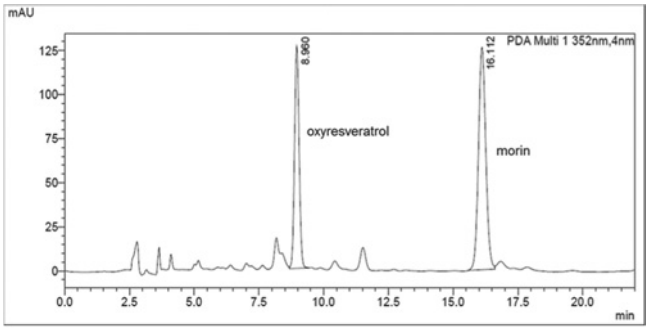
Maclura cochinchinensis (Lour.) Corner. have been used as medicinal plant in tropical and subtropical countries for a long time. The stem of this plant has traditionally been used to treat, skin infection, fever and jaundice. Based on recent study, the compounds in this plant were found to be oxyresveratrol and morin [1,2]. In present study, HPLC was performed using a Luna® 5 µm C18-column eluted with isocretic acetonitrile: 1.5 % formic acid (25:75) with a flow rate of 1 mL / min and detected at 352 nm. The inhibitory effect of the extracts on tyrosinase activity was determined spectrophotometrically with the degree of inhibition of L-DOPA. The HPLC method was illustrated to be precise with RSD < 1.5 %. The average recovery of oxyresveratrol and morin were 100.01 and 99.31 %, respectively. IC_{50} of *M. cochinchinensis* extract, oxyresveratrol, morin, and kojic acid (positive control) were found to be 7.91, 6.54, >500, and 92.25 µg / mL, respectively against tyrosinase. Five samples

from various locations in Thailand of *M. cochinchinensis* stem extracts were analyzed and the average contents of oxyresveratrol and morin were 44.83 and 129.33 mg / g extract, respectively. This work would be useful as a guide for the standardization of *M. cochinchinensis* raw materials and their commercial products for antityrosinase activity.



▶ **Fig. 1** HPLC chromatogram of methanolic extract of *M. cochinchinensis* stem

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P-491 The expression of selected intestinal glucose, fructose and long-chain fatty acid transporters investigated in Caco-2 cells

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Many diseases and metabolic disorders such as Type-2 Diabetes Mellitus show their origin or progress in specific lifestyles, e. g. unhealthy nutrition, physical inactiveness or tobacco use [1]. With regard to unhealthy and excessive food intake, it is an approach to identify new, plant-based substances, which inhibit different transporters in the gut to facilitate patient's therapies. Especially polyphenols are discussed to act as inhibitors on intestinal transporters [2].

At the beginning of our studies, we investigated the expression of glucose (SGLT1, GLUT2), fructose (GLUT5) and long-chain fatty acid (FATP2, FATP4) transporters in the Caco-2 model.

Caco-2 cells have been studied and used as an intestinal barrier model for different investigations [3]. This cell line shows spontaneous differentiation after confluence and expresses a brush border with enzymes and transporters for nutritional uptake [4,5]. We compared the expression of mentioned transporters in Caco-2 cells at different time points within 3 weeks untreated and treated with differentiation reagent Sodium Butyrate. After western blot analysis, the samples were detected by chemiluminescence and we were able to detect the expression of SGLT1, FATP2 and FATP4. Cells treated with Sodium Butyrate showed an approximate expression of each of the transporters earlier after confluence compared to untreated cells at Day 15 or 21 after confluence. Sodium Butyrate is suitable to shorten the differentiation time and still represents the approximate expression of these transporters in the differentiated stage. Therefore, it is a qualified model for further transport studies.

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P-492 The interest of eco-friendly water subcritical grape pomace extract as skin regeneration active ingredient

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LVMH research is committed to reduce our environmental impact. As such, we focus the development of our actives with eco-friendly extraction technologies and co-product uses [1]. This study shows the interest of grape pomace water-subcritical extraction to obtain active ingredient [2]. Different temperatures of extraction were tested; 125 °C which allows interesting extraction yield and polyphenols content with acceptable organoleptic properties was selected for biological screening and phytochemical characterization.

The total polyphenols content of the extract, obtained by Folin Ciocalteu method, is 50 mg_{GAE}/g. The presence of caftaric acid, epicatechin, catechin, pro-anthocyanidins, epicatechin and catechin derivatives was demonstrated by LC-DAD-HRMS analysis.

The extract increases collagen from dermo-epidermal junction synthesis (+23 % collagen VII and +87 % collagen IV) by human keratinocytes. Moreover, this effects on reconstructed skin model are studied. Significative increase of fibronectin, involucrin, integrin α6 is measured, 54 %, 33 % and 41 % respectively. After treatment with extract, the epidermal is also 29 % thicker.

This study shows the interest of eco-friendly water subcritical grape pomace extract as skin regeneration active ingredient.

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P-493 Abstract see SL AR-06

Abstract see on page 1390

Authors' Index