GA – 69th Annual Meeting 2021, Bonn, Germany

Virtual Conference, 5–8 Sept. 2021

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69th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA)

September 5–8, 2021
Global Virtual Meeting
Coordinated from
Bonn, Germany

Congress president:
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Bibliography
Planta Med 2021; 87: 1234–1330
DOI 10.1055/a-1484-9805
ISSN 0032-0943

Georg Thieme Verlag KG, Rüdigerstraße14,
70469 Stuttgart, Germany

The 69th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA) was intended to take place in Bonn, Germany, from September 5–8, 2021. However, due to the pandemic situation across the world the Local Organizing Committee agreed with the Board of the Society to held a competely virtual meeting. Thus, we could offer the opportunity for scientific exchange and a platform for extensive communication amongst GA-members and interested scientists from all over the world – in total more than 400 participants from more than 50 different countries and six continents.

The main scientific topics of the conference were:

- One Health, access and benefit sharing
- Antiinfectives and epidemiology
- Analytics, recent methodology and applications
- Translational natural product pharmacology
- Endophytes and microbes
- Regulation of herbal and traditional medicines
- Medicinal plants and natural products research on traditional medicines
- Animal healthcare and veterinary phytotherapy
- Recent advances in medinal plants and natural product research.

The Meeting started on Sunday, September 5th 2021 with two pre-conference events: the Young Researchers' Workshop and the Workshop on Animal Healthcare and Veterinary Phytotherapy. The scientific program of the Main Conference included 14 keynote lectures, 34 contributed short lectures, award ceremonies and presentation of about 200 posters. The virtual setting provided the unique opportunity to display all posters throughout the conference, a new format with 11 video contribuions in a mediathek was an exciting additional option. Regulatory Affairs topics and a Workshop of the African Research Network were integrated in the timeframe of the Main Conference. The virtual platform gave opportunities to exhibitors and sponsors to present companys, portfolios and visions.

We would like to thank everyone who supported us in setting up an interesting high level prodgram and in realizing this successful virtual meeting, while together exploring new ways of communication and stepping forward into new methodologies of interaction. In particular we want to express our deepest thanks to the GA Executive Board and the GA Board, the members of the Local Organizing Committee and the Scientific Committee, session chairs and of course - all participants for active contributions, discussions or listening. Last, but not least, we want to thank Thieme Publishers for publishing the conference abstracts in Planta Medica.

On behalf of the Local Organizing Committee and GA

Prof. Dr. Werner Knöss

2. Keynote Lectures

KN1 Access and benefit sharing under the Nagoya Protocol – Quo Vadis?

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DOI 10.1055/s-0041-1736737

Access and Benefit Sharing (ABS) have come center stage in the context of research and development on biodiversity and its multiple uses. This is based on a range of international binding agreements and also supported widely in the non-governmental sector. Clearly one of the reasons for this push has been the exploitation of natural resources based on poor practice and thus from lessons learned over centuries. ABS was implicitly included in the Convention on Biodiversity (Rio Convention, 1992), formally adopted in 2002 at the World Summit on Sustainable Development (Johannesburg), and more systematically regulated in the Nagoya Protocol of 2010. Many activities have since been conducted worldwide with the aim of implementing it in individual countries and globally.

Panama was one of the first countries in embracing the ABS with the aim of seeking a sustainable use of its rich biodiversity. Guatemala with a much less well-developed legal framework and having put a temporary halt to the adoption of the Nagoya Protocol, showcases the ongoing need for developing such a framework based on ample participation and representation processes involving the indigenous groups of the country (Heinrich et al 2020, Berger – Gonzalez et al. 2021). In this presentation, we will ask whether the ABS guidelines helped local / indigenous communities? Or have they made it more difficult to take advantage of the benefits of biodiversity? It highlights the long road to a sustainable use of biodiversity based on international, transdisciplinary collaboration. The talk will also call for a strong push to enable the implementation of 'One Health' supporting the fast-changing needs of local communities in increasingly more complex global networks [1, 2].

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KN2 Challenges and opportunities in sourcing for plant secondary metabolite research in the 21st century

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DOI 10.1055/s-0041-1736738

Challenges and Opportunities in Sourcing for Plant Secondary Metabolite Research in the 21st Century Plants have been the source of useful substances since times immemorial. There are an estimated 450.000 flowering plant species on this planet, thousands of which have been used for one purpose or the other in the past and we are currently seeing dramatic improvements in analytical technique: The possibilities of identifying and even predicting the bioactivities of secondary compounds are more sophisticated than ever before. Authentication of plant material has always been a major challenge in secondary metabolite studies, but molecular techniques now theoretically permit the authentication of any plant material as precisely as desired– as long as it contains DNA and requisite reference data are available. Building up these reference data-bases is thus a major current challenge. While there is thus dramatical progress on all technical fronts, legal access to plant material faces new challenges: The Nagoya Protocol seriously limits legal access to plant material for "molecular and biochemical studies". On the other hand, new applications and technical possibilities render major innovations possible based on well-known useful plants. Additionally, major ex-situ collections potentially provide an invaluable source for secondary compound research - an estimated 50.000 plant species are held in the collections of German Botanical Gardens.

KN3 Isolation of CNS active natural products – challenges and opportunities

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A great number of natural products generally found in botanical drugs and in food may affect the central nervous system (CNS) in mammals because they accumulate in the brain to trigger neuropharmacological effects. Since the evaluation of complex mixtures in pre-clinical assays is hampered by an unsolved complexity and a general lack of translation of in vitro data to an in vivo situation, we attempt to isolate CNS active compounds and study their behavior in dose-effect paradigms in different in vivo animal models. Employing versatile techniques like counter current separation (CCC, CPC) coupled to mass spectrometry we isolate bulk amounts of natural products for biological testing. Based on models with zebrafish and mice such as epilepsy-like, learning and memory and anxiety-like we aim to understand the potential neuropharmacological effects of isolated natural products based on pharmacokinetic and pharmacodynamics readouts. As will be shown with examples on ongoing research with selected coumarins, a major challenge, besides the required amounts and purities, lies in the establishment of workflows that allow for rapid and meaningful evaluation neuropharmacologically active natural products.

Funding This work was supported by National Science Center, Poland, grant number 2017/27/B/NZ4/00917.

KN4 After Ten Thousand Years of Domestication, Can Livestock Still Self-Medicate?

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DOI 10.1055/s-0041-1736740

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.

KN5 Comprehensive Analysis and Elaboration of Quality Standard for Traditional Chinese Medicines

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It is of a tremendous challenge for analysis and quality elaboration for traditional Chinese medicine due to its extremely complexity. TCM is an extremely complex system, which contains hundreds or even thousands of chemical components in a single herb, not to mention the compound formulas composed of a handful of even tens of herbs. This generates gigantic hurdle to clarify its chemical composition, which is the fundamental basis for the research of quality control, mechanism, pharmacological and toxicological actions, PK/PD, clinical trial, new drug discovery, etc. Thanks to the new analytical technique advancement, comprehensive analysis of traditional Chinese medicines and other herbal medicines becomes pragmatic and viable. In the past several decades, our research team has devoted to the herbal analysis by using combination of newly developed techniques on the basis of UPLC, 2DLC, HRLCMS, UPC2, etc. to facilitate the comprehensive analysis of chemical components in herbal complex systems. Several typical herbs were exemplified by using this approach. For examples, a series of closely related herbs of Panax species named *Panax ginseng* and its processed products dubbed Red Ginseng, *Panax quinquefolium*, *Panax notoginseng* and their different plant parts were analyzed by LCQ Advantage ion-trap mass spectrometer for the holistic profiling of their ginsenosides and differentiation of their pattern differences. Similarly, other TCM herbs such as *Uncaria rhynchophylla*, *Glycyrrhiza uralensis*, *Alisma orientalis*, Toad Venom, and a few TCM formulas as Dan-qi Tongmai Tablet, and Niuhuang Shangqing Pill were analyzed by multiple neutral loss/precursor ion scanning combined with substructure recognition and statistical analysis. Based on the chemical analysis, metabolic and biological analyses were also approached to further decipher the bioactives in the herbs or herbal combinations, which was named as Trinity Analysis explained as “Chemical-Metabolic-Biological” analysis. Based on aforementioned comprehensive analysis, a holistic quality control model for herbal quality was developed and practiced in herbal drugs and their products therefrom such as dispensing granules, classic formulas and finished combination products. This type of research cemented for elaboration of quality standards. Following this approach, quality standards with tens of TCM herbs and combination products were elaborated and adopted by such main-stream pharamcopoeias as United States Pharmacopoeia, European Pharmacopoeia and Chinese Pharmacopoeia.

KN6 Opportunities and challenges of the One Health approach

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The goal of the “One Health” (OH) concept is to use an interdisciplinary approach for solving health problems. It is based on the evidence that the health and wellbeing of people, animals, plants and the environment are connected and ultimately linked to the state of the entire planet. Synonymous terms such as “eco-health” are used to describe this approach. A range of disciplines are directly relevant to the development of OH solutions. Included are natural sciences such as medicine, microbiology and climate sciences as well as social sciences like economics, behavior sciences and psychology. To productively collaborate across disciplines is both the strength as well as the key challenge of the operationalization of OH. OH is strategically applied in a range of specific problem areas. A prominent example is the global fight against antibiotic resistance (AMR) where a OH approach is promoted across all relevant international organizations [1].

Accordingly, many governments have translated these international recommendations into their country strategies to fight AMR.

Switzerland, for example, has developed the StAR strategy [2], where specific interventions are envisaged for all sectors, including human and veterinary medicine, agriculture and the environment. Strategic measures include the general promotion of animal and public health, preventive measures and the research into alternatives to antimicrobial use, including the use of plant-based therapies.

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KN7 Natural Products at the Interface of Cellular Metabolismus and Stress Response

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DOI 10.1055/s-0041-1736743

Cellular metabolism not only provides energy in form of ATP and building blocks for anabolic reactions, but also turned out to be a major and crucial determinant of cellular behavior and phenotype under stress. For instance, pro-glycolytic Warburg-like bioenergetics has been described as pivotal for pro-inflammatory, mitogenic or promigratory responses in a variety of challenged cell-types, whereas favored fatty acid oxidation could be linked to immunosuppression. Metabolism can drive altered gene expression or protein activity by providing reactive species serving as signaling molecules or supplying metabolites that function as (co)substrates for enzymes and posttranslational (epigenetic) modifications. [1–4] Natural products elicit a variety of bioactivities with the detailed mode of action and immediate target far from being completely understood and dissected. In this context, the role of altered cellular bioenergetics and metabolism had not been exhaustively considered so far.

This lecture will give a synopsis of our findings on how selected natural products can affect cellular motility or inflammatory responses in vitro by shifting or reprogramming the cellular energy metabolism, and how the actual cellular energy state can indeed finetune specific gene transcription programs. It intends to highlight metabolic changes as potential explanation for the observed and often pleiotropic actions of a given natural product and may open up avenues for the discovery of new targets, mode of actions and rationalized “bioenergetic” treatment regimens for natural products.

Funding

The author and her team gratefully acknowledge the funding provided by the Austrian Science Fund (FWF; P23317, P29392, P32600; P33778) that enabled the realization of the presented projects.

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KN9 Medicinal plants and medicinal products and their regulation in Brazil

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DOI 10.1055/s-0041-1736745

Brazil is a megadiverse country. At this moment, 49 992 species are recognized for the Brazilian flora (native, cultivated, and naturalized), being 4993 of Algae, 35 552 of Angiosperms, 1610 of Bryophytes, 6320 of Fungi, 114 of Gymnosperms, and 1403 of Ferns and Lycophyte (FLORA DO BRASIL, 2021). Brazil appears among the countries that most published research on medicinal plants

globally, but it is estimated that less than 15 % of the species have been studied for medicinal use (ZAGO, 2018). Herbal Medicinal Products (HMP) are widely used in Brazil, including the popular and traditional use, or available as compounding or industrialized medicines. But, despite the megadiversity and the acceptance of the population, the number of HMP licensed in Brazil is small, and the number of HMP obtained from native species is even smaller [1, 2].

In order to improve the entire production chain of HMP in Brazil, the Brazilian government published in 2006 the National Policy on Medicinal Plants and Herbal Medicines. According to a process of international convergence, several actions were established, including the constant updating of the herbal medicines legislation.

It is expected that all these factors contribute to the increase in the number of products and information about HMP in Brazil.

Conflict of Interest; Funding (Source, ID)

Statement on behalf of the authors. The author is the only responsible for the material included in this paper.

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KN10 Evaluation of the potential of botanicals and their constituents against the SARS-CoV-2 virus

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DOI 10.1055/s-0041-1736746

The critical challenge that natural products research projects seek to address is identifying biologically active constituents in complex mixtures. The gold standard approach towards this goal is bioassay-guided fractionation, whereby mixtures are successively purified and tested for their ability to achieve a desired biological activity. The success of this approach is reflected by the discovery of many essential drugs, including the antibiotics streptomycin and tetracycline, and the anti-cancer drug Taxol. However, the bioassay-guided fractionation approach is limited by i) its inherent bias towards abundant and easily isolable compounds, and ii) the quality of the biological data used to guide isolation. The Center for High-Throughput Functional Annotation of Natural Products (HiFAN) seeks to address these limitations by developing new tools for the comprehensive evaluation of natural product mixtures. These tools enable the collection of multi-dimensional biological datasets, and the application of untargeted spectrometry metabolomics approaches to comprehensively profile the chemical composition of natural product mixtures. We will highlight the application of these approaches to identify natural product extracts and constituents with potential efficacy against SARS-CoV-2. A panel of botanical extracts and pure natural compounds were screened for blockade of authentic SARS-CoV-2 infection in cell culture. Promising activity was demonstrated by extracts and constituents from the botanical *Stephania tetrandra*, and the chemical and biological datasets were integrated using a multivariate statistical approach to determine which active constituents were most strongly associated with biological activity. We are currently employing HiFAN's gene expression and cytological profiling platforms to derive insight into potential anti-viral and anti-inflammatory mechanisms of action for the bioactive constituents.

Funding Supported by NCCIH grant numbers 3U41AT008718-07S1 and 5U41AT008718-08.

KN11 *Commiphora myrrha:* Novel aspects on medicinal use, pharmacology and sustainable cultivation of an ancient medicinal plant

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DOI 10.1055/s-0041-1736747

Myrrh tree (mainly *Commiphora molmol* Engl., Burseraceae), originating from Eastern Africa and the Arabian Peninsula belongs to the oldest known plants used for medical and cultural applications. Known for its anti-inflammatory and antimicrobial properties, its air-dried resinous gum was applied for medical purposes since ancient times.

Current pharmacological and phytochemical research provides not only scientific reasoning of its historical medicinal use, but also for its application in the treatment of more recent civilization diseases such as irritable bowel syndrome and inflammatory bowel diseases. Clinical studies of the powdered resin as part of a finished herbal combination product confirm these findings.

Ecologically sustainable cultivation and harvesting of pharmaceutical-grade myrrh is one of the key challenges to ensure a sustainable and stable supply chain of the herbal medicinal product in the long term while at the same time preserving biodiversity in the growing region in Ethiopia and the Horn of Africa. Thus, a sustainability project on GACP-compliant cultivation and harvesting of myrrh was implemented by the pharmaceutical manufacturer in Ethiopia with the support of the German Federal Ministry for Economic Cooperation and Development.

Conflict of Interest

C. Vissiennon is employed by Repha GmbH Biologische Arzneimittel

KN12 Unlocking Nature's Pharmacy – from plant to product: An academic odyssey

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DOI 10.1055/s-0041-1736748

For many academic scientists their life's work can be described as an Odyssey – A long journey full of adventures. This lecture presents such an academic journey from discovery of a small bioactive molecule to Phase 1 clinical trials, and describes how this and other research led to, securing funding, to establish a discovery programme 'Unlocking Nature's Pharmacy', and to the foundation of NatPro the TCD Centre for Natural Product research.

Core to this journey is the 'privilaged' indane scaffold, found in many plant and fungal species as well as in several synthetic therapeutic molecules. During our work on the anti-inflammatory activity of naturally occurring indanes, from ferns of Onychium and Basidiomycete species, we discovered a novel indane scaffold that was optimised for both anti-inflammatory activity and bioavailability. Our lead molecule, PH46A, was evaluated in in vivo models of inflammatory bowel disease (IBD), an area of considerable unmet clinical need. The compound significantly reduced histological damage and serum amyloid A (SAA) levels in IL-10−/− colitis mice, was efficacious in the 5 % dextran sulfate sodium (DSS) colitis model and compared favorably with prednisolone in this model and supports its potential use to treat acute exacerbations of the disease. Further, the graded response to the compound may also lend itself to be used at a lower dose to maintain periods of remission. It took approximately 20 years and €13 million to drive the project from academic hypothesis to the clinic. PH46A has progressed and completed Phase 1 human clinical trials [1, 2].

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KN13 The Future of Africa's Natural Products in Drug Discovery and Development: Trends and Challenges

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DOI 10.1055/s-0041-1736749

Africa is faced with six mega challenges which include; population change, changing demographics, urbanization, climate change, land-use change, and transformative technologies. Furthermore, the level of development varies across the African continent which is coupled with inadequate capacity (including human expertise) to handle the drug development process. Despite the existence of the policy and regulatory frameworks in drug development, they are not fine-tuned to support the drug discovery process. Therefore, these challenges pose a threat to natural products drug discovery, and development on the continent. Additionally, drug resistance for example antimicrobial and anticancer drug resistance which renders current drugs ineffective; severe adverse drug reactions (toxicity) of some drugs for example anticancer drugs, ARVs; the high cost of some drugs for example a dose of

Octreotide goes for over USD 250; long-duration therapies for example Antitubercular drugs (6–9 months); incurable diseases for example Cancer, HIV, COVID-19 and the emergency of new diseases like Ebola in Africa and COVID -19 globally drives the search for vaccines and new drugs from natural products which provide a diverse pool for biomolecules with different activities. There are many more challenges to the drug discovery and development process despite the existence of the WHO guidelines which are not domesticated in most African countries. Thus, there is a need to take charge of the different natural products value chains, develop them while bridging the gaps along these chains with the involvement of various actors. This will in turn give room to innovations in drug discovery and development.

KN14 Are gut microbiota the real target of many herbal medicinal products?

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DOI 10.1055/s-0041-1736750

Although many herbal medicinal products can rely on vast experience of clinical efficacy, the mechanisms of action are often not fully understood and the active principles could not yet be elucidated. Moreover, bioavailability of many plant constituents is highly questionable. Therefore, alternative approaches to explain activity should be considered.

Gut microbiota and the human body form a symbiosis which is known to be essential for our health and well-being. Dysbiosis can lead to serious diseases, like systemic inflammation; obesity, asthma, diabetes, and even to certain forms of cancer. Therefore, gut bacteria may be a relevant target for herbal medicinal products, and may help to understand their effects [1].

For example, *Faecalibacterium. prausnitzii* has been considered a major actor of human intestinal health [2], the mucin-degrading bacterium *Akkermansia muciniphila* has been linked with obesity and type 2 diabetes (T2D) [3], and members of the genus *Fusobacterium* have been identified as potential causative agents in colorectal carcinomas [4].

In order to study the interaction of herbal medicinal products with gut microbiota, we have established a research platform, which analyses metabolization of plant extracts by LC-HRMS and microbiome shifts by 16S RNA sequencing [5].

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3. Short Lectures

SL1 An UHPLC-HRMS-based metabolite profiling approach to detect potentially anti-inflammatory active compounds in Chinese Lonicera species

Authors Pferschy-Wenzig M E[1], Ortmann S[1], Atanasov G A[2, 3, 4], Hellauer K[1], Hartler J[1], Kunert O[1], Gold-Binder M[5], Ladurner A[2], Heiß H E[2], Latkolik S[2], Zhao M Y[6], Raab P[1], Monschein M[1], Trummer N[1], Samuel B[1], Crockett S[1], Miao H J[6], Thallinger G G[7, 8], Bochkov [1], Dirsch M V[2], Bauer R[1]

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DOI 10.1055/s-0041-1736751

In this study, the feasibility of a metabolite profiling approach to detect compounds with potential anti-inflammatory activity from Chinese Lonicera species has been assessed.

Ethanolic leaf extracts from eight Chinese Lonicera species (36 accessions) were phytochemically analyzed by UHPLC-HRMS and pharmacologically tested in four different cellular in-vitro assays related to inflammatory processes (NF-κB- and PPARγ-activation, IL8- and NO- production). Phytochemical and pharmacological datasets were correlated by orthogonal projection to latent structures discriminant analysis (OPLS-DA) and candidate compounds potentially related to pharmacological activity were deduced from the respective S-plots. Overall, 65 candidate compounds from different chemical classes were assigned on the basis of mass spectrometry data, and eight of them (one flavone, three bioflavonoids and four long-chain polyhydroxy fatty acids) were isolated from L. hypoglauca leaves. In a test set of 15 candidate compounds, the activities derived from the OPLS-DA models could be partially verified. Several of the tested compounds indeed showed anti-inflammatory activity, but some of them also negatively influenced cell viability. In summary, UHPLC-HRMS-metabolite profiling in combination with OPLS-DA was a feasible strategy for obtaining candidate compounds potentially involved in pharmacological activity, but it may need further optimization to enhance its accuracy.

Funding

The authors gratefully acknowledge the funding provided by the Austrian Science Fund (FWF):
S 10705 and S 10704 (NFN "Drugs from Nature Targeting Inflammation")

SL2 Derivatization-targeted analysis of amino compounds from Cardueae species by liquid chromatography tandem mass spectrometry

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DOI 10.1055/s-0041-1736752

Plants amino compounds (e. g., amino acids, biogenic amines) play important roles for the plant themselves and for human well-being. Reversed-phase liquid chromatography tandem mass spectrometry (RPLC-MS/MS) is one of the most used technique for amines analysis, but due to their structural characteristics, analyzing them in their native form remains challenging. Therefore, a prior derivatization step, to overcome this issue, is necessary [1, 2]. In this work the use of neutral loss scan mode (NLS) is proposed for the first time in the inves-

tigation of amino compounds derivatized with diethyl ethoxymethylenemalonate (DEEMM) and analyzed by RPLC-MS/MS, to obtain an easy and straightforward method for the detection of known and unknown amines. The procedure has been firstly optimized on a model sample containing eight amino acids, with good results in terms of DEEMM derivatives detection and repeatability. The method was then successfully applied to the analysis of five *Cardueae* plant extracts, with 18 amino acids and 3 other amines being putatively identified. A statistical analysis on the collected data revealed a stability in the derivatives profiles among both the different selected species and samples from the same species with different geographical origins. The obtained results confirm the effective use of the proposed approach in the analysis of plants amino compounds for different purposes.

Conflict of Interest; Funding (Source, ID) The authors declare that they have no conflict of interest. This work was supported by the Estonian Research Council grant PUT1589, by the EU through the European Regional Development Fund (TK141 "Advanced materials and high-technology devices for energy recuperation systems") and was carried out using the instrumentation at the Estonian Center of Analytical Chemistry (www.akki.ee).

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SL3 Selfmedicative Behavior and Tanniferous Fodder Plants: Alteration in Taste Perception and Feed Preferences of GIN-Infected Boer Goats

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DOI 10.1055/s-0041-1736753

Nematode infections are a common thread in ruminant livestock and excessive usage of conventional anthelmintics led to emergence of resistant nematode populations. This underlines the relevance of paradigm shift towards a sustainable control approach of nematode infections.

In this study, possible changes in taste perception and in feed preferences of goats were scrutinized to prove ascertained higher feed intake of tanniferous plants by goats in case of nematode-infection.

Feed preferences of 18 Boer goats were analyzed via cafeteria-trial (12 weeks) regarding influence of changes in health status from non-infected to infected. Goats were divided in different groups: I) Non-infected + feeding-trial II) Infected + feeding-trial III) Infected without feeding-trial. The cafeteria-trial was conceptualized with pellets of tanniferous plants (leaves of sainfoin, willow, walnut, blackberry) of various tannin-contents and tannin-free hay pellets. After four weeks a mixed nematode-infection was administered to group II) and III).

Besides feed intake and selection procedure, blood parameters, saliva composition and feces were analyzed on weekly basis in order to make assessments of the course of infection and potential shifts in feed preferences due to changes of taste perception.

Analysis of trial data revealed an alteration from tannin-free (hay) and low tannin-containing feed (sainfoin) to higher tannin-contents (walnut, blackberry) in the course of infection.

Statement on behalf of all authors. On behalf of all authors, the corresponding author states the following: the authors have no conflict of interest.

SL4 *In vitro* antibacterial activity of plant extracts and phytochemicals in comparison with antibiotic growth promoters against intestinal pathogens of farm animals

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DOI 10.1055/s-0041-1736754

From 2022, EU legislation prohibits using all forms of routine veterinary antibiotics, including antibiotic growth promoters (AGPs), for effective farm management of food-producing animals which necessitates searching for their new alternatives.

The purpose of this study was to compare growth-inhibitory effects of plant ethanolic extracts (n = 20) and phytochemicals (n = 4) with AGPs (n = 7) using standard antibiotics (n = 4) as positive control against 12 standard strains of gut bacteria relevant to farm infections by the broth microdilution method following the CLSI protocol.

Amongst tested plants, all three samples of *Embelia ribes* dried fruit extracts showed strongest inhibitory activity against *Bacillus cereus* and *Listeria monocytogenes* with minimum inhibitory concentrations (MICs) ranging from 32 to 128 µg/mL. Phytochemicals namely, 8-hydroxyquinoline and sanguinarine exhibited significant activity against 12 and 8 tested bacteria (MIC 32 – 128 µg/mL), respectively. In between AGPs, carbadox displayed significant activity against 11 tested microbes (MIC 4 – 64 µg/mL). Positive control ciprofloxacin showed substantial effects (MIC 0.0625 – 0.5 µg/mL) against all tested pathogens.

In case of phytochemicals, the results were in correspondence with our previous study [1].

In conclusion, *E. ribes* fruit extracts may have potent antibacterial activity of veterinary importance that need further investigation. Also, 8-hydroxyquinoline and sanguinarine appear promising leads to develop novel agents in this context, although animal trials are needed.

Conflict of Interest; Funding (Source, ID) We declare no conflict of interest. This research was funded by the Czech University of Life Sciences Prague, project IGA 20213109.

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SL5 *Symplocos fasculata* as a Source of Antimicrobial Compounds

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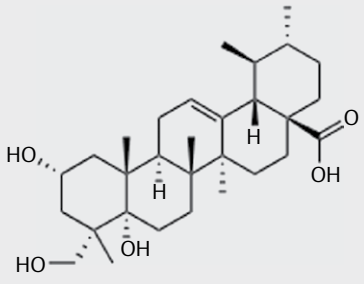
DOI 10.1055/s-0041-1736755

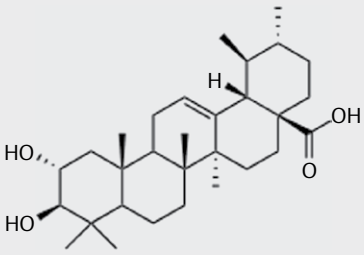
Multidrug-resistant (MDR) bacteria are linked to numerous global medical challenges. Resistance commonly arises due to the acquisition of plasmids encoding multiple antibiotic resistance genes and/or the action of efflux pumps (e. g., NorA, TetK) with wide substrate specificities [1].

The bark of *Symplocos fasculata* Zoll (kindly provided by Dr Stephen Teo, Forest Department Sarawak, Malaysia) was investigated to identify compounds with antimicrobial activities, able to potentiate efflux-mediated resistance or inhibit bacterial conjugation [2]. *Symplocos fasculata* (Symplocaceae) is distributed in tropical and subtropical Asia, America and Malaysia

Minimum inhibitory concentrations (MICs) were determined on plant extracts, fractions and isolated compounds against *Staphylococcus aureus* (ATCC 259233, SA-1199B + NorA and XU212 + TetK) and *Escherichia coli* NCTC10418. Ethyl acetate and acetone extracts had the lowest MIC values (4-8 µg/mL) against *S. aureus*. Two active compounds were isolated and identified using Nuclear magnetic resonance and Mass spectrometrytechniques (Figure 1).



Asiatic acid



Corosolic acid

▶ **Fig. 1** Compounds isolated from an ethyl acetate extract of the bark from *Symplocos fasculata*.

The two compouds = Asiateic acid and Corsolic acid – are pentacylcic triterpenoids. They showed moderate activity against all strains of *S. aureus* used, the MIC value for both compounds was 16 µg/mL.

At sub-inhibitory concentrations, both compounds potentiated the activities of tetracycline and norfloxacin yielding a 2- or 4-fold reduction in MICs against *S. aureus* XU212 + TetK and SA-1199B + NorA, respectively. Extracts and isolated compounds showed moderate activity (10-50 % reduction) against the conjugal transfer of plasmids (R388, pKM101, R6K) to recipient *E. coli* JM109.

Overall, these findings indicate the potential of these compounds as drug leads in the management of bacterial MDR infections.

Statement on behalf of all authors. The abstract is original and not has been submitted to any other peer reviewed journal

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SL6 Increasing hydrophobicity of cyanobacterial puwainaphycins leads to lipopeptide variants with much improved therapeutic index

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DOI 10.1055/s-0041-1736756

Cyclic lipopeptides from cyanobacteria represent an important class of compounds with antifungal properties. Their medical uses are frequently accompanied by cytotoxicity on host cells, so application as antifungal drugs is hampered [1]. In this study we demonstrate that natural lipopeptides known as puwainaphycins (sometimes called Minutissamides) isolated from Cylindrospermum alatosporum [2] with attached variable fatty acid (C10 + n) chain length differ in the kinetics of their cytotoxic effect on human cells and their antifungal effect. Lipopeptides with an extended FA tail showed improved strain-specific antifungal activity against facultative pathogen *Aspergillus fumigatus* (MIC = 0.5–3.8 µM) and plant pathogen *Alternaria alternate* (MIC = 0.1–0.5 µM) but their cytotoxic effect was partially retained (~10 µM). Esterified at position 1' lipopeptides possessed substantially higher antifungal potencies (MIC = 0.2–0.6 µM against *A. alternate*) and greatly reduced (>20 µM) or abolished cytotoxic activity.

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SL7 Switching from inflammation to resolution: what can we learn from medicinal plants?

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DOI 10.1055/s-0041-1736757

Current anti-inflammatory strategies suppress pro-inflammatory lipid mediator biosynthesis but do not engage their pro-resolving counterparts that relieve chronic inflammation [1]. Systematic exploration of our Vietnamese medical plant library led to the discovery of a biflavonoid from *Dracaena cambodiana*, which alleviates inflammation by inducing a lipid mediator class switch from leukotrienes to specialized pro-resolving mediators (SPM) [2]. We elucidated the absolute configuration of the active isomer and identified 5-lipoxygenase as major target. Molecular docking studies suggest that the compound binds to an allosteric cavity at the interface of the catalytic and regulatory 5-lipoxygenase domain. Inhibition of microsomal prostaglandin E2 synthase-1 and other enzymes within lipid mediator biosynthesis was only evident at substantially higher concentrations. Leukotriene production is effectively reduced across human primary innate immune cells (neutrophils, macrophages), in blood and during peritonitis in mice. SPM formation, on the other hand, selectively increases in macrophages of the M2 subtype, is elevated *in vivo* and associated with diminished immune cell infiltration. Together, starting from Vietnamese plants, we identified a biflavonoid that reprograms the lipid mediator profile of innate immune cells towards resolution.

Funding

DFG (SFB 1278, SFB 1127/2, EFRE (2019FGR0095), FWF (S107, T 942 – B30), Tiroler Zukunftsstiftung (AP740021), OEAD.

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SL8 Vioprolide A exerts anti-inflammatory actions in endothelial cells through inhibiting NOP14 and downregulating KPNA2

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DOI 10.1055/s-0041-1736758

Treatment of chronic inflammatory diseases, which are often characterized by overly activated endothelial cells (EC) and persistent leukocyte trafficking from

the blood to the affected tissue, remains a major therapeutic challenge. Thus, the search for novel drugs and drug targets is an ongoing demand.

We have identified the myxobacteria-derived peptide vioprolide A (vioA) to exert anti-inflammatory actions *in vivo* and in ECs *in vitro*. VioA attenuated leukocyte trafficking through the vascular endothelium in the murine cremaster muscle and infiltration of microglia and macrophages during laser-induced murine choroidal neovascularization. Mechanistically, vioA impaired the *de novo* protein synthesis in ECs, thereby decreasing EC adhesion molecule and tumor necrosis factor receptor (TNFR) 1 protein levels. Importantly, downregulation of karyopherin alpha 2 (KPNA2) expression, a carrier protein needed for the translocation of the NF-κB subunit p65 from the cytosol to the nucleus, is a crucial part of the action of vioA causing a decreased NF-κB promotor activity. Knockdown experiments revealed a causal link between the cellular target of vioA, NOP14, and the anti-inflammatory actions observed.

Our results classify the natural product as unique drug lead for anti-inflammatory therapeutics.

Conflict of Interest

The authors declare that there is no conflict of interest

SL10 Simultaneous identification of common synthetic adulterants in slimming aids and sexual enhancers herbal supplements by High-performance Thin Layer Chromatography

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DOI 10.1055/s-0041-1736759

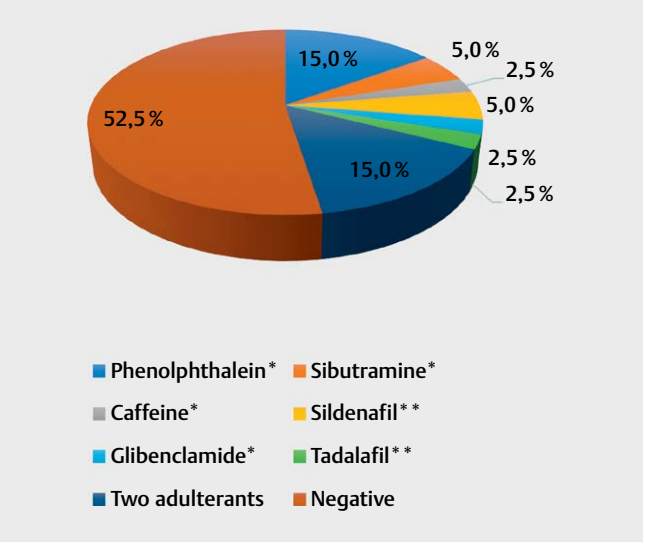
Introduction Herbal supplements are important and widely used health care choices but also pose a global challenge in quality and safety. The lack of regulations and less stringent value chains, for example, result in adulterations with undeclared synthetic substances. The extent and nature of this issue has been unknown in the Middle East.

Besides, analytical methods for easy implementation in laboratories without sophisticated instrumentations and limited resources are always in demand, particularly in developing countries. To the best of our knowledge, all proposed methodologies to date focused on identifying one class of synthetic adulterants, such as erectile dysfunction drugs [1] and anti-obesity drugs [2]. This project aimed to develop and validate a simple, rapid, robust, specific, reliable and cost-effective qualitative and quantitative HPTLC method.

Materials and Methods 40 samples were collected from pharmacies and herbal shops in Iraq, including those promoted for slimming and sexual enhancements. During method development, several steps were optimised, including the choice of stationary phase, mobile phase, samples extraction, injection volumes, detection and derivatisation.

Ethanol solvent system with silica gel as stationary phase was selected. It enabled a good separation of all reference standards (i.e., R_F value ranged from 0.10 to 0.76, and injection volumes varied from 2 to 20 µl).

Results and Discussions The developed method was validated for specificity, precision, repeatability, and linearity. The limit of detection (148-1378µg), the limit of quantification (450-4176µg), and the regression coefficient was calculated**.** Out of the samples analysed, 48 % (n = 19) contained one adulterant; among these, 15 % (n = 6) contained two adulterants. Figure 1 shows the percentage of adulterants identified in herbal supplements promoted for slimming and sexual enhancements (n = 40).



▶ **Fig. 1**

Conclusions The use of supplements promoted for slimming and sexual enhancement could impose a health risk to the consumers due to common adulterations in such products. The method developed enables a efficient one-step detection of common adulterants.

Conflict of Interest

None declared

Funding Schlumberger Foundation Faculty for the Future

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SL11 Metabolic Fate of the Secoiridoids Oleacein and Oleocanthal in an *In Vitro* Continuous Dialysis System with Human Gut Microbiota.

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DOI 10.1055/s-0041-1736760

The olive oil secoiridoids oleacein (OLEA) and oleocanthal (OLEO) bear diverse health promoting and disease preventing abilities; yet, there is a lack of data concerning their ADMET properties, due to their challenging isolation and detection. Furthermore, the beneficial effects of many dietary polyphenols on human health are partly attributed to their biotransformation occurring during colonic metabolism by human gut microbiota.

Since biotransformation studies of OLEO and OLEA are limited, we aimed towards a detailed investigation of their absorption, metabolism and microflora-dependent conversions, using the Gastro-Intestinal Dialysis Model with Colon (GIDM-Colon). This model is an optimized, validated *in vitro* continuous flow dialysis system, simulating the physiological conditions of the human GI tract, including human gut microbiota.

Throughout the GIDM-Colon model, biological samples were collected at crucial timepoints up to 24 h of colonic phase.

Overall, several new metabolites of OLEA and OLEO were identified incorporating statistical, chromatographic and spectrometric tools incorporating an LC-Orbitrap platform. The metabolism patterns seemed to differ greatly among the two compounds. Catechol group of OLEA, was more prone to biotransformation reactions while the elenolic part of both secoiridiods underwent extensive oxidation yielding oleocanthalic acid in both compounds. Oleocanthalic acid was detected during stomach and small intestine phase while managed to survive the 24h of colonic catabolism

Conflict of Interest Statement on behalf of all authors. There is no conflict of interest.

Funding (PlantUp, 5002803)

SL12 Effects of natural compounds on the accuracy of 8-oxo-7,8-dihydro-2'-deoxyguanosine translesion synthesis

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DOI 10.1055/s-0041-1736761

Translesion synthesis (TLS) is a DNA damage tolerance mechanism that relies on a series of specialized DNA polymerases able to bypass a lesion on a DNA template strand during replication or post-repair synthesis. Specialized TLS DNA polymerases pursue replication by inserting a base opposite to this lesion, correctly or incorrectly depending on the lesion nature, involved DNA polymerase(s), sequence context and still unknown factors.

To measure the correct or mutagenic outcome of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) bypass by TLS, a primer-extension assay was performed *in vitro* on a template DNA bearing this lesion in the presence of nuclear proteins extracted from human intestinal epithelial cells (FHs 74 Int cell line); the reaction products were analysed by both denaturing capillary electrophoresis (to measure the yield of translesion elongation) and pyrosequencing (to determine the identity of the nucleotide inserted in front of the lesion). The influence of several natural compounds on the correct or mutagenic outcome of TLS through 8-oxodG was then evaluated, in two experimental conditions, by adding the polyphenol either (i) to the reaction mix during the primer extension assay; or (ii) to the culture medium, 24h before cell harvest and nuclear proteins extraction. Some of the tested compounds significantly influenced the outcome of translesion synthesis, either through an error-free or a mutagenic pathway.

Conflict of Interest None

SL13 Natural products in changing agricultural policies: knowledge sharing and implementation

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DOI 10.1055/s-0041-1736762

The landscape of agricultural policy is changing towards more sustainable systems with minimal use of chemical and antimicrobial products. Natural products, like herbs, can play an essential role in reducing the use of chemical and antimicrobial products in agriculture and animal health care. For this, a government funded project has been started with the aim to distribute and practically implement knowledge about natural products. For this project farmers, veterinarians and their teachers were identified as stakeholders for which an information development and distribution strategy should be developed on the use of natural products. Secondly, an inventory was devised from the different stakeholders by conducting interviews focused on communication needs for transfer of knowledge to students. Building on already available teaching material, like the course for veterinarians and stablebooks, new material is being designed to accommodate these needs. Needs that have so far been identified are: 1) reliable and easily accessible information on safety and efficacy of natural products in animal health care (this has been achieved for example via herd and farm specific health plans) and, 2) to train teachers so they can teach students and other teachers ("train the trainer") on the use of natural products. Finally, distribution of this material is realized by using different options of communication such as interactive group meetings ("knowledge sharing") and online resources ("easily and free accessible information"). First results show that identifying needs and making knowledge (sharing) accessible in multiple ways is helpful in correct implementing and increased usage of natural products by farmers and veterinarians [1, 2].

Conflict of Interest; Funding (Source, ID)

The authors state that there is no conflict of interest, the project is funded by the Dutch Ministery of Agriculture, Nature and Food Quality („Kennis op Maat")

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SL15 New human urine biomarkers associated with hydroxytyrosol consumption and olive-based products

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DOI 10.1055/s-0041-1736763

Hydroxytyrosol (HT) is one of the most characteristic compound of olive products and a highly potent compound with proven positive impact to human health [1]. Moreover, HT is also an endogenous metabolite produced from dopamine. The release of many HT-based products in the market directed the current research efford to the exploration of HT effect in obesity and investigation of its ADMET properties based on a human intervation study.

Therefore, HT was administered as a soft capsule to 28 obese/overweight women in different doses to investigate its anti-obesity effect and the impact on urine metabolome. Towards this purpose, urine samples were collected in three time points (T = 0, T = 3 months, T = 6 months) and analyzed via UPLC-Orbitrap MS using untargeted metabolomics aiming to biomarkers discovery. The three different groups (control, high and low dose) were discriminated according to the administered capsule and dose and 30 statistically significant metabolites were uncovered. Among them, hippuric acid (HA) and phenylacetylglutamine (PAG), two endogenous urine metabolites were distinguished. Additionally, UHPLC-TQ MS methodology using multipole reaction monitoring (MRM) method was used for HA and PAG quantitation in urine samples. HT and homovanillic acid, the most common metabolic derivative of HT, were also quantified. The proposed biomarkers levels verified our initial findings. HA and PAG were associated for the first time with HT consumption and could be utilised as biomarkers.

Authors declare no conflict of interest The present work was carried out and co-funded by the European Regional Development Fund (ERDF) and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH – CREATE - INNOVATE". Authors would like also to thank the Operational Program PlantUP (project code: 5002803).

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SL16 *Albizia julibrissin* DURAZZ. from wild collection – a multivariate data analysis approach

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DOI 10.1055/s-0041-1736764

The aim of our research is to investigate traditional herbal medicines to discover new, plant-based alternatives for the treatment of chronic inflammatory diseases. [1] The bark of *Albizia julibrissin* Durazz., the most valuable candidate emerging from the screening phase, was collected under the guidance of local botanists, in Hubei Province, China, from 9 different locations and 14 different trees. Extraction was performed by successive ultra-sonication of the powdered bark with dichloromethane and methanol, followed by liquid-liquid fractionation of the extracts with dichloromethane, ethyl-acetate, *n*-butanol and water. LPS- and IFNγ- induced RAW 264.7 macrophages and BV2 microglia cells were used to determine the anti-inflammatory effect of the bark extracts via detection of NO with Griess-reagent, while XTT assay was used to determine respective cytotoxicity. [2] Metabolite profiles were analyzed by means of UHPLC-HRMS analysis. Phytochemical profiles and pharmacological data have been correlated by orthogonal projection to latent structures discriminant analysis (OPLS-DA) to deduce potentially active candidate compounds. Identification of candidate compounds by UHPLC-HRMS dereplication and isolation is in progress.

Funding Field research of Raab P was funded by OeAD Marietta Blau-Stipendium (ICM-2019-13445

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SL17 Ethnoveterinary use of medicinal plants in the treatment of equids – a survey in Bavaria

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DOI 10.1055/s-0041-1736765

As pure herbivores, horses and donkeys are predestined for the use of medicinal plants. In 2018 and 2019, we conducted an ethno-veterinary study on Bavarian farms.

A total of 77 interviews were carried out with 101 farmers. Altogether 884 use reports (URs) 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, 34 URs (corresponding to 32 herbal remedy reports (HRs)) with 45 different plant species were specifically linked to diseases of equids (23 URs horses and 11 URs donkeys).

For 23 URs (22 HRs) a single plant was used, 11 URs (10 HRs) were mixtures. The most frequently mentioned plant species in mixtures were: *Calendula officinalis* L. (5 URs), *Achillea millefolium* L. (4 URs) and with each 3 URs: *Carum carvi* L., *Cinnamomum verum* J. PRESL, *Geranium robertianum* L., *Plantago lanceolata* L., and *Pimpinella anisum* L. Among the single plant applications, *Calendula officinals L.* (3 URs) and *Urtica dioica* L. (3 URs) were mentioned most often.

Apart from cinnamon, the most frequently reported plant species are regionally indigenous. Except *Geranium robertianum* L., these plants are well known in the phytotherapeutic use in equids. *Geranium robertianum* L. is increasingly analyzed chemically due to its antioxidant and anti-inflammatory activity [1]. Clinical data and especially applications in equids are still lacking.

Conflict of Interest

The authors declare that they have no competing interests.

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SL18 *In vitro* screening of South African medicinal plants in the pursuit of anti-viral agents against SARS-CoV-2

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DOI 10.1055/s-0041-1736766

The COVID-19 pandemic continues to be a global health concern despite numerous efforts being undertaken to curb it. This has necessitated the urgent need to seek more complementary tools, including drugs, to treat this disease. Inspired by the fact that natural products have historically served as a source of chemical scaffolds for development of drugs, the current study aims to interrogate South African biodiversity in search of novel anti-viral agents against SARS-CoV-2. A dual approach was adopted where *in-silico* screening and a rigorous criterion were used to select pure natural compounds and medicinal plants for the study. Compounds were phenotypically screened *in-vitro* against the main protease and spike protein of SARS-CoV-2. Using a high-throughput fractionation technique, twenty traditionally used plants were extracted and fractionated into semi-pure fractions using hyphenated analytical technologies for an accelerated screening approach.

Of the eight compounds screened, epigallocatechin gallate showed the most pronounced activity in the spike/ACE2 disruptive assay (IC_{50} of 0.44 µg/ml), presenting some inhibition activity at 15 µg/ml in the whole-cell assays. From the twenty plant species screened, four demonstrated good activity (IC_{50} < 10 µg/ml) in the disruption of the spike/ACE2 complex and inhibition of the main protease (> 80 % inhibition at 1 µg/ml). One species additionally exhibited activity in phenotypic screens with the active compound identified as punicalagin (> 75 % plaque reduction at 15 µg/ml). Our data motivates the continued interrogation of South African plants in the search of novel anti-SARS-CoV-2 agents.

Authors declare no conflict of Interest.

Funding NRF SA, DSI SA

SL19 Phytochemical study-Biological activities and application of q 1H-NMR method to determination of paeoniflorin/gnetin in three *Paeonia* species, endemic in Greece

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Paeonia, is the single genus in Paeoniaceae, distributed in Europe and Asia. Peony is named after Paeon, student of Greek father of medicine Asclepius and has been cultivated in China since the Han dynasty named the "king of flowers" and "flowers of richness and honor" [1]. We report herein, the phytochemical study on black fertile seeds (BS) and red infertile ones (RS) of three endemic Greek paeony species: *Paeonia clusii* subsp. *rhodia*, *P. clusii* subsp. *clusii* and *P. mascula* subsp. *Mascula*, not previously studied.

Several stilbene derivatives of resveratrol (*cis*, *trans* forms, viniferin, gnetin H), flavonoid glycosides of luteolin, monoterpene glycoside paeoniflorin, unique in all peonies, etc have been isolated and structurally determined. Moreover,

q1H-NMR methodology has been used to measure peoniflorin and gnetin H content in extracts of studied species and different plant parts: BS, RS, pericarps and leaves. *P. clusii rhodia* (BS) appeared as the richest source of gnetin H (24.96 %). Paeoniflorin was the most abundant metabolite in *P. clusii clusii* (BS) 34.05 %, > (RS) 29.64 %, > leaves 19.11 % > perisperms: 13.26 %. *P. mascula mascula* (BS) 28.91 %, > (RS) 22.5 %, and perisperms 9.02 %. *P. clusii rhodia*: leaves 14.44 % > perisperms 14.76 %. q1H-NMR appeared as essential tool for the quantitative and qualitative analysis of the examined crude extracts.

The total phenolic content was evaluated (Folin-Ciocalteu method) showing extremely high values (103.63–289.97 mg GAE/g), comparably strong antioxidative and anti-tyrosinase properties, in the case of gnetin H higher than kojic acid. Further studies on paeonies are ongoing.

No conflict of interest; This research received no external funding.

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SL20 Innovative Approaches to African Traditional Medicine in analysis of purity, quality and potency of new Phytopharmaceutical extracts.

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DOI 10.1055/s-0041-1736768

A proposed HPTLC technique for *Kigelia pinnata* fruit extract. Commercial extracts of *Kigelia pinnata* HAB19F 30 % EtOh was obtained from suppliers Company 1. and by commercial supplier company 2. Using CAMAG HPTLC Automatic Sampler with HPTLC glass 20x10 cm, Si 60 F254 using solvent system of Ethanol, Chloroform and Ethyl Acetate, developed with 0.5 % anisaldehyde reagent in methanol: acetic acid: sulphuric acid (85:10:5). For purposes of this experiment a known compound found in *Kigelia,* β-Bisobolol was used as a reference standard, the results indicate difference in quality of extracts and purity from different commercial companies it also indicates that further chromatographic analysis needs to be undertaken to show better separation of active ingredients and identify a better reference compound. These results show that further monograph profiling work and HPTLC analysis needs to be undertaken before standardised commercial extracts and a standardised monograph can occur on extracts of *Kigelia pinnata.*

SL21 Safety and efficacy of herbal supplements used for weight loss

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Overweight and obesity are increasing worldwide. Lifestyle change represents the first strategy to counteract the problem, although sometimes a pharmacotherapeutic approach is necessary. People also recourse to herbal products ignoring the real effectiveness and underestimating their safety profile [1]. In this context, the present work aimed at critically evaluating the efficacy and potential risk associated with weight loss botanicals. Only some herbal supplements have been tested for their clinical effectiveness, among these *Ephedra sinica*, *Citrus aurantium*, *Camellia sinensis* (green tea), *Garcinia cambogia,* and *Curcuma longa* (turmeric). Often, the products were characterized by a huge variability, in term of kind of preparation, lacked of standardization or used the purified active compounds, whose effects could be different from that of the phytocomplex [2]. Moreover, the weight loss induced was not clinically significant [3]. Conversely, the same botanicals were sometimes associated with adverse reactions (ARs). *E. sinica* and *C. aurantium* induced cardiovascular ARs, which led in several countries to their restriction in food supplements. Green tea, *G. cambogia* and turmeric showed hepatic ARs. Some green tea-based products have been recalled while *G. cambogia* has never been banned owing to the uncertain causal relationship [4]. Regarding turmeric, supplements involved contained curcumin with enhanced availability or enriched extracts [5], thus a pure substance rather than a traditional preparation whith known safety. Considering the poor clinical evidence supporting the use of botanicals for weight loss, the safety concerns become more relevant. A careful evaluation of the risk/benefits profile of these supplements is necessary also considering they are often used as self-medication.

Conflict of Interest; Funding (Source, ID) None

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SL22 Phyllobilins – ubiquitous natural products with pharmacologically relevant activities

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Phyllobilins are a group of plant-derived bilin-type linear tetrapyrroles, which are generated in the process of chlorophyll degradation. Despite the visibility this biochemical pathway, the as well as the biochemical players involved have remained undetected for a very long time. Only 30 years ago was the first phyllobilin isolated and characterized by spectroscopic methods,which set the stage for the discovery of a variety of phyllobilins with different structural modifications depending on the plant species. Phyllobilins have primarily been regarded as products of a detoxification pathway employed by plants to get rid of phototoxic chlorophyll. Therefore, they have been overlooked as natural product class in terms of a biological role or pharmacological activity of their own. [1] A change of this paradigm, however, is long overdue.

For the first time, we show important pharmacological activities of phyllobilins as ingredients of medicinal plants, comprising potent antioxidative activities,[2] anti-inflammatory activities,[3] and activities on cancer cells.[4] This research sets the stage to systematically investigate the occurrence of phyllobilins and their roles as important phytochemicals.

Conflict of Interest; Funding (Source, ID) The authors declare no conflict of interest. Funding from the German Research Foundation (DFG, Project ID 448289381 to S.M.) is gratefully acknowledged.

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SL23 Artificial Intelligence in the Deployment of Complex tasks in Pharmacology. A case of “Big Data” challenges in Ethnopharmacology

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DOI 10.1055/s-0041-1736771

Traditional Medicine (TM) is an interdisciplinary field of research based both on anthropological and scientific approaches. The database derived from TM prescriptions contains plant extracts and ingredients with unique pharmacological activities. New technologies are nowadays implemented to decode information

entangled in natural product metabolites, selected over millions of years of evolution, and over thousands of years of written records of our civilization. However, the volume and the complexity of sources that need to be monitored and classified, present the so called "big data" challenge. Artificial intelligence (AI) and algorithms of Machine Learning (ML), improve the related research workflows, increasing velocity and precision. In addition to bibliographic databases the necessary information sourced from healers needs to be filtered to obtain fruitful data. The use of personalized data mining techniques that is adaptive in real-time by the experts of ethnopharmacology, is still under evolution. In our model, a ML algorithm is used as an AI system in order to discriminate between relevant and irrelevant documents, biased by decisions provided by a human expert, improving the effectiveness and the efficiency of the identification and retrieval of the appropriate information. The evolution of such a system will contribute to knowledge sharing and scientific advancement in ethnopharmacology.

Conflict of Interest; Funding (Source, ID) The authors declare no conflict of interest; PlantUp (project code: 5002803).

SL24 Sweet cherry (*Prunus avium* L.): valorization of six Italian ancient varieties and recovery of industrial waste by natural deep eutectic solvents

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Prunus avium L. (Rosaceae) fruits, commonly known as sweet cherries, are well known for their health benefit properties mainly in the prevention of chronic degenerative pathologies [1], due to their composition, consisting in a considerable amount of phenolic compounds. In Italy, cherry fruits have a long-held tradition concerning their cultivation and are used both for eating and producing juices, jams, and drinks [2]. Therefore, a consistent amount of waste is generated and its recycle into a new chain of values in terms of circular economy is of increasing demand. In this context, six ancient Italian sweet cherry varieties were investigated by HPLC-MS for their chemical constituents and compared by a bioinformatic approach. All varieties showed fruits rich in flavonol glycosides, anthocyanins and coumaroyl quinic acids, while the petiole chemical composition was characterized by flavonoid glycosides and B type proanthocyanidins. In order to optimize the recovery of these bioactive compounds from cherry waste by using a green and innovative strategy, five different natural deep eutectic solvents (NADESs) have been designed and used as alternative to the classical volatile and toxic organic solvents for the cherry waste extraction. Results were compared to the recovery with classical solvents and the best performing NADES was evaluated in terms of phenol recovered amount.

Funding LG, A Mero gratefully acknoweledge Era-Net Cofund SUSFOOD2 Call 2017 and MIUR.

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SL25 The Story of Xanthohumol C – a Promising Neuro-regenerative Chalcone from Hops

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DOI 10.1055/s-0041-1736773

Humulus lupulus L. – Hops - is a well known medicinal plant. The traditional use for insomnia is related to bitter acids and furthermore an effect on "melancholy" was mentioned in monastic medicine. Since it is known that anti-depressive medication can regulate neuro-regenerative processes concerning adult neuronal stem cells in the human brain, hops was a promising candidate for further investigations.

Using an activity-guided fractionation determining the differentiation inducing activity in mouse embryonic forebrain-derived neural precursors (DCX-reporter as well as staining experiments), Xanthohumol C (ENDF1), was found as potent neuro-regenerative chroman-like chalcone [1], more effective than other flavonoids tested. The effect is probably not mediated via the estrogen [2] or the TRKA-pathway [3].

A structure-activity study revealed the pyrano ring as responsible structural characteristic. A cyclodextrin-based extraction process, which leads directly to a more water-soluble and bioavailable formulation opens the way for phytopharmaceutical applications [4].

Furthermore, Xanthohumol C can overrule central nervous system growth inhibitors e. g., semaphorine and facilitates regeneration of neurons [3] making it suitable for a possible treatment of spinal cord injury.

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SL26 *Abietane Diterpenoids from Plectranthus spp. as a starting tool in Cancer Research*

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DOI 10.1055/s-0041-1736774

Natural products from medicinal plants represent an important source of novel therapeutic substances to fight severe diseases including cancer. The *Plectranthus* genus is commonly used in traditional medicine due to its potential to treat several illnesses. Diterpenoids are commonly found in *Plectranthus* spp., and have a widespread spectrum of biological activity, namely anticancer properties.

Protein kinase C (PKC) family isoforms have been the focus of intense research, and are recognized as therapeutic targets in anticancer drug development. In this work, a small library of abietane derivatives was studied for their ability to activate PKC isoforms. To obtain the lead molecules, several extraction methods were tested to optimize the extraction of the bioactive diterpenoid 7α-acetoxy-6β-hydroxyroyleanone (Roy). The results obtained revealed potent activators of PKC family proteins, namely: a selective activator of PKCδ, the 7α-acetoxy-6β-benzoyloxy-12-*O*-benzoylroyleanone (RoyBz). The patented diterpenoid RoyBz was prepared using Roy as starting material. RoyBz potently inhibited the proliferation of colon cancer cells by inducing a PKCδ-dependent mitochondrial apoptotic pathway involving caspase-3 activation. The results indicate that RoyBz targets drug resistant cancer stem cells, in HCT116 colon cancer cells, preventing tumor dissemination and recurrence. These results point to promising activators of PKCs with high potency and isoform-selectivity that may emerge from the exploitation of this new family of abietane diterpenoids. Molecular docking studies are currently ongoing to further identify new selective abietane diterpenoids as new PKC modulators.

Funding: This work was supported by FCT UIDB/04567/2020 and UIDP/04567/2020.

SL27 Isolation of cytotoxic compounds from the underground parts of Valeriana sisymbriifolia Vahl. through activity-guided fractionation

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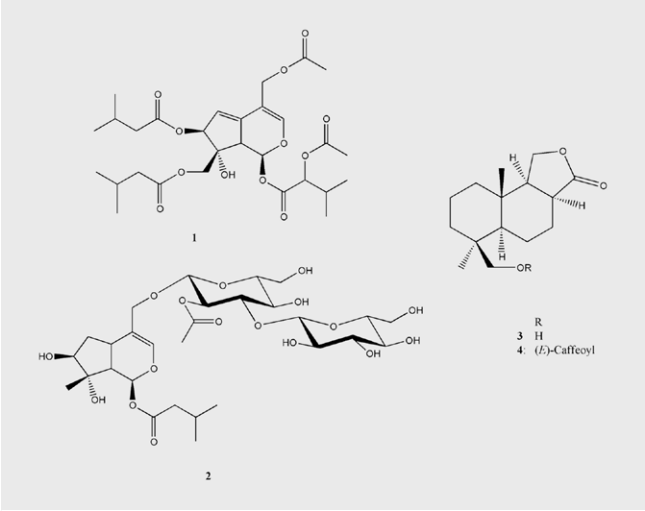
DOI 10.1055/s-0041-1736775

Valeriana sisymbriifolia is a perennial herb that is particularly distributed in Turkey, Iran and Iraq [1]. Previous studies on *Valeriana* species indicated sedative, anxiolytic, neuroprotective and cytotoxic activities which are mainly attributed to its iridoids and sesquiterpenes [2]. In this study, we aimed to isolate the cytotoxic compounds from underground parts of *V. sisymbriifolia* through *in vitro* activity-guided fractionation technique. The EtOH extract, its fractions and isolates were evaluated for their cytotoxic activities against four cancer cell lines (MCF-7, HGC-27, PC-3, A549) and against HUVEC cell line by MTS assay. The $CHCl_3$ and EtOAc fractions exhibited remarkable cytotoxic activity against the tested cancer cell lines (IC_{50}: 2.4-16.3 µg/mL). Thus, $CHCl_3$ and EtOAc fractions were separated by chromatographic methods to give two new iridoids (**1** and **2**) and two new sesquiterpene lactones (**3** and **4**) together with nine known compounds, didrovaltrate (**5**), valtrate (**6**), 7-homovaltrate (**7**), 1-α-acevaltrate (**8**), baldrinal (**9**), 11-ethoxyviburtinal (**10**), patriscabrol (**11**), bornyl caffeate (**12**) and caproleic acid (**13**). The structures of the isolates were elucidated by NMR and HR-MS analyses. **6** showed strong cytotoxic activity on A549 cell line with IC_{50} value of 7.5 µM while **5**, **6**, **7**, and **8** exhibited significant cytotoxic activities on MCF-7 cell line with IC_{50} values ranging from 2.5 to 5.9 µM. Also, **6** and **7** indicated the best cytotoxic activities against HGC-27 cell line (IC_{50}: 2.3 and 3.7 µM respectively). **6**, **7** and **8** showed noteworthy cytotoxic activity against PC-3 (IC_{50}: 2.3-9.7 µM). The mechanisms behind the cytotoxic activities are underway.



▶ **Fig. 1**

Acknowledgements This study was supported by TÜBİTAK (Project No: 119Z094)

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SL28 The Botanical Safety Consortium: a public-private partnership to enhance the botanical safety toolkit

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DOI 10.1055/s-0041-1736776

The Botanical Safety Consortium (BSC) is a public-private partnership aimed at enhancing the toolkit for conducting the safety evaluation of botanicals. The BSC serves as a global forum for scientists from government, academia, industry, & NGOS to work collaboratively on developing & integrating new methods based into routine botanical safety assessments. The objectives of the BSC are to: engage with a broad group of global stakeholders to leverage the safety approaches; establish appropriate levels of chemical characterization for botanicals; identify fit-for-purpose in vitro & in silico assays to evaluate botanical safety; evaluate the application of these tools via comparison to the currently available information; & integrate these tools & approaches into a framework that can facilitate robust evaluation of botanicals. Initial endpoints of focus are genotoxicity, hepatotoxicity, ADME, developmental & reproductive toxicity, cardiotoxicity, neurotoxicity, & systemic toxicity. Groups addressing pharmacognosy, chemical analysis & data analysis have also been initiated.

This presentation will provide an overview on the structure, goals, & strategies of this initiative & early insights regarding our first objectives, namely the selection & prioritization of botanicals based on putative toxicological properties, & pilot work regarding level of chemical characterization of candidate botanicals to be used for testing.

The authors declare no conflict of interest. The funding for the BSC is from the US FDA/NIH and member companies.

SL29 The LOTUS initiative for knowledge sharing in Natural Products research

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DOI 10.1055/s-0041-1736777

With the recent explosion of information, Natural Products (NP) research critically needs efficient ways to access and share knowledge, also to save precious knowledge being lost [1]. The reporting and sharing of NP occurrences in biological organisms are relevant to numerous scientific fields ranging from drug discovery to chemical ecology or chemotaxonomy.

Through the LOTUS initiative, we aim to offer better knowledge sharing in NP research. We established a data harmonization, curation, and validation pipeline to gather and appropriately document structure-organism pairs. These pairs are then shared through the Wikidata platform. Wikidata is particularly indicated for the sharing of knowledge in life sciences as demonstrated in [2]. We made 700,000 + referenced structure-organism pairs available on Wikidata. As an example, it is, now possible to retrieve biological organisms contain-

ing chemical compounds described as anti-infective (https://w.wiki/vo9). This offers exciting perspectives, linking information gained over different, sometimes disconnected, fields of investigation.

Fundings CRSII5_189921, CRC1127, LM2018131, G-2019-11458, U41 AT008706 and AT000155

The authors declare no competing interest.

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SL30 RCT with a phytodrug combination of nasturtium herb and horseradish root for uncomplicated acute rhinosinusitis

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DOI 10.1055/s-0041-1736778

Purpose A combination herbal remedy containing nasturtium (Tropaeoli majoris herba, 200 mg powder/filmtablet) and horseradish (Armoraciae rusticanae radix, 80 mg powder/filmtablet) has been used as licensed drug in Germany for more than 40 years (Angocin® Anti-Infekt N, Repha, Germany), mainly for the treatment of respiratory infections. This clinical trial was to investigate into its efficacy and safety for uncomplicated acute rhinosinusitis.

Methods 380 patients (m/f, aged 18-75) with nasal obstruction and facial pain for ≤ 3 days were to be randomized in a double-blinded, placebo-controlled, multicenter phase IV study and treated with the recommended dose of 3x4 tablets over 14 days. Primary endpoint was MRSSinv/MRSSpat documented between day 6 and day 10, computed as AUC, assessed by ANCOVA with day 3 as covariate.

Results 380 patients were randomized, 238 were included in the FAS for statistical analysis. Treatment with the herbal combination revealed a significant smaller AUC (14.99) compared to placebo (18.52, $p = 0.0003$, χ2-test). At visit 3, responder rates were significantly higher for patients receiving the herbal combination compared to placebo (92.1 % vs. 83.3 %, $p = 0.0418$). Adverse events occurred in 21.9 % and 18.6 % of participants receiving the herbal combination and placebo, respectively. Most common adverse events were headaches and gastrointestinal complaints.

Conclusion The criterium for efficacy of the primary outcome was fulfilled with good tolerance and safety. Further research should concentrate on differential activity by microbiological etiology, reduction of recurrence rates and effects on specific symptoms of rhinosinusitis.

SL31 Gut microbiota and phytochemical constituents of natural products – pharmacological and metabolomic approaches

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Introduction The human microbiota plays a tremendous role in mediating the health benefits of substances from plants, not only in nutrition, but also in case of herbal medicinal products. It has been shown that some substance groups, such as flavonoids and polyphenols, exert their health benefits only after metabolic activation by the gut microbiota and absorption in the large intestine. These interconnections are of special relevance in case of natural products in therapeutic use.

Methods and results The interconnections are exemplified with some characteristic studies. The role of microbiota on the action of the flavonoids kaempferol and quercetin was shown in male C57BL/6 mice that their anxiolytic and antidepressant action depends on their microbial metabolism, thus indicating that these are just only prodrugs [1]. For herbs used in GI diseases, the role of the microbiota in metabolizing phytochemical components was shown by studies in human fecal suspensions using PCoA [2], with a high interindividual variability of metabolism of key constituents, e. g., liquiritigenin.

Summary and conclusions The action of natural products and their phytochemical constituents is to a large extent depending from the gut microbiota [3], and is mostly still unexplored, as recent reviews show [4]. Despite already a big number of studies is available, there is still a large need of further studies.

Conflict of Interest; Funding (Source, ID) OK is employee of Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany, MTK has been receiving honoraries from of Steigerwald Arzneimittelwerk GmbH.

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SL32 Quercetin obstructs Triple Negative Breast Cancer (TNBC) progression by targeting HuR

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Hu-antigen R (HuR) or embryonic lethal, abnormal vision, Drosophila (ELAV)-like protein-1 (ELAVL-1) has been implicated in various cancers [1]. HuR expression has been associated with treatment resistance of TNBCs, and impairing HuR signaling re-sensitizes TNBC cells towards chemo-as well as radiotherapies. In the present study, we investigated the clinical significance of HuR protein and it's targeting by quercetin in breast cancer.

Using an online meta-analysis tool, we found that HuR expression positively correlated with reduced overall survival of TNBC patients. Furthermore, we showed that the TNBC breast cancer cell lines i.e., MDA-MB-231 and MDA-MB-468 are good model systems to study HuR protein, as they both exhibit a significant amount of cytoplasmic HuR (active form). We showed that quercetin treatment significantly inhibited the cytoplasmic HuR in both TNBC cell lines. By using specific HuR siRNA, we established that quercetin-mediated inhibition of adhesion and migration of TNBC cells is dependent on HuR. Overall, the present results demonstrate that increased HuR levels are associated with TNBC progression and relapse, and the ability of quercetin to inhibit active HuR protein provides a rationale for using it as an anticancer agent for the treatment of aggressive TNBCs.

Conflict of Interest; Funding (Source, ID) All Authors declare no conflict of interests. Funding (AIIMS Intramural, Grant No. A-515)

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SL33 Biochemometry as a tool to unravel the anti-inflammatory activity of the Ayurvedic remedy red sandalwood

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Pterocarpus santalinus L.f., is an endemic species native to the Southern parts of India. Its heartwood is used as an anti-inflammatory Ayurvedic remedy [1]. Indeed, a CH_2CL_2-MeOH heartwood extract (PS-E) significantly suppressed the expression of cell adhesion molecules (VCAM-1, E-selectin) and other pro-inflammatory molecules (CX3CL1, GM-CSF) in IL-1 stimulated endothelial cells. These data provide first insights into its molecular mode of action. The aim of this study was a fast identification and targeted isolation of constituents contributing to the observed effects. For this purpose, we applied the biochemometric approach ELINA [2, 3]. PS-E was separated into 35 microfractions with a quantitative variance of constituents over several consecutive fractions. This was achieved by a unique hyphenation of a flash chromatography instrument to a high-performance counter-current chromatographic device. ^{1}H NMR data and bioactivity data of all microfractions were then correlated by heterocovariance analyses [4] and statistical total correlation spectroscopy. Complemented by LC-MS-ELSD data, ELINA differentiated between active and inactive constituents prior to isolation. The follow-up of these results led to one stilbene, five isoflavanoides, two isoflavanes and three pterocarpans as potential inhibitors of inflammatory gene expression.

Funding FWF Project P 34028

The authors declare no conflict of interest

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SL34 Antiviral activity of Ugandan medicinal plants used by herbalists against human immunodeficiency virus type-1 (HIV-1)

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DOI 10.1055/s-0041-1736782

access to antiretroviral drugs (ARVs) than those eligible in Sub Saharan Africa. Many PLHIV use medicinal plants which have not been scientifically evaluated. The aim of this study was to investigate the anti-HIV-1 activity of selected one widely used medicinal plant species for treating AIDS PLHIV in Uganda. A total of 22 plant extracts (ethanol & Dimethyl Sulphoxide) were tested for their antiviral activity against HIV-1 Pseudovirions using the human glioblastoma cell line; U87.CD4.CXCR4, which was transfected with the luciferase firefly reporter gene. The selective inhibition of the growth of HIV-1 PV was measured using the selectivity index (SI). The ethanol extract of *Psorospermum febrifugium* Spach exhibited the highest selective antiviral activity against HIV-1 (SI = 165.8, CC_{50} = 99.45 µg/ml) without cytotoxicity to the U87.CD4.CXCR4 cells at a concentration of 8 µg/ml. The other species with relatively high SI were *Bridelia micrantha* (Hochst.) (120.0), *Warbugia ugandensis* Sprague (76), *Securidaca longipendunculata* Fresen (72), *Albizia coriaria* Oliv (64.0). However, both the ethanol and DMSO extracts of *A. coriaria* (CC_{50} of 6.4 and < 4 µg/ml) and *W. ugandensis* (7.6 and 1.5 µg/ml) were also highly cytotoxic to the cells. Some of the plant extracts tested have potential antiviral activity against HIV-1 as shown by the high SI and lower cytotoxicity. Fewer people living with HIV/AIDS (PLHIV) have

4. Young Researchers Workshop

YRW0 Impulse Lecture How specialized metabolites mediate interactions between plants and other organisms

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DOI 10.1055/s-0041-1736783

Small molecular weight organic compounds are common across the galaxy and transcend all known biological interactions. Plants, in particular, have evolved a remarkable capacity to produce diverse sets of so-called specialized metabolites from a few simple, inorganic precursors. Already in 1977, Rhoades argued that plant specialized metabolites are likely multifunctional, i.e. that they serve multiple purposes. Multifunctionality may render the production of specialized metabolites more cost effective and may explain their abundance and tight spatiotemporal control in plants. Work over the last decades confirms that specialized metabolites often have a broad range of functions, from growth and development to defense [1]. However, our understanding of how this multifunctionality affects the interactions between plants and other organisms, including herbivores and their natural enemies is limited. In my presentation, I will explore the importance of multifunctional plant metabolites for plant-environment interactions by discussing our work on benzoxazinoids, the most abundant specialized metabolites in grasses such as wheat and maize. We find that benzoxazinoids act as direct defenses, within-plant defense signaling molecules, microbiome modulators and siderophores. At the same time, the western corn rootworm, a specialist maize pest and important agricultural pest, exploits benzoxazinoids as foraging cues, protective agents and micronutrient providers. Thus, the multifunctionality of plant specialized metabolites is mirrored in the adaptations of a specialist herbivore, resulting in a tightly interlocked metabolism. These findings have implications for our understanding of the ecology and evolution of plant specialized metabolites, and for their use in agriculture and medicine.

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YRW1 Silver birch (*Betula pendula* Roth) – phytochemical characterization of the constituents and their metabolites present in the urine

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DOI 10.1055/s-0041-1736784

Traditionally-used medical plants, such as silver birch, might be useful in supporting antimicrobial therapy and the prevention of urinary tract infections (UTI) [1]. Their constituents, metabolized and excreted in the urine, might act therapeutically on the infected urinary tract or even get activated by uropathogens' own enzymes [2]. Presented research is focused on describing urine metabolites of birch leaves infusion.

The UHPLC-DAD-MS^n was used for the sample analysis. The lyophilized water infusion of leaves of *Betula pendula* was examined. Volunteers on the low polyphenol diet were drinking *B. pendula* infusion for several days and delivered the urine samples. The urine was purified with SPE, and metabolites' fraction

was obtained. Metabolites were incubated with uropathogenic *Escherichia coli* (UPEC).

Based on the phytochemical screening of the plant extract, the major groups of constituents are flavonoids and depsides. The main group of metabolites were various glucuronides. Additional compounds were detected after incubation with UPEC.

The *B. pendula* infusion contains various compounds of established anti-UTI activity. The presence of their metabolites in the urine was confirmed. Some of them are metabolized furtherly by UPEC.

Conflict of Interest The authors declare no conflict of interest. Funding: the Polish National Science Centre research grant OPUS 15 No. 2018/29/B/NZ7/01873

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YRW4 Mining the effects of a *Bacillus* sp. olive tree endophyte-derived lipopeptide extract on the metabolism of *Colletotrichum acutatum* applying GC/MS and ^{1}H NMR metabolomics

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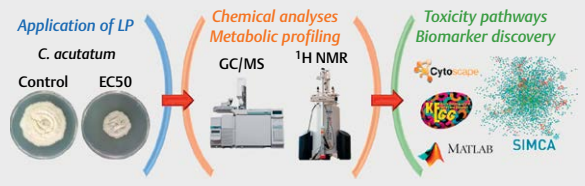
DOI 10.1055/s-0041-1736785

The issues that the plant protection sector is facing dictate the need for the discovery of improved sources of bioactivity as plant protection products. Within this context, endophytes have become the focus of the research based on their capacity to synthesize compounds with unique bioactivity.

Here, the effect of a previously isolated lipopeptide extract (LP) of a *Bacillus* sp. olive tree endophyte on the metabolism of *Colletotrichum acutatum* [1] was investigated (Figure 1). In the analyses, GC/EI/MS and NMR platforms were employed performing metabolomics.

A large portion of the fungal metabolome was recorded, including various carboxylic, amino, and fatty acids, carbohydrates and phenolic compounds. Based on multivariate analysis, metabolites-biomarkers of the toxicity of the applied LP were discovered; α,α-trehalose, L-proline, and phenylacetate were amongst the metabolites with the highest leverage on the observed toxicity, that also, play central role in fungal metabolism. The latter, is associated to the pathogenicity of the fungus, indicating an antipathogenic activity of the LP.



▶ **Fig. 1** Pipeline of the metabolomics study.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. The work was funded by the project "Plant Up: Upgrading the Plant Capital" MIS 5002803

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YRW6 Biologically active phenanthrenes from four *Juncus* species native to Hungary

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Phenanthrenes are aromatic plant metabolites derived mainly from stilbenes by oxidative coupling. Such compounds occur only in few plant families. Phenanthrenes have diverse structures and they possess different pharmacological activities. Because of their rare occurrence and specific substituents, they can be served as chemotaxonomic markers [1, 2].

The aim of our work was the phytochemical and pharmacological investigation of Juncaceae species occurring in the Carpathian Basin in order to isolate their phenanthrene constituents. In the course of this work, four *Juncus* species (*J. atratus*, *J. ensifolius*, *J. gerardii*, and *J. maritimus*) were investigated.

The dried and ground plant materials were extracted with methanol. After concentration, the extracts were dissolved in 50 % aqueous methanol, and solvent-solvent partitions were performed with hexane, chloroform and ethyl acetate. Phenanthrenes are enriched in the chloroform phase; therefore, these fractions were separated at first by column chromatography and then further purified by Sephadex LH-20 gel chromatography and medium pressure liquid chromatography. As final purifications, high-performance liquid chromatography and preparative thin layer
chromatography were used. The structures of the components were determined by NMR spectroscopy, and MS measurements. All compounds were tested for antiproliferative activity *in vitro*.

Altogether 52 phenanthrenes were identified from the four species, 31 of them are new natural products. The compounds are methyl-, hydroxyl-, hydroxymethyl-, formyl-, methoxy-, metoxy-methyl-, acetyl-, acetylene- and vinyl-substituted mono- and diphenanthrenes. All compounds were determined for the first time from the plants. Most of the isolated phenanthrenes possessed antiproliferative activity on one or more cancer cell lines.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. The work was supported by the National Research, Development and Innovation Office, Hungary (NKFIH; K128963), the UNKP-20-3 New National Excellence Program of the Ministry of Human capacities and EFOP 3.6.3-VEKOP-16-2017-00009.

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YRW7 Quest for New Lead Compounds Against Malaria Based on Natural Prodrugs Present in *Nauclea pobeguinii* and Their Metabolites

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DOI 10.1055/s-0041-1736787

The phytochemical composition of *N. pobeguinii*, used for treatment of malaria, was comprehensively characterized using UHPLC-UV-HRMS data. A diversity of compounds was detected, mainly alkaloids and saponins. Previous studies on strictosamide, the putative active constituent, showed no *in vitro* activity while the extract showed moderate *in vitro* activity [1]. It is suggested that metabolites of phytochemicals present in *N. pobeguinii*, most likely alkaloids, are responsible for its medicinal effect. An *in vitro* gastrointestinal model was used to simulate *in vivo* biotransformation of an extract of the plant and strictosamide itself. Analysis of these samples allowed the monitoring of the relative

abundances of individual compounds over time. XCMS and EDGE were used to extract significant differential profiles from the raw longitudinal multiclass LC-MS data. An interactive Shiny app in R was used to rate the quality of the resulting features.

These ratings were used to train a random forest model. In general, glycosylated alkaloids showed a decrease in intensity over time. Alkaloids containing no sugar moieties, including angustine-type alkaloids, showed no gastrointestinal biotransformation. Prominent differences were observed between biotransformation of strictosamide present as a pure compound and the compound present in the extract. The characterized biotransformed extract is purified and tested for *in vitro* activity against malaria.

Conflict of Interest; Funding (Source, ID)

No Conflict of Interest; Special Fund for Research of the University of Antwerp (Concerted Action), no. 30732.

The authors declare that they have no known competing interests that could have appeared to influence the work

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YRW8 Evaluation of endophytes on *Albizia julibrissin* DURAZZ. from wild collection

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DOI 10.1055/s-0041-1736788

The aim of our research is to discover new, plant-based alternatives for the treatment of chronic inflammatory diseases, from the treasures of traditional herbal medicines. [1] The bark extracts of *Albizia julibrissin* Durazz., showed anti-inflammatory activity in LPS- and IFNγ- induced RAW 264.7 macrophages and BV2 microglia cells in a screening phase, however the active compounds have not yet been identified. It has been reported, that Albiziae cortex is usually covered in patches of lichen and in the last few years, several plant endophytes have drawn considerable attention as a source of biologically active compounds. [2] To identify possible endophytes of A. *julibrissin* bark and their potential influence on the anti-inflammatory activity of Albiziae cortex, samples from wild collection were dissected, surface sterilized and incubated at 28 °C on different growth media. [3] Hyphae from the primary inoculation were picked and transferred to new agar plates to obtain a pure culture, which was used for direct PCR. Blast analysis was done to assign endophyte strains. Phytochemical analysis of active constituents is currently in progress.

Funding

Field research of Raab P. was funded by OeAD Marietta Blau-Stipendium

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YRW9 Antidiabetic and insulinotropic properties of bark of Heritiera fomes: inhibits starch digestion, protein glycation, DPP-IV activity, and glucose absorption in gut.

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DOI 10.1055/s-0041-1736789

Heritiera fomes (Sundari) have been accepted as a supplement for the treatment of pre and post diabetes. The present study investigated the effects of ethanol extract of *H. fomes* (EEHF) on insulin release from clonal pancreatic β-cells and mouse islets and protein glycation, starch digestion, glucose diffusion, DPP-IV enzyme activity *in vitro* and glucose homeostasis in type 2 diabetic rats. EEHF, significantly stimulated insulin release in concentration-dependent manner from BRIN-BD11 cells and isolated mouse islets with stimulatory effects equivalent in magnitude to 1μM GLP-1. Insulinotropic effects of *H. fomes* (200μg/ml) on BRIN-BD11 cells were partly inhibited by verapamil, diazoxide and calcium free conditions showing importance of ion channels and Ca^{2+} in mechanism of action. Secretion was further potentiated by activation of augmentation pathways revealed using IBMX, tolbutamide and KCl. At 200μg/ml, EEHF induced membrane depolarization and increased intracellular Ca^{2+}. Extract significantly inhibited starch digestion, DPP-IV activity, and decreased protein glycation. To investigate the possible effects on carbohydrate digestion, the sucrose content of the gastrointestinal tract was observed in 24h fasted rats after an oral sucrose load (2 · 5g/kg bw). The extract significantly inhibited postprandial hyperglycaemia after sucrose load and inversely increased unabsorbed sucrose content throughout the gut. The extract also significantly suppressed the absorption of glucose during in situ gut perfusion with glucose. Furthermore, the extract improved glucose tolerance and gut motility. This investigation hypothesized insulin release is the mechanism of action to control diabetes indeed and reassures to carry on to establish the plant for diabetes treatment.

YRW10 Development of a phytotherapeutic prototype based on triterpenes encapsulated in nanocarriers for the treatment of obesity and T2DM.

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DOI 10.1055/s-0041-1736790

Obesity is fueling a rise in type 2 diabetes mellitus (T2DM), with more than 700 million people worldwide estimated to suffer it by the year 2045 [1]. Previous studies have revealed that a triterpene-rich fraction from *Eucalyptus tereticornis* leaves reduced fasting blood glucose and improved glucose tolerance and insulin sensitivity in a T2DM murine model by intraperitoneal administration [2], but with limitations across oral administration. This work aimed to develop a polymeric nanocarrier (PN) to transport triterpene-rich fractions orally through the gastrointestinal tract in pre-diabetic mice and control the effects of obesity and T2DM to overcome such limitations.

The PN was formulated with polylactic-co-glycolic acid (PLGA) polymer encapsulating triterpenes, self-assembled by the solvent evaporation method and physicochemically characterized by HPLC, DLS, DSC and SEM. Triterpene release on an *in-vitro* human gastrointestinal model (GIM), *in-vivo* assays in a C57BL/6

mouse model, and metabolic biomarkers were assessed, compared and analyzed by ANOVA.

The resultant PN had 165 nm average size, -35,1 zeta potential, 0,18 polydispersity index, 3,5 % loading capacity and 98 % encapsulation efficiency. The GIM showed a progressive release of triterpenes, lower in the stomach reactor and up to 75 % in the intestine. The in-vivo assay demonstrated a decrease in the mice's weight and glucose measured in the blood and changes in metabolic biomarkers related to obesity and T2DM. Overall, these PN administrated orally open the way towards developing new nanoformulations based on natural products to decrease pre-diabetes and obesity in mice with an approximation on human GIM.

Conflict of Interest; Funding (Source, ID)

Funding from Minciencias Colombia (grant 807-2018, ID 63027).

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YRW11 Phyloactivity-based screening of coumarin-containing plants against Trypanosoma cruzi and target identification

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DOI 10.1055/s-0041-1736791

Chagas disease is caused by the protozoan parasite, *Trypanosoma cruzi*. It is a neglected tropical disease prevalent in Latin America. Chemotherapy with benznidazole or nifurtimox is effective when administered in the acute stage but the efficacy is lower in the chronic phase of the disease and these drugs become toxic [1]. Among rural communities with no accessibility to conventional medicine, intervention with botanical drugs seems to be a possible alternative against parasitic diseases [2]. We performed a systematic analysis to challenge this hypothesis starting with an ethnobotanical/pharmacological fieldwork among indigenous groups in the Bolivian Chaco where Chagas disease is hyper endemic. From the antichagasic plant taxa and chemotaxonomic clusters, we identified several natural product scaffolds via bioactivity-guided isolation [3]. We are currently interested in the antichagasic coumarin-containing plants and aim to identify the potential targets of coumarins in *T. cruzi*. To this aim, a library of 23 coumarins-containing plant taxa were tested. To assess the anti-trypanosomal activity, we have established an *in vitro* parasite release assay to quantitate the trypomastigotes released from infected host cells by flow cytometry. We have synthesized clickable coumarin probes to identify the possible targets in *T. cruzi* trypomastigotes.

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YRW12 Insights into biomimetic oxidized resveratrol metabolite mixtures

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DOI 10.1055/s-0041-1736792

Resveratrol, a well-known natural polyphenol, has a plethora of pharmacological activities. It has low bioavailability and undergoes extensive metabolism; numerous reports are available on the bioactivities of glucuronidated and sulfated resveratrol conjugates [1]. Relatively little is known on its potentially bioactive oxidized metabolites. Resveratrol may directly scavenge free radicals in living systems, which can lead to the generation of a wide range of new metabolites with altered bioactivities [2]. Our studies aimed at evaluating some potential pharmacological implications of this phenomenon. Oxidation of resveratrol through various chemical reactions, including biomimetic approaches, resulted in several mixtures that exhibited greater bioactivities compared to the parent compound. Mixtures were tested for in vitro antioxidant activities (DPPH, ORAC), and inhibitory action on lipoxygenase, xanthine oxidase, acetylcholinesterase and angiotensin converting enzymes. Spectral analysis and metabolomic profiling against a library of resveratrol oligomers showed a diverse group of compounds including dimers, iodine-, methoxy- and benzofuran-substituted derivatives. Using a multi-step chromatographic isolation procedure, 20 compounds were obtained in pure form. Antioxidant and enzyme inhibitory studies show that iodine-substituted compounds and dimers exhibited greatest bioactivities. Additional studies to determine inhibition kinetics and molecular docking simulations are currently ongoing.

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YRW13 Analytical determination of Chios mastic gum triterpenoids in human plasma and fecal samples by UPLC-ESI-TripleTOF-HRMS

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DOI 10.1055/s-0041-1736793

Chios mastic gum (CMG) is the resin obtained from *Pistacia lentiscus* L. var. *Chia*, which is endemic to the Greek island of Chios. CMG has a rich ethnopharmacological history, deeply rooted in the Mediterranean populations' phytotherapeutic tradition, while, in 2015, CMG was officially recognized by EMA as a traditional herbal medicinal product. From a phytochemical standpoint, CMG is rich in triterpenes, with masticadienonic acid (MNA) and isomasticadienonic acid (IMNA), being its characteristic constituents [1]. In a continuation study [2, 3], aiming to assess the effectiveness of CMG as a novel phytotherapeutic supplement for the treatment of NAFLD/NASH, a UPLC-ESI-TripleTOF-HRMS method was developed and validated for the determination of MNA and IMNA in plasma and stool samples of volunteers receiving a daily dose of CMG. Different extraction protocols were applied to each sample matrix and parameters, such as linearity, specificity, LOD, LOQ and accuracy, were evaluated. Finally, the method was applied to more than 200 plasma and 80 stool samples for the quantitation of MNA and IMNA, revealing a considerable inter-individual variability in their levels. This method can be utilized in any pharmacological study examining CMG's bioactivity in a human cohort.

Conflict of Interest; Funding

There is no conflict of interest. EV Mikropoulou is thankful to the Stavros Niarchos Foundation (KA: 14320). The authors are grateful to "PlantUP" (project code: 5002803).

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5. Animal Healthcare and Veterinary Workshop

VEW1 Animal origins of ethnomedicinal plants: folklore revisited from an animal self-medication perspective

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DOI 10.1055/s-0041-1736794

Folklore from around the world suggests that animals have been providing inspiration for the discovery of medicinal plants for the treatment of disease in humans and their livestock for a very long time. Looking to animals for medicinal knowledge points to a widespread recognition of their ability to self-medicate using plants to prevent or actively treat disease. The interdisciplinary field of animal self-medication is providing scientific evidence for this ability in species across the animal kingdom and lends support to animal-origin medicinal plant folklore and recent ethnomedicinal information. Here, several case studies of purported animal-inspired plant medicines used by cultures around the world are presented together with ethnomedicinal and pharmacological evidence. Based on this evidence the diversity and potential mode of self-medicative behaviors are considered. Over 20 animal species, including llama, sloth and jaguar in South America, reindeer and yak in Eurasia, langur and macaque in Asia, and chimpanzee, wild boar, porcupine and elephant in Africa, are linked to these case studies, representing a variety of potential preventative or therapeutic self-medicative behaviors. These examples provide an important perspective on what is likely to have been a much wider practice in the development of human traditional medicine. A role for animal self-medication research in the rejuvenation of old therapies and possible new discoveries of phytotherapies for human and livestock health is encouraged.

Conflict of Interest; Funding
The author has no conflict of interest and recieved no funding for this contribution.

VEW2 Phytotherapy in Dental Disease of Companion Animals

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DOI 10.1055/s-0041-1736795

Gingivitis and periodontal disease are common in cats in dogs. They are frequently diagnosed late in the course of the disease and are often undertreated. Phytotherapy – employing herbs or plant extracts can augment conventional dentacare and may provide opportunities for mitigating the pathogenesis of gingivitis and periodontal disease and optimising dental health. This update examines a selection of herbs including calendula and turmeric, outlining their proposed mechanisms of action and potential use in veterinary medicine.

VEW3 Ethnoveterinary uses of some Yemeni plants: A review of the scientific evidence

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DOI 10.1055/s-0041-1736796

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 literature review on their bioactivities and toxicological properties. Searching the scientific literature has revealed various pharmacological activities that may support the claimed healing activities of eleven (**Table 1**) out of fourteen plant species for some of their ethnoveterinary utilization. Moreover, three remedies were found to demonstrate toxic effects in experimental studies (**Table 1**). It can be concluded that our work has provided valuable scientific information on the biological and toxic activities of some Yemeni ethnoveterinary remedies that could be utilized for the benefit of farmers to rational the use of these remedies and avoiding their toxicity.

Conflict of Interest; Funding (Source, ID)
Statement on behalf of all authors. The authors declare not to have any conflict of interest.

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VEW4 Herbal Remedies for coccidiosis in poultry

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Coccidiosis is an economically important disease in the poultry industry. *Eimeria* species are protozoan parasites that infect the intestinal guts and cause symptoms and even death in poultry. Apart from anticoccidial chemicals and vaccines, plants and their compounds are emerging as an attractive alternative to control coccidiosis in poultry. In this presentation, we update and highlight recent advances in the functions and mechanisms of anticoccidial plants, including biological and chemical properties of the plants and compounds [1]. Emphasis is placed on the modes of action of the anticoccidial plants and compounds such as interference with the life cycle of *Eimeria*, regulation of host immunity to *Eimeria*, growth regulation of gut bacteria and/or multiple mechanisms. Biological actions, mechanisms and prophylactic/therapeutic potential of the compounds and extracts of plant origin in coccidiosis are summarized and discussed.

Finally, we include and discuss successful application of herbal remedies against coccidisois on the markets worldwide [2].

Conflict of Interest; Funding (Source, ID)
On behalf of all authors, we declare no conflict of interest except that Wen-Chin Yang is an co-inventor of a anticoccidial formulation, Rotam-CS. This work was financially supported by 110AS-22.1.2-L1(8), AS-KPQ-110-ITAR-14, and MOST 107-2321-B-001-038 -MY3 (Taiwan).

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VEW5 Obliquumol, a novel compound with patented activity against Candida albicans could also have useful applications in animal health

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In a search for extracts active acetone tree leaf extracts against Candida albicans of more than 500 tree species, Ptaeroxylon obliquum extracts had very good activity. The active novel compound named obliquumol was isolated. It had higher activity and cellular safety than the positive control amphotericin B and

represented a new framework for the potential development of antifungal compounds. The compound also had very good activity against two important animal fungal pathogens Aspergillus neoformans and Cryptococcus neoformans (MICs of 16 and 8 µg/mL) with a low cytotoxicity CC50 > 200 µg/mL against human liver (C3A) cells and Vero monkey kidney cells. It is known that the anthelminthic benzimidasoles are also active against fungi. We therefore decided to investigate the activity of obliquumol against Haemonchus contortus obtained from infected sheep against egg hatching and larval development. The compound had reasonably good activity in the larval development (LC50 0.22mg/mL) and egg hatching (LC50 0.095 mg/mL) assays. When we determined the acute toxicity in an animal experiment the compound was not toxic at the highest dose tested based on OECD guidelines. It therefore appears that obliquumol could also be useful as an antifungal and anthelmintic agent in protecting animals. Animal studies to investigate the in vivo activity appears to be a reasonable next step.

VEW6 Dietary Natural Extracts in Intensive Pig Farming: A Rational Alternative to Irrational Use of Antibiotics

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The incorporation of natural feed additives in intensive pig farming has acquired considerable relevance during the last years, as increasing restrictions to the use of antibiotics have been imposed around the world. Nevertheless, the use of antibiotic growth promoters and the prophylactic administration of oral broad spectrum antibiotics to large numbers of animals, at therapeutic doses, are still common practices in many countries. This irrational management has led to the development of antimicrobial resistance posing catastrophic animal health issues and contributing to the undergoing global antimicrobial resistance emergency. In search for rational alternatives to antibiotics, dietary natural extracts have aroused as tools to overcome challenging periods of the pigs' productive cycle. This is particularly important during the post weaning stage, when piglets are subjected to major stressful events that negatively affect the gastrointestinal tract at different levels. Essential oils (carvacrol, thymol, etc.) and other herbal extracts (Cynara, olive, yucca, etc.) have shown to increase nutrient digestibility, improve immune status, exert antioxidant and antiinflammatory effects, reduce urease activity and modulate intestinal ecosystem *in vivo* and *in vitro*. Moreover, improved performance parameters have been associated with the utilization of dietary plant-derived products. In some cases, the outcomes were similar to those obtained by antibiotics. Even if results are not always conclusive and there is a great deal of controversy, mostly due to the diversity of plant species and formulations evaluated, overall findings are very promising indeed. The increasing scientific research focused on natural products offers the pig sector an opportunity to incorporate a healthier and more responsible production system [1, 2].

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6. African Network Session

ANS1 Nutraceutical Potential of *Fadogia ancylantha*, Indigenous Knowledge and Intellectual Property Rights.

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Africa is endowed with a variety of floral biodiversity and unique indigenous knowledge systems regarding the utilization of plants among communities. However, Africa's contribution to global bioeconomy remains minimal, largely due to lack of investment in research and development (R&D), human capacity development and poor propriety regulations in most African countries. The objectives of this paper are three-fold: i) to present the phytoconstituents isolated from *Fadogia ancylantha* that have shown antidiabetic activities in C2C12 and Chang myocytes, ii) to examine the role of intellectual property rights in promoting products derived from natural resources and indigenous knowledge, and iii) to discuss barriers and enablers associated with phytomedicine research in Africa. Using bioassay-guided fractionation, bioactive compounds were isolated and characterized by various spectroscopic methods.

The polar active component provided four compounds: acetylchalcone, glucosidic chalcone, hydrocoumarin and glucosidic coumarin. Equimolar of pure compounds in C2C12 cells gave relative glucose uptakes of 151.06 ± 0.11, 143.88 ± 0.11, 170.47 ± 0.08 and 153.76 ± 0.09 %, respectively; which were statistically higher ($p < 0.05$) than 130.36 ± 0.06 and 137.47 ± 0.06 % obtained for insulin and metformin, respectively. These results were consistent with the traditional use of the plant. Except for the Nagoya Protocol on Genetic Resources, exploitation of innovations based on indigenous knowledge appear to face legal challenges when subjected to most trade agreements. Globally, IP refers to the claim of ownership, which does not necessarily apply to communities who act as custodians and keepers of the knowledge. Therefore, African countries need to be capacitated to ratify the Nagoya Protocol for improved economic benefits from biodiversity [1, 2].

Conflict of Interest Authors declare no conflict of interest.

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ANS2 Panel discussion: Key Challenges for Natural Products Research – Perspectives from African Scientists.

DOI 10.1055/s-0041-1736801

Africa has long since been troubled by the spreading of communicable diseases such as TB and HIV but has also in recent years become an epicentre for diseases such as cancer. Traditional knowledge of medicinal plants has been at the core of treatment for these diseases for many on the continent. The advancement of research into the true efficacy and related activity of the continent's indigenous flora has been lacking and many reasons for this have been brought to the forefront. During the panel discussion, experts in the field of natural products research will delve into topics such as intellectual property, bioprospecting and funding opportunities available for African researchers. The hope is that this discussion will highlight the problems faced by the African researchers of today but also try to bring forth solutions and a way forward. Many times, it has been said that scientific research is one of the main drivers for the economy and decisions needed to be taken by policymakers. The time

has come for Africa to come together and to undertake ground breaking scientific endeavours to understand and utilize the endless possibilities that exist in the soil and flora found on our continent.

7. Video Contributions

VC1 The flavonolignan silibinin from *Silybum marianum* targets hepatic lipid biosynthesis and elevates the hepatic biotransformation capacity

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DOI 10.1055/s-0041-1736802

Silibinin is considered as major active component of *Silybum marianum* (milk thistle) extracts and is traditionally used for the treatment of toxic liver damage and as unique therapy for intoxication with *Amanita phalloides*. However, the molecular mechanisms of silbinin remained enigmatic. Membrane-stabilizing properties of silibinin, modulation of the function of membrane proteins as well as effects on metabolism have been discussed since decades. Here, we show that silibinin and even more pronounced the milk thistle-containing phytopharmaceutical Silimarit® significantly increase the content of major phospholipid classes in human monocytes and hepatocellular carcinoma cells *in vitro* as well as in murine liver *in vivo*. The accumulation of phospholipids arises from intracellular membranes and could partially be ascribed to changes in the expression and activation of enzymes in fatty acid metabolism. The enrichment of hepatic phospholipids and expansion of intracellular membranes is associated with an increased expression of ER-localized metabolizing enzymes and elevated biotransformation capacity of mouse liver. Taken together, we provide a link between hepatic fatty acid/phospholipid biosynthesis and biotransformation, which might contribute to the liver-protective properties of silibinin related to intoxication as well as metabolic diseases such as steatosis and cirrhosis.

Conflict of Interest; Funding (Source, ID) The study was supported by Bionorica SE

VC3 SFx – How fast and robust can a rose root analysis be?

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Supercritical fluid (SFx) techniques have been recently introduced for the extraction and separation of natural compounds of a wide polarity range [1]. The advantages of these methods are unique: they are ecofriendly and allow for a targeted extraction and separation by adaption of the polarity using different co-solvents. Since centuries, extracts of the dried roots and rhizomes of *Rhodiola rosea* L. (rose root) are used for their adaptogenic and health beneficial properties. For the quality assessment of herbal products, a robust and precise analytical method is a prerequisite. The analysis and quantitation of key secondary metabolites of rose root is particularly crucial due to the heterogeneity of the used plant material.With the aim to establish and validate a fast and robust SFx workflow for rose root samples, seven diverse secondary metabolites have been successfully extracted and quantified within this study. Eight commercial *R. rosea* preparations and 24 herbal drugs have been extracted using 60 % CO_2 and 40 % methanol. The SFE extracts were separated on an UPC² instrument in a remarkable short time below 3.5 minutes. The robustness is underlined by good results in terms of linearity, accuracy and precision on a QDa mass detector. The investigated herbal drugs showed a high heterogeneity concerning their quantitative composition. The fact that only one of the commercial products met the declared content of rosavins and salidroside, further highlights the need for a reliable method to assess the quality of *R. rosea* [2].

Conflict of Interest The authors declare no conflict of interest.
Funding NATVANTAGE Grant 2018 (Wilhelm Doerenkamp Foundation), FWF Project P 34028.

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VC4 Celastrol: a natural compound with antibacterial properties. Mode of action against Staphylococcus epidermidis

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DOI 10.1055/s-0041-1736804

The methylene quinone celastrol is a natural compound present in plant species of the Celastraceae family that has shown antibacterial properties against *Staphylococcus epidermidis*[1]. This Coagulase-negative Staphylococci (CoNS) is commonly found as a skin commensal and is considered the primary cause of CoNS-related infections, particularly in nosocomial settings [2]. This work presents a first approach to elucidate the mechanism of action of celastrol on *S. epidermidis*. Exposure of *S. epidermidis* cultures to celastrol revealed a bactericidal effect that was positively associated with drug concentration, inversely related to the size of the bacterial inoculum, and conditioned by the chemical composition of the growing media. The electron micrographs, Back-light test, and the measurement of oxygen consumption did not show notable damage to external structures such as the cell wall or the plasma membrane, although treated cultures showed alterations in the formation of septa during cell division. The evaluation of the macromolecular synthesis, and the uptake of its precursors showed that celastrol at 15 µg/ml had the most severe effect on peptidoglycan synthesis by inhibiting the incorporation and uptake of *N*-Acetylglucosamine by > 50 % in 2 and 10 minutes, respectively. Glucose uptake was also immediately inhibited by celastrol by > 60 % within 2 minutes. These data suggest that celastrol could primarily target cell wall synthesis and compromise cell division. This study raises a first hypothesis about the effect of celastrol against *S. epidermidis*, although additional evaluations are necessary to obtain a more precise understanding of its mechanism of action.

Funding
This study was supported by RTI2018-094356-B-C21 Spanish MINECO co-funded by the European Regional Development Fund (FEDER) projects.

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VC5 Biocompatible electrospun CA and PHB wound dressings containing Alkannins & Shikonins

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In order to develop a potential dermal medical device for effectively treating skin wounds, we advanced our research by focusing on two biomaterials – cel-

lulose acetate (CA) and polyhydroxybutyrate (PHB) – in which we incorporated a mixture of the established wound healing molecules, alkannin and shikonin (A/S) and their derivatives. We present here the biological assessment of the electrospun CA and PHB fiber mats loaded with the A/S mixture.

Neat and drug-loaded scaffolds were fabricated by electrospinning. Circular discs of the prepared samples were cultured for 3 and 7 days, upon seeding Hs27 fibroblast cells on top of the scaffolds. Cytocompatibility was evaluated in terms of cell adhesion, morphology and viability. Additionally, the antibacterial activity was tested against staphylococci.

CA and PHB scaffolds containing low drug contents of the A/S mixture (< 1 wt. %) favored the adhesion, spreading and proliferation of fibroblasts, while the attached cells on the scaffolds surface showed an increased viability. Regarding the antibacterial assay, samples with high drug loadings (> 1 wt. %) managed to inhibit the growth of bacteria around the edges of the scaffolds. Further, SAR studies of the A/S mixture constituents were performed.

The findings from the biological assessment of the A/S-loaded scaffolds highlight their advantages as potential wound dressings.

Funding

This work was supported by the project 'MICROMETABOLITE' funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie No 721365.

VC6 Balms of selected species of Pinaceae and their influence on keratinocyte re-epithelialization *in vitro*

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DOI 10.1055/s-0041-1736806

Exudates of Pinaceae are used in traditional medicine to treat wounds [1, 2]. The balms of Picea abies, Larix decidua and Pinus nigra were tested in a HaCaT keratinocyte based in vitro assay for their potential to boost re-epithelialization as measure for wound closure. As positive control served the pure compound lysophosphatidic acid (LPA, 10 µM) and extracts of several medicinal plants well known for their wound healing properties (birch, marigold, St. John's wort, manuka honey). The cytotoxicity of the extracts was estimated by evaluating the metabolic activity and cell mass via the resazurin and crystal violet assay. Re-epithelialization was determined by disrupting a keratinocyte monolayer to simulate an injury. The cell free area was monitored after 24h exposure of three different sample concentrations (1, 3 and 10 µg/mL). Picea abies and Pinus nigra showed dose dependency, significant activity was measured for Larix decidua at 3 µg/mL.

Qualitative comparison of the three Pinaceae exudates is shown presenting TLC, LC-UV/DAD-MS and SFC-MS as suitable tools for quality assessment of these medicinally used plant products.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflicts of interest. This work was supported by the Austrian Federal Forest by supplying the crude plant material of Picea abies and by the Vienna Anniversary Foundation for Higher Education [grant number H-246128/2016].

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VC7 Nutraceutical properties and health benefits of bioactive molecules derived from *Spirulina* enriched functional food

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Immunomodulation, angiotensin converting enzyme inhibition, anti-oxidative effects are just a few examples of the different biological activities suggested for metabolites from *Arthrospira platensis*, better known as '*Spirulina platensis*'[1, 2]. Therefore, this micro-alga in considered a super-food. The aim of our study was to characterize different foods enriched with *Spirulina* (i.e. sorbets and pasta) in terms of amount of alga actually incorporated and of its effective bioavailability, since the efficacy of such products as a source of bioactive compounds is strictly dependent on these parameters. The first step was the study of the *Spirulina* matrix in order to identify the most relevant metabolites. Therefore, we proceeded developing an extraction method suitable to obtain a representative extract, which was subjected to a liquid chromatography separation coupled with high resolution mass spectrometry analysis (hrLC-MS/MS). A number of compounds, covering a large chemical space were identified (i.e. atty acids (linoleic and gamma linoleic acid), pheoforbides and derivates and monogalactosil monoacylglycerol and derivates) to which different health benefits have been attributed [3]. We also identified proteins and peptides playing an important role in beneficial effects on human health; in fact, the amino acid composition suggests the existence of significant peptides encrypted within the sequences of parent proteins -mainly phycocianins [4]. Most of the active metabolites and peptides were detected also in the analyzed functional foods, thus confirming their suitability as nutraceutics. However, some interesting differences emerged from our analyses, which should be taken into account to generate more effective healthy products.

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VC8 Gaseous methyl jasmonate can increase hypericins content in Hypericum perforatum aseptic plants

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Hypericum perforatum is an important medicinal plant which is traditionally used to treat diseases of the digestive tract. Recently its ability to improve mood and fight depression was shown. This effect is caused mainly by hypericins and hyperforin, the substances with a wide range of biological activities. The aim of this study was to investigate the effect of methyl jasmonate on the production of hypericins, using the elicitor in a gaseous form. This is a natural way of methyl jasmonate transfer from plant to plant but such form of the elicitor application is underestimated in biotechnological practice.

Aseptic plants of H.perforatum were used. Methyl jasmonate treatment was performed using 4 elicitor concentrations. The plants were cultivated for 3 days, after that the shoots were harvested, lyophilized, extracted with ethanol, and hypericins content was determined by HPLC.

It follows from the obtained data that airborne methyl jasmonate can significantly increase the content of hypericins in the shoots of St. John's wort. Thus, the addition of 2 µl methyl jasmonate diluted in ethanol in a ratio of 1: 10000 can increase the content of hypericins in the shoots up to 50 %, diluted in a ratio of 1: 1000000 - by 30 %. Undiluted methyl jasmonate slightly reduced the content of the studied substances.

It can be assumed that such a simple and convenient way of treating in vitro cultivated plants with methyl jasmonate may be highly suitable for increasing the content of hypericins as well as other biologically active secondary metabolites

Conflicts of Interest; Funding (Source, ID) Statement on behalf of all authors. The authors declare they have no conflicts of interest

VC9 Barcode High Resolution Melting (Bar-HRM) analysis for authentication of *Echinacea* products

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DOI 10.1055/s-0041-1736809

Echinacea purpurea products are among the top-selling herbal products and used for reducing many symptoms of cold, flu and some other conditions. The market for herbal products has increased rapidly over the last few decades, but this has, in turn, increased the opportunities for malpractices such as contamination or substitution of products with alternative plant species. A study into *Echinacea* herbal products showed that 11 % had no *Echinacea* present [1]. DNA barcoding is a technique used for authentication of herbal medicines due to its potential as a highly specific and cost-effective tool, but as of yet there is no a validated standard procedure for *E. purpurea* authentication, and this project aims to fill this gap.

The full chloroplast genomes of the nine Echinacea species present on the NCBI database have been studied to find differences and potential regions for primers design to be used for Barcode High Resolution Melting (Bar-HRM) analysis. Six out of nine *Echinacea* species have been sourced and grown from seeds to test the specificity of the primers. The newly designed primers have been tested against DNA extracted from these plants and against over the counter *Echinacea* products.

The results showed that Bar-HRM assay could be used to identify *Echinacea* at a species level. It also proved the potential for these techniques to be used in not just pure samples but also in processed samples with multiple plant components.

No conflict of Interest; this project was supported by De Montfort University HEIF funds

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VC10 Damiana for the treatment of female sexual interest/arousal disorder (FSIAD) – Results of a multicentre non-interventional study

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Damiana (*Turnera diffusa*) is a widely used aphrodisiac with more than 300 years of application experience [1]. In Germany, Damiana extracts are the only pharmaceutical therapy option for Female Sexual Interest/Arousal Disorder (FSIAD; ICD-10 Code: F52.22). However, there is still lack of clinical data regarding Damianas use in FSIAD.

This multicenter non-interventional study was conducted to gain further insights into the effectiveness and tolerability of a herbal medicinal product, containing Damiana leaf dry extract, in the treatment of FSIAD. 70 patients with FSIAD (according to diagnosis criteria A and B of the DSM-5 [2]), were enrolled by six privately practicing gynecologists. The application period was eight weeks, followed by a four-week follow-up period.

Patients who finished the study (n = 35, 46.1 ± 10.9 years) showed a significant increase in Female Sexual Function Index (FSFI-d, $p < 0.01$). The score of the Female Sexual Distress Scale – Revised (FSDS-R) decreased significantly ($p < 0.01$) while quality of life (measured by Münchner Lebensqualitäts-Dimensionen Liste (MLDL)) tended to increase.

We were able to show, that in patients with FSIAD, the Damiana extract contributes to the individual symptoms as well as to the experienced mental distress, respectively.

Conflict of Interest; Funding (Source, ID)

This study was conducted with libiLoges® and sponsored by Dr. Loges + Co. GmbH, Winsen/Luhe, Germany.

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8. Poster Contributions

8.1 One Health, access and benefit sharing

PC1-1 Unlocking Nature's Pharmacy - the story of an Irish Bog Lichen

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DOI 10.1055/s-0041-1736811

Ireland is home to almost 250 recorded lichen species [1] including 57 species of genus *Cladonia* [2]. Irish Bogs harbour *Cladonia portentosa* as one of the most abundant lichen, located on the west coast and acidic boglands. A lichen is a symbiotic combination of photobiont algae and mycobiont fungi. Algal part undergoes primary production (photosynthesis) to produce nutrients, that are taken up by fungi to produce a class of secondary metabolites called lichenic acids. Lichenic acids exhibit a broad-spectrum bioactivity and their composition and proportion varies with maturity and micro- and macro-environments of lichens viz. geographical locations, seasons and nutrient availability.

Samples of *C. portentosa* were collected from six different locations on Irish boglands and were subjected to three different extraction methods (soxhlet, ultra-sonication and maceration) using methanol as solvent, to yield 18 extracts. Chemical profiling of these extracts was performed by TLC, HPLC and NMR. Samples were prepared in duplicate for NMR measurements, and data was analysed using a metabolomic approach to study the variation of metabolites in same species from different locations. Soxhlet extraction proved to be the most efficient method of extraction, giving the highest yields and extracting greater number of metabolites as revealed by TLC and HPLC.

results. Metabolomic analysis of NMR data differentiate the metabolite profiles of *C portentosa* from different locations. The structures of the principal metabolites common to all the *C. portentosa* samples from all regions, will be reported.

Conflict of Interest; Funding (Source, ID)

No conflict of interest. Funding: Unlocking nature's Pharmacy from bogland species. Philanthropy.

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8.2 Animal Healthcare and Veterinary Phytotherapy: Science and Practice

PC2-2 Antiviral Medicinal Plants of Veterinary Importance (A Literature Review)

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Today, there is an ever-increasing need for substances with antiviral effects since specific therapies against viral infections often appear unsatisfying and limited. Treatment with antiviral drugs often leads to the formation of new genetic virus mutations, which are resistant to drug therapy. Hence, medicinal plants with their diversified secondary compounds provide a promising alternative solution. Plant substances that are effective against animal viruses have been the subject of intensive research over the last few decades. The objective of this systematic review was to survey plant species with activities against viruses causing serious animal diseases, particularly those with high mortality rate or substantial economic losses. The methods were based on the recommendations of the PRISMA statement and the AMSTAR measurement tool. The kind of extract, fraction, or compound exhibiting the antiviral activity of the plants and the design of the trial were particularly considered. In total, 130 plant species that exhibit antiviral activity on 37 different virus species of the following families – *Herpesviridae, Orthomyxoviridae, Paramyxoviridae, Parvoviridae, Poxviridae, Nimaviridae, Coronaviridae, Reoviridae,* and *Rhabdoviridae* were compiled in this systematic review. These species cause infections, among others, in poultry, cattle, pigs, horses, shrimps, and fish. Overall, 30 plant species exhibited activity against various influenza viruses, and 30 plant species were noted to be active against the Newcastle disease virus. In addition, several plant species were effective against the bovine herpes virus and the fowlpox virus. Some of these plant compounds are promising candidates for the development of new antiviral remedies, which are urgently required.

PC2-3 *In vitro* effects of a phytogenic feed additive in a co-culture model of the piglet gut

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Animal health, welfare, and performance are essential concerns of the livestock industry, influenced by a multitude of complex factors. For example, an intact gut barrier is essential for animal gut health and correlates directly with systemic and local health issues. Phytogenic feed additives may play an essential role in positively boosting animal gut health. Recently, a novel *in vitro* co-culture model of the piglet gut [1] to assess gut barrier integrity has been described and used to evaluate the effects of phytogenic essential oil mixtures such as oregano oil. Intestinal porcine epithelial cells (IPEC-J2) and peripheral blood mononuclear cells (PBMC) are co-cultivated, as a model for apical and basolateral sides of the porcine intestinal epithelium. PBMCs were stimulated with 1.25 µg/mL Concanavalin A (ConA) to simulate an inflammation response *in vitro*, indicated by decreased transepithelial electrical resistance (TEER) that is measured using chopstick electrodes. In this trial, a phytogenic feed additive (BIOMIN Holding GmbH) including carvacrol, menthol, and methyl salicylate, was applied to the test system at 150 µg/mL. Effects on barrier integrity were evaluated in the absence or presence of the challenge stimulus over 48 hours. Basolateral addition of the phytogenic feed additive to the test system mitigated the effects of the applied ConA stimulus and increased TEER by 12.4 %, compared with the stimulated control ($n = 4$, $P < 0.05$). This indicates positive effects of the phytogenic feed additive on intestinal epithelial barrier integrity *in vitro* in the presence of an inflammatory stimulus.

Conflict of Interest / Funding
The authors declare no conflict of interest. Funding by the Austrian Research Promotion Agency (FFG) is gratefully acknowledged

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PC2-4 Efficacy of Norway spruce ointments and bacterial and fungal alterations in the treatment of castration wounds in piglets

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DOI 10.1055/s-0041-1736814

Our study aimed to evaluate the efficacy of Norway spruce ointments on wound healing of castration wounds in piglets. We included 95 pigs and randomly divided them into five treatment groups: Norway spruce balm (Vulpuran), Norway spruce resin (Abilar), pork lard (ointment base of Vulpuran), no treatment (negative control) and antibiotic blue spray (Cyclo spray, positive control). Wound healing parameters (healing time, wound size, reddening of wound edges and surrounding, swelling, secretion and wound contamination), microbiological status and haptoglobin level were investigated. Some positive effects on wound healing parameters were found in the Norway spruce groups. Vulpuran treatment led to significantly lower wound secretion than the untreated control did ($p = 0.019$). After the study, the highest rates of completely closed wounds were found in the Norway spruce groups (Abilar 44.8 %, Vulpuran 34.2 %) compared with the rates in the control (31.6 %), blue spray (23.7 %) and lard (15.8 %) groups. Bacterial isolates at the family level belonged to *Staphylococcaceae, Corynebacteriaceae, Enterobacteriaceae, Streptococcaceae*, *Aerococcaceae* and *Morganellaceae*. More than half of the fungal colonies were classified into genus *Candida* (67 %). A comparison of the five treatment groups on day 3 revealed that Norway spruce led to the lowest rate of wounds colonised with fungi (Vulpuran 70 %, Abilar 77 %) in comparison with blue spray (88.9 %), lard (100 %) and no treatment (100 %). Fungi could only be detected in one of the 13 samples treated with Vulpuran on day 8, which nearly reached significance ($p = 0.055$).

Conflict of Interest; Funding (Source, ID)
Statement on behalf of all authors. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

PC2-5 Effect of encapsulated grape dry extract supplementation on the immunity of young ruminants during a vaccination period.

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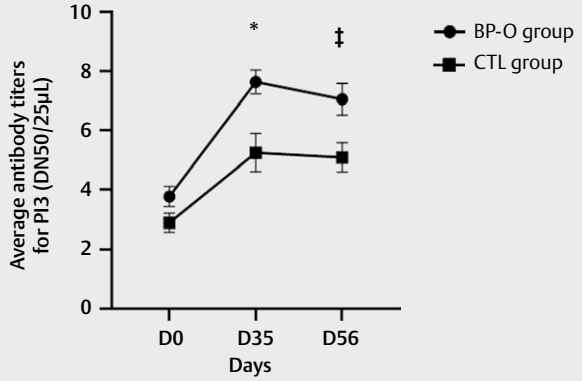
Vaccination is an important mainstay of biosecurity and disease prevention in breeding. Vaccination failures represent an economic burden for the farmer. Polyphenol supplementation, known for its antioxidant properties, would help reduce oxidative damage and improve the success of vaccination [1]. We eval-

uated the effect of an encapsulated dry grape extract supplementation around vaccination on the immune response in young ruminants.
22 young female cattle (aged 6 to 8 months), born in the same dairy farm, were randomly divided into 2 groups. One group (BP-O, n = 11) was supplemented with an encapsulated grape dry extract (Nor-Grape® BP-O, Nor-Feed, France), whilst a control group (CTL, n = 11) was not. All animals were vaccinated (D14) with an inactivated vaccine against PI-3V and BRSV. A booster was given 3 weeks later (D35). Supplementation began 15 days before vaccination (D0) and ended 15 days after the last injection (D49).
Antibody titers were measured by virus neutralization test. Total antioxidant capacity was measured by the TEAC method. These measurements were performed on D0, D35 and D56.
Results show that BP-O group had a greater overall antibody response to BRSV and PI3 ($P < 0.05$ at D35), and a greater total antioxidant capacity ($P < 0.05$ at D56) compared with the CTL group.



▶ **Fig. 1** Comparison of PI3 neutralizing antibody titers between groups after vaccination.

* $P < 0.05$; ‡ $0.05 \leq P \leq 0.10$

Hence, this supplementation strategy could be of interest to enhance the immune response during a vaccination episode by reducing oxidative stress. Supplemented animals became seropositive more quickly and long-term immunity appears to be improved.

Financial support for this research was provided by Nor-Feed.

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PC2-6 Alpine Sow-Thistle Against Nematodes: A Dual Approach to Anthelmintic Discovery

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DOI 10.1055/s-0041-1736816

The nematode *Ascaridia galli* (AG) is a notable parasite afflicting chicken livestock [1]. As anthelmintic (AH) resistance occurs among established drugs, plant natural products are explored as alternative treatments.
In course of the EUREGIO project 'HERBAL', ten alpine grown plants were selected based on ethnopharmacological use. Among them, *Cicerbita alpina* was the most active and consequently submitted to an activity-guided isolation procedure. For activity testing, an *in vitro* AG embryo development (ED) assay was utilized, showing that at 0.5 mg/ml, polar fractions of CA had an effect with 42 % and 43 % ED respectively. Additionally, an IR-Based worm motility assay with *Caenorhabditis elegans* offered another life-cycle stage for screening. Active fractions were further separated via FCC and SEC with compounds postulated via MS/NMR. Furthermore, pharmacophore models were developed for the known AH β-tubulin target. Using bioassay-guided isolation and *in silico* methods, this dual approach searches for natural active principles against nematodes.
EUREGIO (Interregional Project Networks) (IPN 119) 'HERBAL'. The authors declare no conflict of interest.

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PC2-7 *Melissa officinalis* as gut contractility modifier in swine – *ex vivo* study

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DOI 10.1055/s-0041-1736817

Melissa officinalis has a long history of being used in traditional medicine for the treatment of gastrointestinal tract disorders and in reducing stress. This study aimed to develop knowledge about *Melissa* extract and its main active substances in regards to swine intestine contractility.Materials and methods: The experiments were conducted on circular and longitudinal colon samples collected from routinely slaughtered pigs. The effect of standardized *Melissa officinalis* extract (Nor-Balm®) [1], rosmarinic and lithospermic acids on spontaneous and ACh-induced activity was evaluated under isometric conditions [2].
Results The results revealed significant and dose-dependent potency of *Melissa* extract to decrease the magnitude of acetylcholine-induced contraction. The impact was slightly stronger on longitudinal than circular colon smooth muscle. Besides, the extract enhanced spontaneous contractility in longitudinal muscle layer. In case of rosmarinic and lithospermic acids the spontaneous colon motility was dose-dependently increased. Rosmarinic acid inhibited remarkably the contraction induced by ACh in both muscle types, whereas litospermic caused increased and decreased response to ACh in circular and longitudinal colon muscles, respectively.
Conclusions The results of the performed study indicate that *Melissa officinalis* can be used to control gastrointestinal motility. Its ability to limit the size of ACh-induced contractility might be beneficial in hypermotility disorders in pigs. Moreover, it seems that rosmarinic acid contributes largely to the final effect of the plant. The final effect of Melissa extract is not the sum of tested acids activity what proves more complex interaction and probable contribution of other phytoconstituents.
The authors declare no conflict of interest. Sorphon Suor-Cherer and Mohammed el Amine Benarbia work in the R&D department of Nor-Feed.
The study was financed by SGGW and NF.
Statement on behalf of all authors. I agree to publish the abstract in an issue of Planta Medica

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PC2-8 Bioassay guided fractionation of Senna singueana and its potential for development of poultry phytogenic feed additives

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DOI 10.1055/s-0041-1736818

Plant based products are potential alternatives to in-feed antibiotics. The aim of this study was to evaluate antibacterial, anti-lipoxygenase and antioxidant activity, and cytotoxicity of *Senna singueana* fractions and isolated compounds. Solvent-solvent partitioning of the methanol leaf extract of *S. singueana* was followed by column chromatography with the ethyl acetate fraction. Antibacterial efficacy was determined against poultry pathogens including *Escherichia coli, Staphylococcus aureus* and *Salmonella enteritidis* using a two-fold serial microdilution assay. Anti-lipoxygenase activity was evaluated using the ferrous oxidation-xylenol orange (FOX) method. Antioxidant activity was assessed using radical scavenging assays. Fractions had significant to weak antibacterial activity with MIC values ranging from 50 – 938 µg/ml.

The ethyl acetate fraction and the seven subsequent column fractions had potent anti-lipoxygenase activity with IC_{50} values of ≤ 2.5 µg/ml, lower than that of quercetin (positive control). The ethyl acetate fraction and some sub-fractions had powerful antioxidant activity with IC_{50} values of ≤ 5 µg/ml in the ABTS assay. Bioassay-guided isolation led to isolation of a bioactive compound, luteolin.

Based on the good antibacterial activity, potent antioxidant and anti-inflammatory activity as well as low cytotoxicity of its fractions, *S. singueana* is a promising candidate for the development of poultry phytogenic feed additives.

Conflict of Interest; Funding

The authors declare no conflict of interest. The National Research Foundation (NRF, South Africa) is acknowledged for research funding (UID 111945 to LJM).

PC2-9 Effect of a phytogenic feed additive on survival and performance of shrimps receiving a fish meal reduced diet

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Nutrient sparing concepts in aquatic diets are recognized as reasonable approach to support sustainable and efficient use of marine resources and thereby contribute against overfishing. Simultaneously, the reduction in marine protein source (MPS) often causes reduced health status and thus increased mortality of animals grown in aquaculture. The objective of the current study was to investigate the impact of a phytogenic feed additive (PFA) on shrimp performance in a nutrient sparing concept over a period of 57 days. A total of 240 white-leg shrimps *Litopenaeus vannamei* (1.5 – 2.0 g body weight (BW)) were randomly allocated to 12 tanks (RAS system with appropriate water quality parameters) which were divided into two experimental groups (n = 6). Both groups received a diet based on soybean meal, wheat and corn, including 15 % MPS. One group additionally received 0.2 g/kg feed of the PFA, an encapsulated blend of essential oils with thymol as major component. Shrimp health was monitored through observation and water quality parameters were recorded daily. BW as well as feed consumption were determined at day 0, 28 and 57. Hepatopancreas weight was measured at trial end. Statistical analysis comprised Student's t-test and Kolmogorov-Smirnov test. Over the entire trial period, the PFA group showed increased biomass ($p < 0.001$) and weight gain per tank ($p < 0.001$), survival ($p = 0.004$) and decreased feed conversion ratio (FCR, $p = 0.001$). Especially the improved FCR demonstrates how PFAs can promote a better efficacy in nutrient utilization which ultimately yields an improved health status, increased survival whilst contributing to improved sustainability through reduced effluents.

PC2-10 Ethnoveterinary use of medicinal plants for treatment of dogs – a survey in Bavaria

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DOI 10.1055/s-0041-1736820

Interest in the use of medicinal plants in small animal veterinary practice is growing increasingly in German-speaking countries [1]. In 2018 and 2019, an ethnoveterinary study was conducted on Bavarian farms. This included the documentation of medicinal plant applications for all animals living on the farm, also dogs.

A total of 77 interviews were carried out of which 28 interview partners kept dogs. Altogether 884 use reports (URs) were recorded, comprising detailed information about plant species, plant part used, the manufacturing process for the end-product, dosing, administration, and therapeutic intention. Six interview partners reported 17 URs with 14 different plant species specifically linked to the treatment of dogs.

We were able to determine a daily dosage in dry plant equivalent per kg metabolic body weight ($g/kg^{0.75}$) for each of the following five plant species: *Artemisia abrotanum* L. (herb) 0.4; *Elymus repens* L. (root) 1.8; *Ricinus communis* L. (oil) 7.1; *Rubus idaeus* L.(leaves) 0.3 and *Sambucus nigra* L. (flowers) 0.2.

Rubus ideaeus L. was given to the female dogs during lactation, whereas the other four plant species were used in cases of gastrointestinal disorders and parasites. Instead of castor oil more effective laxatives with far fewer side effects are recommended for constipation nowadays [2]. The other four species are well known medicinal plants in humans and animals. However, clinical studies in dogs are rare.

Conflict of Interest; Funding (Source, ID)

The authors declare that they have no competing interests.

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PC2-11 Anthelmintic potential of Phyllanthus urinaria L. (Phyllanthaceae) in the model organism Caenorhabditis elegans.

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DOI 10.1055/s-0041-1736821

Soil-transmitted helminthiases continue to pose great economic burden on human and animal health. The emergence and spread of resistant parasites coupled with fewer, unavailable or high-cost drugs make the control of parasitic worms challenging. The need for new and effective anthelmintic agents is

therefore critical, if the 2030 targets for this neglected tropical disease are to be attained [1]. Tanniferous plants are recognized as potential sources of active metabolites against helminthiases. Unlike condensed tannins (proanthocyanidins) whose anthelmintic activity is widely studied, little is known about the hydrolysable tannins [2]. The goal of this study is to investigate the constituents of *Phyllanthus urinaria* L. (Phyllanthaceae), an anthelmintic plant from Ghana, which contains mainly ellagitannins [3]. Aqueous acetone (70 %v/v) extract of the whole plant and its water and n-hexane fractions were tested in the model organism *Caenorhabditis elegans* (L4 larvae). The crude extract produced moderate activity (IC_{50} of 2.6 ± 0.4 mg/ml) after 72 hours of incubation. Whereas the n-hexane fraction showed no significant activity, the water fraction was active (IC_{50} of 3.0 ± 0.6 mg/ml). This was fractionated on Sephadex LH-20 gel (Methanol : water 1:9 to 1:0) and further purified by chromatography on MCI gel®, preparative HPLC and RP 18 flash column to yield gallic acid (105 mg), ellagitannins including geraniin (302 mg), chebulagic acid (112 mg), corilagin (235 mg), repandusinic acid A (35 mg), and the flavonoid, rutin (22 mg). Investigations are currently ongoing to ascertain the activity of these compounds in both *C. elegans* and animal intestinal parasites.

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PC2-12 Some scientific rationale for the use of the bark of *Ptaeroxylon obliquum* (Rutaceae) as anthrax remedy in South African Ethnoveterinary medicine

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DOI 10.1055/s-0041-1736822

Asides its use as a biological weapon, anthrax causes high morbidity and mortality in domestic and wildlife animal populations globally resulting in great economic loses. Anthrax-infected humans presents with mild skin or respiratory symptoms highlighting its zoonotic potential. The bark of Ptaeroxylon obliquum (Rutaceae) is used in South African Ethnoveterinary medicine as a remedy against anthrax. Quest for empirical evidences as basis for indigenous use of the plant motivated the determination of its activity against Bacillus anthracis, the aetiology of anthrax. Different Ptaeroxylon obliquum bark extracts were prepared and their minimum inhibitory concentration (MIC) were tested against vegetative and sporulated forms of B. anthracis Sterne vaccine strain using a 2-fold microdilution test while cytotoxicity of extracts was tested against Vero kidney cells using the MTT assay. Possible active compounds in the acetone crude extract were detected using GC-MS. The extracts had excellent activity with MICs ranging from 4,88 – 19,53 µg/mL for both forms with moderate to low cytoxicity. Moreover, the extracts had excellent selectivity index against the bacteria with the methanol extract being the best (12) against B. anthracis sporulated form. This indicates their selectivity for toxicity against the B. anthracis than against normal cells. The acetone extract houses important bioactive compounds such as neophytadiene and resorcinol that may be partly responsible for the observed activity. The study provided some scientific rationale to the use of P. obliquum in traditional medicine. The extracts may hold potential as decontaminants and as topical remedies against disease symptoms in humans.

Conflict of Interest; Funding (Source, ID)

National Research Foundation (NRF, South Africa, grant 105993 to LJM) and Medical Research Council (SIR to LJM) are acknowledged for research funding. The University of Pretoria funded the postdoctoral study of IMF.

PC2-13 Antimicrobial in vitro activity of eleven essential oils against Pasteurella multocida isolated from respiratory tract of calves

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DOI 10.1055/s-0041-1736823

Essential oils (EOs) as natural plant products with antimicrobial activity might be an alternative to antibiotic treatment of *Pasteurella multocida (PM)* in bovine respiratory disease (BRD). The aim of this study was analyses of the *in vitro* antimicrobial activity of eleven EOs against *PM* isolated from respiratory tract of calves. Bacteria (n = 10) were sampled within the "outdoor veal calf" project [1] and agar disc diffusion (ADD) with determination of inhibition zone radius (IZR) and microdilution with determination of minmimum inhibitory and bactericidal concentration (MIC, MBC) was performed. Cinnamon cassia and lemongrass oil showed strongest activity (MIC 0.0078 %, 0.0313 %, IZR 22.25 mm, 30.12 mm) followed by coriander, winter savory, thyme, clove, and peppermint oil (MIC 0.125 – 0.25 %, IZR 18.5 – 11.25 mm). Eucalyptus, spruce, and star anise oil had the weakest activity (MIC 0.5 – ≥ 1.0 %, IZR 7.5 - 5.0 mm). ADD leads to an underestimation of cinnamon cassia activity and gives conflicting results for wintergreen oil (MIC 0.5 %, IZR 14.5 mm). The MBC equaled the MIC for thyme, winter savory, and eucalyptus oil, but was one two-fold dilution above the MIC in 10 - 67 % of cases for most other EOs. In conclusion, especially cinnamon cassia and lemongrass, but also coriander, winter savory, and thyme oil are promising candidates for treatment of PM infections in BRD.

Conflict of Interest; Funding

P. Mayer, L. Nau and V. Becher are employees of SaluVet GmbH. Funding by SaluVet GmbH and Laboklin GmbH & Co. KG.

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PC2-14 Evaluation of the metabolic and vascular effects of a nutritional mixture of Berberine – Citrus – Apple extracts in a rat model of experimental metabolic syndrome

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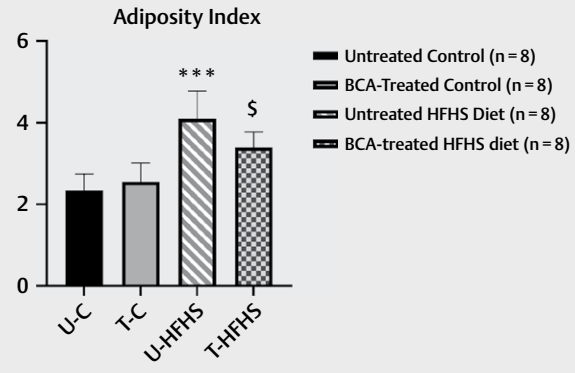
DOI 10.1055/s-0041-1736824

Nutraceuticals help prevent various metabolic disorders such as those related to metabolic syndrome (MetS). The latter is generally caused by a sedentary lifestyle and the consumption of ultra-processed products [1, 2]. Here, we evaluated the beneficial effects of a nutritional mixture based on Berberine and Citrus (Citrus pericarp-35 % bioflavonoids-aqueous extraction) and Apple (phloridzin-5 %-hydro-ethanolic extraction) (BCA) extracts in a rat model of MetS.

Thirty-two 8-week-old Wistar rats were randomly divided into 4 groups: untreated control (U-C;n = 8), BCA-treated control (T-C;n = 8), untreated rats fed with High Fat High Sugar Diet (HFHS) (U-HFHS;n = 8) and BCA-treated rats fed with HFHS diet (T-HFHS;n = 8). All rats received daily water or BCA mixture (100mg/kg po) for 13 weeks. Morphological and metabolic parameters were measured at the beginning and the end of the treatment. Concentration-relaxation curves to insulin and acetylcholine were also determined on isolated thoracic aorta.

Compared with the control group, rats fed with HFHS showed an increase in body weight, abdominal circumference, and adiposity index calculated from perirenal and peri-epididymal fat ($P < 0.001$). The HFHS diet induced atrophy

of the ceacum (P < 0.001). The BCA treatment did not modify weight gain or food intake but significantly decreased visceral fat weight (Figure 1) and reduced ceacal atrophy (P < 0.01) compared to the HFHS untreated group. Relaxing effects to acetylcholine showed no difference between the four groups. By contrast, in the HFHS-fed group, insulin-mediated relaxation was slightly impaired and this impairment was counteracted by BCA treatment.



▶ **Fig. 1** Effect of BCA mixture on adiposity index in control rats and rats fed with HFHS diet. Values are expressed as mean ± SEM
***: P<0.001 vs. U-, $: P<0.05 vs. U-HFHS.

We found that the BCA nutritional mixture improved insulin-mediated relaxation and decreased visceral adiposity likely through energy intake-independent mechanisms that require further investigation.

Financial support for this research was provided by La Région Pays de la Loire.

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PC2-15 Antioxidant and anti-inflammatory effects of the association of an apple extract with dihydroberberine: study in isolated rat aorta and in canine whole blood culture

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DOI 10.1055/s-0041-1736825

The high prevalence of overweight in pets led to consider news strategies to address all the obesity-associated comorbidities, especially to reduce oxidative stress and inflammation. Some plant-derived molecules appear to be good candidates for this purpose. Apple and berberine have already individually demonstrated antioxidant and anti-inflammatory properties *in vitro*[1, 2] and *in vivo*[3] in human and mouse. In this study, we evaluated their combined effect on *ex vivo* models using lipopolysaccharide (LPS) of *E. Coli* to mimic oxidative and inflammation processes characterizing obesity. Different concentrations of dihydroberberine and apple extract (phloridzin-5 %-hydro-ethanolic extraction) were first tested to select those actually effective, using a DPPH assay. Then we evaluated whether the effective antioxidant concentrations were able to prevent LPS-induced inflammatory and oxidative responses in isolated rat aortic rings (*measured by electronic paramagnetic resonance method*) and in canine whole blood cultures (*by ELISA test*).

Dihydroberberine (5 µg/mL) and apple extract (200 µg/mL) alone or combined, reduced the LPS-induced superoxide anion and nitric oxide levels in isolated thoracic aortas. By contrast all treatments were unable to prevent the LPS-induced production of both IL-6 and TNF-α in canine whole blood cultures, while dihydroberberine alone or in association with the apple extract decreased IL-10 level.

We evidenced that dihydroberberine and the apple extract, at the concentrations tested, exhibited antioxidant and anti-inflammatory properties in rat aortic rings by mechanisms that remain to be determined. Nevertheless failure to find pro-inflammatory cytokine reduction in the canine blood cultures requires further investigations.

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PC2-16 Biological activity of the ethanolic extracts and essential oils of four species of the genus *Lippia* from Guatemala, against the aquaculture pathogen *Aeromonas hydrophila*

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Fish are susceptible to different bacterial infections when they are raised in conditions of high population density, causing high mortality rates and decreased productivity in aquaculture. *Aeromonas hydrophila*, the most common pathogen in tilapia (*Oreochromis* spp.) culture in Guatemala, causes the syndrome of bacterial hemorrhagic septicemia (BHS) in tilapia causing considerable economic losses to fish farmers [1]. To evaluate the potential of native Guatemalan plants as nutraceuticals in substitution of antibiotics in the prevention of diseases caused by this pathogen, the antibacterial activity of the ethanolic extracts and essential oils of four species of the genus *Lippia* (*L. chiapasensis*, *L. salamensis*, *L. dulcis* and *L. graveolens*) known by its biological activity [2], was assayed. Leaves of the four species were collected in different provinces of Guatemala during 2020. The dried leaves of the four species were subjected to maceration for 48 h with three consecutive portions of 70 % ethanol, then filtered and evaporated. The essential oils were obtained by hydrodistillation of the dried leaves for 2 h. The ethanolic extracts of the four species showed antibacterial activity against *A. hydrophila* (8.2-13.8 mm inhibition diameter) by the disk diffusion assay. The *L. salamensis* extract showed the highest inhibition (8.7 to 13.8 mm). The essential oil of *L. graveolens* was the oil that showed the highest inhibition of *A. hydrophyla* (28.3-30.1 mm) comparable to that of the oxytetracycline control. Thus, *L salamensis* and *L. graveolens* will be subjected to assays against other bacteria to assess its potential as nutraceuticals for aquaculture.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest.

This research "Actividad biológica de fracciones preparativas de extractos de seis plantas del género *Lippia* nativas de Guatemala contra patógenos acuícolas " was co-financed by Fondo de Investigación de la Dirección General de Investigación, de la Universidad de San Carlos de Guatemala, Digi/Usac2020, parti-

da presupuestaria 2020-11300060-12-00-000-001-000-022-001-22-0000-0000 Investigación Básica No. de proyecto DES7-2020”

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PC2-17 Effects of *Echinacea purpurea* (L.) Moench extract in calves

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A randomized double-blinded placebo-controlled field trial was conducted to investigate effects of *Echinacea purpurea* L. extract (EP, 0.5 g (EPL) and 5 g (EPH) daily) given *per os* for four weeks (second to fifth week of life (WOL)) to calves during the challenging period (a) of Bluetongue-virus (BTV) vaccination and (b) transport to and acclimatization on a second farm after four WOL [1]. The 27 calves from one Swiss dairy farm were observed for eight weeks. Clinical parameters were recorded daily. Blood samples were analyzed for IgG, white and red blood cell counts and mRNA abundance of various inflammatory markers in leukocytes. Compared to placebo, both dosages of EP slightly increased body temperature after rehousing and led to an elevated mRNA abundance of prostaglandin E synthase 24h after vaccination. EPL reduced diarrhea days by 44 % (EPL: 7.5 days, placebo: 13.6 days; p = 0.03), but ECH decreased levels of hemoglobin and hematocrit. This is the first study about EP in calves and further investigations need to be performed.

Conflict of Interest; Funding (Source, ID)

We thank the Swiss retailer Migros for funding this study. The funding institution was neither involved in the study design, collection, analysis and interpretation of the obtained data nor in writing the manuscript.

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PC2-18 A multicomponent herbal extract to treat subclinical ketosis of dairy cows

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A multicomponent herbal extract was investigated in a field study in early lactating cows to treat subclinical ketosis in 16 Swiss dairy herds. Effects of the orally administered herbal extract (HE), containing *Camellia sinensis*, *Cichorium intybus*, *Gentiana lutea*, *Glycyrrhiza glabra*, *Taraxacum officinale*, *Trigonella foenum-graecum* and *Zingiber officinale*, were compared to placebo and a positive control (sodium propionate (SP)). A total of 123 cows received one of the blinded preparations twice a day for five days. Three successive cases of the same ketosis severity within the same farm were distributed randomly to one of the three treatments. Cows were followed up for 14 days, starting at the 1st treatment. Milking time and treatment showed a significant interaction for milk acetone as ketosis indicator: SP led to an immediate decrease, whereas HE resulted in a delayed decrease, starting on day 7, reaching significantly lower milk acetone values on day 14 (3.17 mg/L, p = 0.003) compared to placebo (4.89 mg/L). SP and placebo did not differ on day 14 (SP: 3.57, p = 0.10). In conclusion, HE and SP are both likely to improve subclinical ketosis in dairy cows, however, probably by different modes of action [1].

Conflict of Interest; Funding (Source, ID)

The study was funded by SaluVet GmbH. The funding institution was neither involved in data collection nor in data analysis. Two of the authors are staff of SaluVet GmbH.

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PC2-19 Indigenous diagnostic methods and ethnobotanical knowledge used against cattle diseases in Ramotshere Moiloa Local Municipality, South Africa

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Ethnoveterinary medicine is a component of indigenous knowledge systems that requires preservation and conservation due to its importance for health and well-being of cattle in rural areas. The study aim was to document the indigenous diagnostic and ethnobotanical knowledge utilized in the treatment of cattle diseases in Ramotshere Moiloa Local Municipality, South Africa. Data was gathered using a semi-structured interview guide and field observations. Using the snowball technique, 90 participants were purposefully identified to participate in the study. The most preferred diagnostic skills among the participants included behavioural observation, seasonal change, and physical change. A total of 69 plants belonging to 35 families were used to treat 26 cattle diseases which mainly include wounds, constipation, discomfort, and intestinal parasites. The most common plants were *Solanum lichtensteinii* Willd., *Purslane portulaca*, *Opuntia ficus-indica* (L), *Gomphocarpus fruticosus* (L.) W.T.Aiton and *Schkuhria pinnata* (Lam.) Kuntze ex Thell. Compositae, Leguminosae, and Asparagaceae were the predominant plant families. Leaves (37 %) and roots (28 %) were the most used plant components. Decoction (50 %) and Infusion (21 %) were the most preferred method of preparation.

The administration method was distributed between oral (70 %) and topical (30 %). The current study contributes to the documentation of important indigenous knowledge among local communities. It is envisaged that plants with high usage frequency may be potential candidates for bio-prospecting in the drive toward establishing their ethnopharmacological relevance.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. This work is based on the research supported in part by the National Research Foundation of South Africa (Grant Numbers: 118585). The opinions, conclusions/recommendations herein this study are based on the findings of the authors, therefore, the funders (NRF, NWU and KYC) accept no liability whatsoever in this regard

8.3 Antiinfectives and Epidemiology

PC3-1 *In vitro* Synergistic Interactions of Phytochemicals and Their Synthetic Analogs with Tetracycline Against Diarrhoeal Causing Bacteria

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The prevalence of infectious diarrhoea is increasing, posing serious threat to both human and animal lives, as well as causing large cost to society worldwide.

Additionally, treatment of pathogens causing diarrhoea has become complicated due to alarming increase in bacterial antimicrobial resistance [1]. Combination of standard antibiotics with phytochemicals and their synthetic analogs seems to be promising direction for development of new preparations with synergistic growth-inhibitory activity against diarrheagenic bacteria. For example, sanguinarine, a benzylisoquinoline alkaloid isolated from *Sanguinaria canadensis* has been reported to show *in vitro* synergistic effect in combination with streptomycin against clinical isolates of *E. coli* [2]. In this study, we determined *in vitro* synergistic effect of phytochemicals (sanguinarine and tannic acid) and their synthetic analogs (nitroxoline and zinc pyrithione) with tetracycline against standard diarrhoeal causing pathogens (*Enterococcus faecalis*, *Listeria monocytogenes*, *Shigella flexneri* and *Yersinia enterocolitica*). 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 synergistic activity was evaluated according to the sum of fractional inhibitory concentration (ΣFIC) indices obtained by chequerboard method [4]. Several synergistic effects were observed against most of the bacteria tested in this study with ΣFICI ranging from 0.156 to 0.500, whereas the significant ΣFICI (0.156) was shown on combination of tetracycline with zinc pyrithione against *S. flexneri*. These results can be used for development of new antidiarrhoeal preparations for humans and animals.

PC3-2 *Origanum vulgare* L. essential oil effect against *Candida* spp. biofilm formation

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Targeting biofilms formation, which is a major virulence attributes of Candida spp. [1], could be used not only to decrease pathogenicity, but also to potentiate conventional antifungals decreasing drug resistance, doses and toxicity. Essential oils, have emerged as a novel strategy to inhibit biofilms and to potentiate conventional antifungals [2]. In this study we evaluated the antivirulence effects of Origanum vulgare L. essential oil (O-EO) against Candida spp. and its combination with fluconazole and nystatin against biofilms.
First, we obtained de minimal inhibitory concentration (MIC) for Candida albicans strains ATTCC 90029 (0.01 mg/L) and ATCC 10231 (0.97 mg/L) and for non-albicans strains ATCC CD36 (2.6 mg/L) and ATCC 6258 (5.3 mg/L). We evaluated the effect on adhesion (through crystal violet assay), changes in morphogenesis and the antibiofilm effect on the maturation stage (through a modified scratch assay and viability by MTT reduction assay), and combination studies between fluconazole/nystatin and O-EO (by the microdilution checkerboard assay). O-EO significantly inhibited morphogenesis for the four strains assayed by at least 50 % and inhibited adhesion more than 60 %. In the scratch assay, O-EO inhibited recolonization almost completely for ATCC 90029 strain (97 % inhibition) and by 50 % in ATCC 10231. The IC50 for viability of biofilms in ATCC 90029 and ATCC10231 strains was 7.4 and 2.8 mg/L, respectively. And the checkerboard assay showed that O-EO was able to synergistically interact with fluconazole and nystatin. This study demonstrates that the use of O. vulgare could be a strategy against virulence patterns and to potentiate the effect of antifungals.

PC3-3 Macromycetes metabolites to fight multi-drug resistant bacteria

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Antibiotic resistance is becoming one of the major concern for the world public health [1]. As the armamentarium dries up, promising natural resources must be explored for the discovery of new antibiotics. In this regard, we chose to explore the antibacterial potential of wild mushrooms [2]. Seventy two extracts of macromycetes were screened on 5 pathogenic bacterial strains using a direct bioautography on thin layer chromatography (TLC-DB) technique. The most potent extracts (regarding the number of antibacterial zones and the spectrum of activity) were then screened on 8 multidrug resistant bacterial strain, highlighting the potential of several extracts against antibiotic resistance. Active metabolites revealed by bioautography were identified as fatty acids such as stearic acid after high resolution mass spectrometry (HRMS) and NMR. Characterization of other bioactive metabolites is still in progress. The chosen approach combining bioautography with various strains followed by desorption on TLC and further structural characterization allowed us to accelerate the identification of antibacterial constituents from mushrooms extracts. Further assays are mandatory to confirm the antibiotic potential of these compounds.
Authors declare they have no conflicts of interest related to this project.
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PC3-4 Chemical characterization and antimicrobial activity of saponins isolated from S*aponaria Cypria,* an endemic species of Cyprus.

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Introduction Saponaria plants are rich in active molecules called saponins, known to inhibit bacteria. Saponins may therefore be utilized as an alternative source of active components with antimicrobial activity.
Materials and Methods Saponin extracts were isolated from *Saponaria officinalis (S. officinalis)* and *Saponaria cypria (S. cypria),* by maceration [1]. The fraction of saponins was quantified with the vanillin-sulphuric Acid calorimetric Assay [2] and its chemical composition was further characterized with the use of TOF-MS [3]. The antioxidant activity of saponins was evaluated using DPPH colorimetric assay [4]. The antimicrobial activity of saponin extracts was tested against bacteria using the MIC/MBC microbroth method and the time-kill assay [4].
Results Extraction protocols revealed higher saponin yield in *S. cypria* as compared to *S. officinalis*. Identification and characterisation of the main saponins, showed significant differences in the composition of the active saponin molecules between the two species. Comparison of the antioxidant activity demonstrated a higher TEAC activity in *S. cypria*, probably attributed to the higher content of saponins present. The antimicrobial activity of both species showed a MIC range of 0.39-6.25 mg/ml with a preference to *Staphylococcus aureus* and with *S. cypria* exhibiting higher antimicrobial activity in all bacteria tested. Time kill assay revealed the inhibition of all bacterial growth for both species.
Conclusions Our data demonstrate differences both in the saponin composition and antimicrobial activity, between the two *Saponaria* species. This might be

attributed to the different local environmental growth conditions which may exert an impact on the expression of active components in the endemic species.

Conflict of Interest; The authors declare no conflict of interest.

Funding: This work was financially supported by RIF Cyprus (Restart 2016-2020, Call 0618, Project 0176)

All authors have read and agreed to the published version of the abstract.

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PC3-5 The chemical structure- antimicrobial activity relationship of monoterpenoids evaluated in vapour phase

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Our investigation was focused on the chemical structure-antimicrobial relationship of new anti-infective agents. This study used the broth microdilution volatilization method [1] to test the growth-inhibitory effect of selected monoterpenoids in liquid and vapour phase against standard strains of respiratory pathogens such as *Haemophilus influenzae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pyogenes*. Thymoquinone (p-menthane) possessed the strongest antibacterial activity against all bacteria tested except *P. aeruginosa* with MICs ranging from 8 to 16 µg/mL in liquid and from 16 to 32 µg/mL in vapour phase. In addition, MICs of thymol, carvacrol (p-menthanes), and β-thujaplicin (cycloheptane monoterpenoid) against all pathogens tested vary between 32 to 1024 µg/mL and 128 to 1024 µg/mL in liquid and vapour phase, respectively. Representatives of acyclic monoterpenoids, m-menthanes, cyclohexane monoterpenoids, camphanes, fenchanes, pinanes, caranes, and thujanes did not show relevant antimicrobial activity. The results suggest, that the monoterpenoid subgroups p-menthanes and cycloheptane monoterpenoids characterized by a ring structure with hydroxyl- and/or keto groups have antimicrobial activity in liquid and vapour phase.

The authors declare no conflict of interest. This research was financially supported by the Czech University of Life Sciences, Prague [IGA 20213109].

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PC3-6 Identification of anti-parasitic agents in *Cichorium intybus*.

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As the focus on animal welfare and environmental hazards increases, organic farming becomes more prominent. With the prolonged grassing time, cattle now have a much higher exposure to gastrointestinal (GI) parasites, which affects animal growth[1], resulting in markedly economic consequences for the farmer. Long periods on grass also mean long periods of anti-parasitic treatment, leading to a higher risk of resistance. As the treatment of parasites in cattle currently relies on few anti-parasitic drugs, the resistance is increasing, and it is crucial that we find alternative treatment methods. Cattle grazing on chicory (Cichorium intybus) has lower Ostertagia ostertagi infection levels, indicating that chicory could be a promising anthelmintic agent [2]. Until recently, the responsible, active compounds were unknown. Five chicory cultivar were investigated for anthelmntic activity using a model in vitro system based on the mortality of the swine nematode Ascaris suum exposed to chicory extracts. With a combination of metabolic networking and bio-guided fractionation, this study conclud that a combination of sesquiterpene lactones (SL's) are the compounds associated with the anti-parasitic activity of chicory. Five SL's were purified and identified through NMR. The synergistic analysis further identified 8-deoxylactucin as the main active compound, acting synergistically with the other SL's. Metabolic research is devoted to determining the lethal mechanism of action of anti-parasitic SL's in Caenorhabditis elegans.

Conflict of Interest M de Roode is an employee of Sensus b.v. The other authors have no financial or other conflicts of interest. Funding (This work was funded by the Danish Council for Independent Research (Grant DFF–6111-00394). Fractionation and purification of compounds were further supported by the Green Development and Demonstration Program (GUDP) (Project No. 34009-17-1220). MPE was supported by CONICYT Chile (FONDECYT Postdoctorado #3170875).)

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PC3-7 Antibacterial properties of selected Nordic medicinal plants

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Knowledge on the use of medicinal plants is vulnerable to loss in today's acculturated societies. Nature retains a large potential for novel pharmaceutical lead compounds, and with the re-emergence of infectious diseases it is therefore of particular importance to preserve this knowledge. Nordic herbal medicine is rich in species and cures, but its potential has not been systematically explored, and this project focus on Norwegian plant species that have been historically neglected and not extensively studied. Historical literature and material on medicinal plants and medical treatments with focus on plants used in treatment of diseases related to the gastrointestinal system and infectious diseases have been analyzed. A search in Sci-Finder and Pubmed was performed in order to select plants that have not previously been extensively studied, which resulted in a list consisting of 23 plants. The plants were extracted in water, as the traditional modes of preparation in most cases would be in the form of plant decoctions. Components from the water extracts were further isolated through ethanol precipitation and solid phase extraction (SPE). The crude water extracts, the SPE-fractions and polysaccharide fractions were screened for antibacterial effects by disc diffusion assay. The following bacterial strains were used: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. The results indicated that the SPE-fractions from *Antennaria dioica*, *Potentilla erecta* and *Alnus incana* exhibited highest anti-bacterial activity.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflicts of intereset.

PC3-8 Anti-influenza A virus activity of acetone crude leaf extracts of three understudied South African *Syzygium* (Myrtaceae) species

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Influenza virus induced illnesses are of One Health importance globally with high morbidity and mortality in humans and animals. Therefore, effective antiviral and adjunct therapy is required. Growing resistances to available antiviral agents poses a challenge to effective therapy. Plants are putative sources of novel antiviral compounds. Here, the anti-influenza activity of acetone crude leaf extracts of three South African medicinal plants, namely *Syzygium legatii*, *Syzygium masukuense*, *Syzygium* sp. were determined *in vitro*.

Cytotoxicity was determined by the 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT) assay against Madin-Darby Canine Kidney (MDCK) cells. The concentrations below 50 % cytotoxic concentration (CC_{50}) values were tested against influenza A virus in different combination treatments. Effect of extracts on viral titre and viral surface glycoproteins were tested by hemagglutination (HA) and hemagglutination inhibition (HI) assays, respectively.

S. legatii had the best CC_{50} (421.7 µg/ml) while *S.* sp. had the best 50 % effective concentration (EC_{50} = 40.6 µg/ml) against induced viral cytopathic effect on MDCK cells, followed by *S. masukuense* (EC_{50} = 42.4 µg/ml). *Syzygium legatii* and *S.* sp. had the best selectivity index (CC_{50}/EC_{50}) of 4, showing selective viral toxicity. Log_{10} HA titer from the HA assay in combined treatments with virus compared to the virus control group showed that *S. legatii* and *S. masukuense* had remarkable activity, especially in the co-penetration treatment. The extracts were as active as the positive control (Oseltamivir carboxylate).

The plant extracts had anti-influenza activity. The compounds responsible for the observed anti-influenza activity and their mechanism(s) of action are the focus of future studies.

Conflict of Interest; Funding (Source, ID)
The authors declare no conflict of interest. National Research Foundation (NRF, South Africa, grant 105993 to LJM) and Medical Research Council (SIR to LJM) are acknowledged for research funding. The University of Pretoria funded the postdoctoral study of IMF

PC3-9 Comparison of epidemiological data on functional GI diseases under an herbal therapy from non-interventional studies in the US and Germany

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DOI 10.1055/s-0041-1736838

The comparison of epidemiological data from different countries can help to improve the understanding of patient's needs and of therapeutic approaches globally. In two recent non-interventional studies (NIS), one from the US and the other from Germany [1–3], data from patients with functional GI symptoms using an herbal medicine [4, 5] have been collected. The US study was conducted by Grundmann et al. by an internet questionnaire. The study from Germany was a NIS in patients who used natural products (PhytoVIS) and carried out through interviews in pharmacies and doctor's offices. Original data on users of the same natural product, STW 5, were selected from both studies and evaluated using descriptive statistics.

The number of patients meeting the inclusion criteria in the US study was 50, in German (DE) 1515. 88 % of the US resp. 70 % of DE participants were female. Dyspepsia-like symptoms were the largest group in both studies, while IBS could be assigned to 15.6 % resp. 4.0 % of patients. 50.7 % resp. 63.1 % rated the treatment very effective, 24.5 % resp. 32.4 % moderate, 5.9 % resp. 5.6 % not effective, and 21.7 % resp. 0 % did not know.

There are, accordingly, considerable similarities, e. g., in the predominance of female patients and the rating of the treatment effect. Overall, these studies turn out to be a valuable source of data on the use of an herbal treatment and for assessing differences and similarities between the patient populations in the US and Germany.

Conflict of Interest; Funding (Source, ID)
JM has been intern, OK is employee of Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany. KN and ER received fees from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.
Many thanks to Annika Lindner for the analysis of the data.
The study was supported by Kooperation Phytopharmaka, Bonn, Germany.

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PC3-10 Gender data from the PhytoVIS study, a pharmaco-epidemiological NIS more than 20.000 patients

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DOI 10.1055/s-0041-1736839

Background Gender pharmacology is a field attracting increasing attention, but for medicinal plants and natural products, data are rare [1]. Purpose: To address this gap, data from the PhytoVIS study, presumably the world's largest pharmacoepidemiological study on the use of herbal medicinal products [2], were assessed. Methods: The PhytoVIS data set contains information on epidemiology of patients and therapeutic indications, efficacy and tolerability of herbal medicinal products used by them, which have been captured in pharmacies and in doctor's practices in Germany, in compliance to the ENCePP Code of Conduct [3], and was evaluated. Results: Overall, 24056 men and women were evaluated, thereof 16443 patients were women and 7613 were men. The efficacy of the therapy in women/men was rated very good in 45.9/42.5 %, good to moderate in 38.2/41.5 %, minimal in 11.6/11.5 % and unchanged to worsened in 4.2/4.6 %. The tolerability was good in 91.5 % of women and 90.6 % of men. Besides differences in medicines for menopausal/prostate complaints, the proportion of men taking herbal products for colds and joint pain was higher, while women were more likely to take herbal products for anxiety, sleep disturbance, and bladder dysfunction. Conclusion: The data shed light to a field of gender pharmacology in which up to now data has been rare [1] and gives a picture of the use of herbal medicines in an unselected cohort of patients. Despite clear differences in few fields of application, men and women were similar in the use of herbal preparations in general.

Conflict of Interest; Funding (Source, ID)
TA-S and JM have been interns, OK is employee of Steigerwald Arzneimittelwerk GmbH. KN received remunerations for research projects from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.

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PC3-11 Echinacea as a Potential Weapon against Coronavirus Infections?: A Mini-Review of randomized controlled trials

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DOI 10.1055/s-0041-1736840

Echinacea purpurea broadly inhibits coronaviruses and SARS-CoV-2 *in* vitro [1]. This review discusses the available evidence from human studies. A literature search on PubMed and EMBASE yielded two RCTs with results on coronavirus infections during prevention with *Echinacea purpurea*. Incidence and viral loads were measured by reversed transcriptase polymerase chain reaction (RT-PCR). Jawad (2012) collected nasopharyngeal swabs from N = 755 adults over 4 months of prevention. Overall, 24 and 47 enveloped virus infections occurred, including 21 and 33 coronavirus detections [229E; HKU1; OC43] with Echinaforce [2'400mg daily] and placebo, respectively (p = 0.0114). No significant difference was observed on symptom level. Ogal (2021) administered Echinaforce extract [1'200mg] or control for 4 months to N = 203 children (4 – 12 years). Echinacea reduced the incidence from 47 to 29 enveloped virus infections (p = 0.0038) and 11/13 coronavirus detections [229E, OC43, NL63] were counted (p > 0.05). Viral loads in nasal secretions were significantly diminished by 98.5 %, with Ct-values 31.1 [95 % CI 26.3; 35.9] versus 25.0 [95 % CI 20.5; 29.5] (p = 0.0479). Finally, respiratory symptoms during coronavirus infections were significantly reduced with area-under-curve AUC = 75.8 (+/-50.24) versus 21.1 (+/-21.27) score points (p = 0.0036). Echinacea's broad antiviral spectrum was confirmed in two clinical trials suggesting its potential for prevention of infections by respiratory pathogens, including coronavirus [2, 3].

Conflict of Interest; Funding (none)

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8.4 Analytics, recent methodology and applications

PC4-1 A new alkaloid from *Pancratium maritimum* L.

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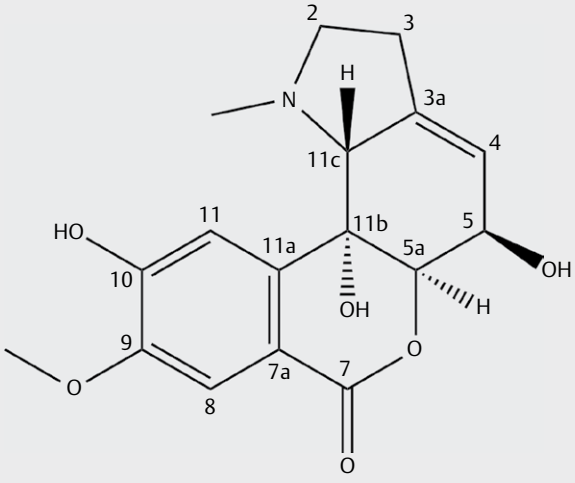
DOI 10.1055/s-0041-1736841

Pancratium maritimum L. (sea daffodil) is one of the most studied plant species in the Amaryllidaceae family [1]. Recently, due to the advancement of new probabilistic methods (DP4, DP4+, DP4-*J*) and spectroscopic techniques (VCD), the potential of the phytochemical investigation on isomeric compounds has been significantly improved. In this study, application of those techniques in combination with conventional phytochemical analysis led to the isolation and identification of a new alkaloid (**1**) from *P. maritimum*, together with 15 known compounds. Compound (**1**) was found to be a diastereomer of 2α-10bα-dihydroxy-9-*O*-demethylhomolycorine [2]. Using the DP4 and DP4+ probabilities, the relative configuration of (**1**) was assigned as either RRRR or SSSS and was in agreement with observed NOE effects. Finally, the absolute configuration of the new compound was confirmed to be RRRR by VCD spectroscopy. The novel compound was named (5R,5aR,11bR,11cR)-5,11b-dihydroxy-10-*O*-demethylhomolycorine.



▶ **Fig. 1**

Conflict of Interest; Funding (Source, ID)
The authors declare no conflict of interest. We are grateful to the HPC core facility CalcUA of the University of Antwerp and VSC (Flemish Supercomputer Center), funded by the Research Foundation - Flanders (FWO) and the Flemish Government.

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PC4-3 Integrated NMR and LC-MS metabolite profiling data for the quality control of table olives

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DOI 10.1055/s-0041-1736842

Foodomics combine food & nutrition with advanced techniques and bioinformatics in the research for food profiling, authenticity control and biomarker identification. NMR and LC-MS are widely used in the field, individually to the greatest extent.

The aim of the present study was to employ both LC-HRMS & NMR platforms towards the quality control of table olives, exploring cultivar, geographical origin and debittering method. To ensure the integrity of these assessments, steps prior to analysis were identical for both, while acquired data were subjected to statistical analysis in parallel or in an integrated manner through Statistical HeterospectroscopY (SHY).The sensitivity of HRMS led to the identification of almost 10-fold tentative markers compared to NMR [1], yet the superiority of the latter in identification confidence and estimation of concentration levels cannot be overlooked. The statistical models of the HRMS data showed less dispersion, higher robustness, and improved classification parameters, most probably due to the higher number of detected features. Nevertheless, the similarity in the fluctuation of the concentration levels of tentative markers was evident.

It seems that the two techniques are complementary, while SHY proved to be a valuable aid in the identification of tentative biomarkers.

Conflict of Interest; Funding

The authors declare no conflict of interest. This research was financed by the Emblematic Action "The Olive Roads" (project code: 2018ΣΕ01300000). The authors would also like to thank the project PlantUp (project code: 5002803).

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PC4-4 Comparing multiple batches of finished *Ginkgo biloba* products from international markets including EGb 761® by principal component analysis based on NMR spectroscopic data

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DOI 10.1055/s-0041-1736843

Extracts of *Ginkgo biloba* are highly complex mixtures of a multitude of substances. Although medicinal products based on *Ginkgo biloba* extracts comply with quality standards defined by regional pharmacopoeias, it is common ground that the currently known and quantified active ingredients flavonol glycosides and terpene trilactones are not exclusively relevant for therapeutic efficacy of the quantified extract. For a comprehensive assessment of the therapeutic efficacy, the involvement of other compounds also need to be considered. In the present study, multiple batches of finished Ginkgo biloba products from international markets including products containing EGb 761® were analyzed by NMR spectroscopy using an untargeted approach to collect quality data beyond the standard parameters defined by pharmacopoeias. NMR data were statistically evaluated by principal component analysis using the software Bruker AMIX v.3.9.15 to assess batch to batch consistency and compare product composition from different manufacturers.

The analysis revealed a high batch to batch consistency of products based on EGb 761® and major variations between products based on EGb 761® and products of other manufacturers, respectively.

Since finished *Ginkgo biloba* products of different manufacturers vary in their composition assessed by NMR spectroscopy, it is an open question whether therapeutic efficacy data may be fully transferrable if solely based upon equal contents of flavonol glycosides and terpene trilactones.

Conflict of Interest All authors are employees of Dr. Willmar Schwabe GmbH & Co. KG, Germany.

PC4-5 NMR-based metabolite profiling and application of STOCSY in honey quality control

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DOI 10.1055/s-0041-1736844

Food quality, authenticity and safety constitute an important issue globally. Due to its short supply and high demand, honey has become a prime target for economically motivated adulteration. As for other food commodities, NMR-based methods have been employed in the quality control of honey. However, a challenging step remains the identification of biomarkers, mainly due to the scarcity of databases, as well as the samples' complexity and variability.

Therefore, the aim of the current study was to apply NMR-based metabolite profiling in the quality control of Greek honey. Moreover, a parallel goal was to evaluate the impact of **S**tatistical **To**tal **C**orrelation **S**pectroscop**y** (STOCSY) in the biomarker identification process [1]. STOCSY correlates signals of the same biomarker based on the variance of its concentration levels across the samples on their respective spectra.

Thus, using MVA on NMR data, it was possible to discriminate honey samples of different geographical and botanical origin from Greek Eastern Aegean islands. Furthermore, 5-hydroxymethylfurfural and methyl syringate stood out as indicative biomarkers of botanical origin identified via STOCSY.

In conclusion, NMR-based metabolite profiling is an effective method in honey quality and authenticity assessment, while STOCSY is a valuable statistical tool for biomarkers' identification, with the present being its maiden application in honey. Nevertheless, its implementation in food is still in early stages.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. The authors would like to thank PlantUp (project code: 5002803).

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PC4-6 Dereplication of extracts from nutraceutical mushrooms Pleurotus using molecular network approach

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DOI 10.1055/s-0041-1736845

Pleurotus is an edible mushroom and represents a well-known genus of basidiomycetes being the third most consumed on a global scale besides to presents several biological activities. This genus is described by presence of steroids, fatty acids and polysaccharides. In addition, *Pleurotus* has become increasingly appealing as functional foods, known as nutraceuticals, due to their health benefits. Dereplication techniques aided by high resolution mass spectrometry and Global Natural Products Social Molecular Network (GNPS) were used to analyze the chemical composition of methanolic extracts of six *Pleurotus* species (*P. sapidus, P. ostreaus, P. ostreaus* var. Florida, *P. djamor, P. citrinopileatus* and *P. pulmonarius*). The used approach pointed towards different molecular families including the annotation of 8 essential fatty acids and 7 steroids, being 6 of them suggested according to the molecular network correlated nodes. This work shows an approach of dereplication for extracts as a relatively rapid tool for characterization of fungi species and determined its nutraceutical value [1–3].

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. The authors thank FAPESP (grants 2016/24985-4 PS, 2018/21492-2 DCZ and 2018/25646-4 LAC) for financial support for the development of this work.

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PC4-7 Development and validation of amino acids analysis method using LC-triple-TOF and aTRAQ labeling protocol. Application in human plasma samples

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DOI 10.1055/s-0041-1736846

Amino Acids (AA) analysis is of great importance in many areas of biochemical, pharmaceutical and clinical research. Currently, there are evidences that the supplementation of foods with specific AA influence appetite and energy intake especially with arginine (L-arg). The aim of this study is to develop and validate a method for absolute quantitation of AA in human plasma incorporating a triple-TOF (AB Sciex 5600$^+$) platform and the aTRAQ labeling protocol as well as to demonstrate their effective compatibility. aTRAQ reagents consists of a reporter and a reactive tag that couples with the primary or secondary amine group of each AA. Ion-pair chromatography (IPC) with HFBA as ion-pair reagent was used for the separation of AA while SWATH scan type, in positive ESI mode was employed for MS acquisition. The method was validated according to EMEA. Moreover, the newly developed method was applied in plasma samples obtained from a 12-week, two-arm parallel dietary intervention in which overweight volunteers consumed a wheat biscuit enriched with L-arg or a conventional wheat biscuitOverall, with the new method 45 AA were accurately quantified in human plasma while it was possible to monitor the L-arg plasma levels between (or in the two?) groups.

Conflict of Interest; Funding (PlantUp, 5002803, OREKTOSPRION, T1EDK-03747) Statement on behalf of all authors. There is no conflict of interest

PC4-8 Analysis of triterpene acids in *Boswellia serrata* by Supercritical Fluid Chromatography

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DOI 10.1055/s-0041-1736847

The gum resin of *Boswellia serrata*, also known as Indian frankincense, is a commonly used traditional medicine with many desired pharmacological, especially anti-inflammatory, effects. They are due to specific acidic triterpenes in the plant, boswellic acids (BAs) [1]. Even though many techniques have been evaluated for their analysis, the use of Supercritical Fluid Chromatography has never been considered before. In this study, the baseline separation of six boswellic acids, all derivatives of α- and β-BA, in less than 6 min by SFC is described, using a Viridis HSS C18 SB column and a mobile phase comprising carbon dioxide and a mixture of methanol, acetonitrile and ammonium hydroxide. In respect to speed this surpasses all other currently known analytical approaches. The method was successfully validated following ICH guidelines (e. g., $R^2 \geq 0.999$, LOD ≤ 5.5 mg/mL) and could easily be hyphenated to mass spectrometry. This permitted the tentative assignment of further triterpene acids (mainly tirucallic acid derivatives), together with significantly improved sensitivity.The practical applicability of the method was confirmed by analyzing commercially available dietary supplements containing *B. serrata* gum resin or extracts thereof. Although the investigated products all exhibited β-boswellic acid as major constituent, the total concentration of BAs was extremely variable (2,61-43,24 %).

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PC4-9 Development of innovative products based on hydrodistillation by-products of plants of the Greek flora

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DOI 10.1055/s-0041-1736848

Cretan Iama is an innovative food supplement which contributes in strengthening the body's defense. It is based on the unique biodiversity and the beneficial effects of plants of the Greek flora on human health and well-being. Cretan Iama is produced by the hydrodistillation of three aromatic plants, Greek dittany (*Origanum dictamnus*), thyme (*Coridothymus capitatus*) and sage (*Salvia fruticosa*) [1, 2]. Various by-products are produced during the procedure, among them hydrosols and aqueous plant extracts that contain bioactive polar substances and could be of further exploitation. The materials (hydrosols and aqueous plant extracts) were treated with resin chromatography and afterwards separated with CPC to isolate their main compounds. The chemical profile of the obtained extracts and fractions was determined using modern chromatographic and spectroscopic techniques (HPTLC, HPLC-DAD, LC-MS and NMR). The phytochemical study showed that especially the aqueous extracts were very rich in phenolic compounds and flavonoids. Their antioxidant properties were also evaluated and again the aqueous extracts and especially the fractions obtained after resin chromatography had low IC_{50} values. The study showed that the by-products had promising chemical profiles containing bioactive substances and could be developed to new innovative phytotherapeutic products and/or food supplements.

The authors declare no conflict of interest;

Funding EPAnEK 2014-2020, Hydraroma, T2ΕΔΚ-02951

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PC4-10 Phytochemical analysis and evaluation of the antioxidant activity of edible green plants growing in Greece

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DOI 10.1055/s-0041-1736849

The Mediterranean diet is renowned for its beneficial properties on human health, since it has been proven to contribute to decreased rates of heart disease, cancer and neurodegenerative diseases and therefore provides an excellent field for the discovery of new bioactive agents [1, 2]. Leafy green vegetables are an integral part of the Greek dietary regime. Herein, seven samples of plant species belonging to the family Compositae were studied for their content in bioactive compounds and antioxidant activity. Specifically, *Centaurea raphanina*, *Cichorium intybus*, *Sonchus tenerrimus*, *Sonchus oleraceus* and *Sonchus asper* were collected, extracted and analyzed for their chemical composition with HPTLC, HPLC-DAD and LC-MS. The plants possess a complex and interesting chemical profile, containing bioactive constituents, like flavonoids and phenolic acids. The extracts were evaluated for cytotoxicity and antioxidant activity by performing cell-based assays. All the examined extracts showed no significant toxicity to BJ normal human skin fibroblasts and HaCaT immortalized human keratinocytes The extracts of *C. raphanina* were found to reduce the intracel-

lular oxidative load and upregulate the expression levels of antioxidant responses-related genes in BJ fibroblasts or HaCaT keratinocytes. Additionally, the extracts of *S. asper* exerted significant antioxidant activity in human keratinocytes. The results showed that the studied plants could be promising candidates for the development of protective or therapeutic agents, like food supplements.

The authors declare no conflict of interest;

Funding Bilateral and Multilateral S&T Cooperation Greece-China, 2018, NutraFood, T7ΔΚΙ-00296

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PC4-11 Isolation and identification of urine metabolites after hydroxytyrosol supplementation in humans

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DOI 10.1055/s-0041-1736850

Hydroxytyrosol (HT) is amongst the most potent natural scavengers of olive products found also in a plethora of foodstuffs, nutraceuticals and supplements. Critical factor for HT potency is its metabolisation fate in human system. Usually, the interpretation of the biochemical pathway that determines the bioavailability and activity is mainly based on plasma and urine analysis via LC–MS. However, the lack of appropriate standards, poses the risk of vague or imprecise identification and systematic quantification inaccuracies.

Thus, the goal of the present study is the isolation of HT metabolites from urine and unambiguous identification thereof with NMR. For this purpose, urine samples from women after HT supplementation for a six-month period were used [1].

Urine is a matrix easily obtained in large volumes, providing information about individuals dietary exposure. Urine samples after solid phase extraction-(SPE) treatment were subjected to fractionation using Centrifugal Partition Chromatography-(CPC). CPC lacks solid support, which makes it an excellent choise for biological substrates analysis. After several trials, the best separation was achieved with the biphasic solvent system methyl-t-butyl ether/n-butanol/acetonitrile/water (2:3:1:4, with 8 % AA), in ascending mode. For further purification of metabolites semi-preparative RP-HPLC was employed in certain fractions. For the identification both LC-HRMS and NMR were employed. So far, several HT metabolites together with endogenous ones have been identified, in high amount and purity. The unabiguous identification of metabolites and their correlation offer a better insight in HT bioavailability and mechanism of action.

Authors declare no conflict of interest

The current work is funded gy General Secretariat for Research andInnovation (GSRI)/MIA-RTDI (Project code: T1ΕΔΚ-04267). The present work was co-funded by the European Union (ERDF) and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "STRENGTHENING RESEARCH AND INNOVATION INFRASTRUCTURES" (project code:5002803).

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PC4-12 Quantification of *Citrus limon* (L.) Burmin in complete feed with UHPLC-MS/MS using a double "one-point" standard addition

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DOI 10.1055/s-0041-1736851

A specific and sensitive method for quantification of *Citrus limon* (L.) Burmin in complete feed based on UHPLC-MS/MS has been developed and validated. The additive studied is a natural commercial feed additive (Citrozest®, Nor-Feed, France) used in animal nutrition, consisting of a standardized extract of *Citrus limon* (L.) Burmin in which eriocitrin has been identified and quantified. Eriocitrin was used as phytomarker for the quantification of the additive with a level of 67 ng/g of phytomarker in complete feed. Eriocitrin quantification is performed by the standard addition method prior HPLC-MS/MS analysis. To compensate for the loss of eriocitrin during the extraction step, cameliaside A is used as internal standard and the recovery is also determined by standard addition method. Standard addition method may be too much time consuming for routine analysis. To decrease the workload of the analysis, a simplified approach based on the "one-point" addition was used for both standard additions permitting the utilization of the method in routine. The method was developed and validated in-house in accordance with the guidelines recommended by IUPAC [1] for the quantification of *Citrus limon* (L.) Burmin in complete feed. This method of analysis has all the prerequisites to be used by European authorities as part of the registration of a feed additive [2].

Conflict of Interest; Funding (Source, ID)

The authors declare that they have no conflict of interest. This study was financed by Nor-Feed and Agence Nationale de la Recherche (ANR).

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PC4-13 An accurate and cost-effective method for the quantification of total triterpenoid and steroidal saponins in raw plant materials

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DOI 10.1055/s-0041-1736852

Saponins are heterosides widely distributed in the plant kingdom. Their properties are used in many industrial sectors such as food, cosmetics, agricultural, pharmaceutical and their use tend to increase due to the tendency of the market to use natural ingredients [1]. Although many techniques exist to quantify saponins: gravimetric, foaming, spectrophotometric or chromatographic [2], none of these methods makes it possible to be at the same time accurate, fast, inexpensive with a response similar for the triterpenoid and steroidal saponins. A colorimetric method constituted of *p*-anisaldehyde and sulfuric acid was developed and avoids all the above disadvantages. Parameters used in this method permit to obtain a response similar for steroidal and triterpenoid saponins with a high specificity in complex matrices reducing the sample preparation step. The method was compared with an HPLC-ELSD method over four saponins plants: *Camellia oleifera, Chenopodium quinoa, Trigonella foenum-graecum* and *Yucca schidigera*. The rapid and cost-effective method gave result similar in term of precision and accuracy that is suitable for routine industrial analyses.

Conflict of Interest; Funding (Source, ID)

The authors declare that they have no conflict of interest. This study was financed by Nor-Feed and Agence Nationale de la Recherche (ANR).

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PC4-14 Potential adaptation of GC-EI-MS method to determine the residue of natural EOs in eggs

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Natural essential oils (EOs) are employed as efficient natural red mite repellents in egg production. EO residue in eggs and the method for detection are rarely discussed in the literature. This study presents an adaptation of GC-EI-MS method to identify and quantify the residue of EOs in eggs laid by in feed EO-supplemented hens, for 10 months compared to eggs from unsupplemented hens. 2 major aromatic compounds, geraniol and eugenol, originated from *Cymbopogon nardus* and *Eugenia caryophyllus* EOs respectively, were targeted.

This protocol was adapted based on the GC-EI-MS method developed by X. Li *et al.*,2019 [1]. Briefly, raw white and yolk were taken for analysis. Frist, acetonitrile (ACN) was used for protein precipitation. Next, ionic strength effect of NaCl was employed to increase analytes distribution into organic solvent. A delipidation step then followed with hexane. Finally, decanted ACN layer was injected into the GC-MS system.

5 repetitions were carried out. The extraction procedure was verified by submitting a standard of geraniol to the complete protocol.

The verification of extraction revealed that 70 % of geraniol was retrieved in decanted ACN. No aromatic compound was detected in all eggs from both tested groups. Indicating that the likelihood for EOs residues accumulation in eggs laid by hens regularly supplemented in feed with EOs for a duration of 10 months was poor. Further experiments are recommended to validate the efficiency and robustness of this method adaptation to determine aromatic compounds from EOs in eggs.

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PC4-15 Semisynthetic ecdysteroid-cinnamic derivatives against *Trypanosoma cruzi*

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Chagas disease is a serious tropical disease that affects millions of people. It is caused by *Trypanosoma cruzi*, a protozoan parasite. Common transmission occurs via stings of Triatominae bugs as vectors [1]. In this work, our aim was to prepare new bioactive ester derivatives of 20-hydroxyecdysone (20E), and test against *T. Cruzi.* 20E was reacted with cinnamic acid, EDCI and DMAP in mol. sieve. DCM for 4.5 days [2]. The crude products were purified via flash chromatography and RP-HPLC. Four ecdysteroid esters including 2 new compounds were obtained, and their structures were confirmed via NMR as the 2-, 2,3-, 2,22, and the 3,22-cinnamate of 20E. Together with the new 20E cinnamates, 52 previously prepared ecdysteroid derivatives were tested against *Trypanosoma cruzi* epimastigotes. 2,22- and 3,22 phenylpropanoid esters and 6-*O*-*E* and *Z*-*tert*-buthyl-oximether of 20-hydroxyecdysone diacetonide demonstrated promising, and parasite selective activity at 5 µM. Synthesis of the caffeate and ferulate derivatives of 20E are currently ongoing.

Conflict of Interest; Funding NKFIH K-134704; EFOP 3.6.3-VEKOP-16-2017-00009

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PC4-16 Phytochemical assessment of *Dendrobium fimbriatum* Hook (Orchidaceae)

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The *Dendrobium* genus contains central medicinal and ornamental orchids and is an important resource for the discovery and development of new drugs and cosmetics [1]. In this context, we aimed to assess the chemical composition of the hydroalcoholic extract of the whole plant *D. fimbriatum* Hook. After a compilation of the molecules previously described in the literature, we employed a dereplication strategy named CARAMEL based on CPC and NMR analyses. 21 compounds (mainly primary metabolites, phenolic compounds, and organic acids) were identified. To further chemically characterize the extract, a purification procedure was performed by preparative HPLC-DAD-ELSD, resulting in the identification of 4 additional phenanthrenes: hircinol, plicatol A and B, and the new compound plicatol D (4, 10 dimethoxy-2,5-phenanthrenediol), together with one furostanol saponin: protodioscin. In total 26 compounds were described in *D. fimbriatum* Hook in this study. Among them, 22 were identified for the first time in this species.

This research was financially supported by Guerlain to contribute to the understanding and preservation of orchids. Authors declare they have no financial interests and no conflicts of interest that are relevant to the content of this publication.

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PC4-17 Multivariate Data Analysis of LC/MS profiles of *Buxus sempervirens* L.: Seasonal Variability of Alkaloid profiles and Identification of Antiprotozoal Compounds

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The *nor*-triterpene alkaloids of *Buxus semper-virens* have shown conspicuous in vitro activity against the causative agents of malaria tropica and East African sleeping sickness [1]. To characterize the temporal variability in the alkaloid profile and to identify further antiprotozoal compounds of this plant, 30 different extracts of *B. sempervirens* as well as 20 fractions, exhibiting a wide range of in vitro bioactivity, were analysed by UHPLC/ + ESI-QqTOF-MS/MS. The analytical profiles were investigated by multivariate data analysis (MVDA).

The resulting principal component analysis (PCA) model elucidates the seasonal variation in the alkaloid profile. Partial least squares (PLS) regression highlighted several compounds as mainly responsible for the antiprotozoal activity.

These compounds were dereplicated on grounds of their mass spectra, so that several further congeners with strong antiprotozoal activity may now be isolated.

In conclusion, the MVDA models give detailed information on temporal variability in the alkaloid profile of two different varieties and organs (leaf vs. twig) of *B. sempervirens* and allow to identify the optimal harvesting time for the isolation of compounds with strong antiprotozoal activity.

The authors declare no conflict of interest; LU Szabó received a doctoral fellowship from Apotheker-stiftung Westfalen-Lippe.

All authors have read and agreed to the published version of the conference abstract.

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PC4-18 Herba Epimedii biotransformation in *in vitro* simulated gastrointestinal digestion and faecal fermentation systems

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Epimedium has been widely used in ethnopharmacological practices, and is rich in a variety of beneficial active ingredients, however, its biotransformation in simulated gastrointestinal digestion remains unclear. The dynamic changes of components of Epimedium during the simulated gastrointestinal digestion and subsequent human faecal fermentation were investigated by UPLC-MS, HPLC-DAD and multivariate data analysis. Epimedium samples were subjected to HPLC-fingerprints and 60 peaks (compounds) of digestive products of different stages were collected to analyze the effects of Epimedium's gastrointestinal metabolisms. The major bioactive flavonoids ingredients, epimedin C, icariin and baohuoside I, were identified among the compounds. Application of PCA to HPLC data showed that there were obvious differences among the samples after gastric, saliva, intestinal digestion, colonic fermentation at 0.5 h and other times (24 h, 48 h and 72 h). The contents of epimedin C, icariin and baohuoside I all decreased significantly after gastric digestion, while increased significantly after intestinal digestion. UPLC-MS analysis indicated difference between the samples after gastric digestion and saliva digestion in component, possibly due to a series of gastric environmental factors such as low pH and pepsin etc. Additionally, after colonic fermentation simulation stage, the components of Epimedium changed obviously, this may be due to the fact that most of the glycosylated flavonoids of Epimedium were decomposed into phenolic acids. This study provides scientific basis for rational assessment and application of Epimedium.

Conflict of Interest Not applicable; Funding (Incubation Project on State Key Laboratory of Biological Resources and Ecological Environment of Qinba Areas, SLGPT2019KF04-04) Statement on behalf of all authors: All authors have read and approved the abstract.

PC4-20 Analytical and chemometric approaches for quality characterization of *Cannabis sativa* L. with focus on cannabinoids

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The quality characterization of *Cannabis sativa* L., especially with respect to its containing cannabinoids, is associated with many analytical challenges. Within this context, the power of chemometrics is recognized for interpretation and optimization of analytical procedures [1], as was undertaken in this work. Initially, Design of Experiments was employed for the optimization of extraction conditions following rational comparison of extraction techniques [2]. In parallel, effective chromatographic methods based on UPLC-PDA and GC-MS were developed for quantitative purposes and characterization of extracts. Pattern recognition techniques were then applied on HPTLC chromatograms to investigate the effect of extraction solvent on metabolite fingerprints and cannabinoid yield. Furthermore, classification and discrimination of hemp samples from different Greek regions and cultivars was attempted through chemometric processing. To this end, both first- and second-order data from LC-PDA were analyzed with multivariate techniques, also exploiting the multi-way nature of data. Finally, an NMR-based workflow was developed for metabolic profiling of *C. sativa* samples and identification of potential biomarker compounds.

There is no conflict of interest Funding from Stavros Niarchos Foundation (grant number KA 14320), European Union (ERDF) and Greek national funds (Codes 5002803 & T1EDK-04301).

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PC4-21 Royal Jelly encapsulation in a combinatorial system consisting of liposomes and cyclodextrins – skin functionality and controlled release

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DOI 10.1055/s-0041-1736859

Royal jelly is a white viscous substance with a gel texture secreted by the hypopharyngeal glands of young workers bees. Owing to its excellent biological properties, royal jelly is widely used in the food, supplement, and cosmetics industry. Numerous studies have shown anti-aging, anti-inflammatory, antimicrobial, anticancer and antidiabetic properties. Royal jelly exhibits physicochemical instability depending on time and storage temperature. Ideal storage temperature of royal jelly is -20 ° C while higher temperatures cause color change and component degradation [1].

In the present work fresh Greek royal jelly was incorporated in a combinatorial system consisting of liposomes and beta-hydroxypropyl cyclodextrins, achieving 95 % encapsulation efficiency, referring to 10-hydroxydecenoic acid (10-HDA). The system was proven to be physicochemically and microbiologically stable during a 6-month period, while it preserves the biofunctionality of royal jelly – as depicted by the stable levels of 10-HDA. The system releases 10-HDA in a time-controlled manner, while it was shown to exhibit significant skin bioactivity: it induces miRNA 129 expression in skin fibroblasts, a gene that has a main function the protection of fibroblasts from aging process. Furthermore, the system Increases cell proliferation and cell viability in human fibroblasts and promotes mitochondrial fusion which is related to the increase of cellular metabolism and energy production. The above results show promise for the use of the system in dermal applications, having the benefits of controlled release and of storage at room temperature.

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PC4-22 Quantitative and qualitative evaluation of 60 Labiatae species, growing in Greece, regarding the content of selected abietane-type diterpenes using 1H-qNMR.

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Carnosic acid (CA) and carnosol (CS) are abietane-type diterpenes with plenty of biologic properties [1–2]. Quantitative and qualitative evaluation of 60 plant species of Lamiaceae, growing in Greece, regarding their content in CA, 12-methoxy-carnosic acid (12MOCA), CS and 7-methoxy-epirosmanol (7MER) using ^{1}H-qNMR is presented.The methanolic extracts from leaves of species: *Ballota, Hyssopus, Lavandula, Marubium, Nepeta, Phlomis, Ocimum, Origanum, Rosmarinus, Salvia, Stachys, Coridothymus, Mentha, Melissa, Sideritis, Satureja, Teucrium*, Lamium and *Betonica*, were analysed using as standards the isolated and fully structurally characterized metabolites by our group. At least one of the target compounds was detected in 29 species: *Rosmarinus* (2), *Salvia* (23), *Melissa*, *Ocimum*, *Mentha* (2). 7MER has not been determined in any of the extracts. All three CA, CS and 12OMCA, were identified only in *Salvia (9) and Rosmarinus (2).* The richest source in CA was *S. pomifera subsp. pomifera (Cretan sage)* (21.6 ± 0.6 mg/g dry leaves) > *S. fruticosa (Greek sage)* (12.5 ± 1.6 mg/g) > *S. officinalis L. (Common sage)* (11.7 ± 4.3 mg/g), in CS was *S. officinalis* (1.1 ± 0.4 mg/g) > *R. officinalis L.* (1.1 ± 0.1 mg/g) > *S. canariensis* (1.1 ± 0.3 mg/g) while in 12OMCA was *S. microphylla* (10.7 ± 3.3 mg/g) > *S. officinalis* (6.1 ± 2.3 mg/g) > *S. fruticosa* (3.1 ± 0.3 mg/g). *Salvia* and *Rosemary* revealed as best sources for CA, CS and 12MOCA among the studied species. ^{1}H-qNMR spectroscopy offers a useful tool for the quantitative and qualitative analysis of the examined compounds in complex extracts, in a simple, rapid and direct way without sample deterioration.

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PC4-23 Optimization of *Rosmarinus officinalis* distillation and study of essential oil's chemical composition using Headspace GC-MS

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Rosmarinus officinalis (commonly known as rosemary) is a widely used medicinal and aromatic herb. It has been used since Hippocrates for its soothing and beneficial effects and has antibacterial, anti-inflammatory and antioxidant properties. Aim of the present work was to study the chemical composition of essential oil (EO) of rosemary cultivated in Greece, as well as to define the pilot scale distillation conditions that maximize its yield and optimize specific bioactive compounds' abundance. All distillations were conducted at an industrial distillery, implementing a Face-Centered Composite experimental design, in order to optimize the yield and relative abundance of compounds of interest, by varying the process parameters of steam distillation: time, temperature and pressure. EOs' composition was determined by Headspace GC-MS analysis. Among the chemical compounds identified in the rosemary EO, *a*-pinene is the most prominent constituent under all distillation conditions applied. Data processing showed that the factor that mainly affects the yield is time. Optimal region of the independent variables was determined by conducting three-dimensional response surface analysis of the independent and dependent variables, choosing yield, eucalyptol and camphor abundance, as optimization criteria.

Funding
This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-0417

PC4-24 Cannabinoid content in industrial hemp (*Cannabis sativa* L.) varieties grown in Slovenia

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Hemp (*Cannabis sativa* L., subsp. sativa) contains cannabinoids that are of increasing scientific interest, the most known among them are cannabidiol (CBD) and (-)-trans-Δ9-tetrahydrocannabinol (Δ9-THC) with known therapeutic activities [1]. Fifteen varieties of common hemp from the 'Common catalogue of varieties of agricultural plant species' were analyzed: Fedora 17, KC Dora, Monoica, USO 3, Helena, Santhica 27, Tisza, Tiborszallasi, Antal, Carmagnola, Finola, Kompolti hibrid TC, Ferimon, Novosadska, Marina. Contents of cannabinoids Δ8-THC, Δ9-THCA, Δ9-THC, CBC, CBDA, CBD, CBDVA, CBDV, CBGA, CBG, CBL, CBN, THCV were determined by a validated HPLC method in bracts, remaining parts of inflorescences and whole inflorescences of each plant (n = 3). Contents of cannabinoids were highest in bracts. Contents of total CBD + CBDA in whole inflorescences were highest in: Helena (2,4 %), Tisza (1,9 %), Antal (1,8 %), Fedora 17 (1,6 %) and Tiborszallasi (1,6 %). The highest Δ9-THC + Δ9-THCA content was found in KC Dora (0,9 %), but lower than 0,2 % in other varieties. CBG + CBGA prevailed in Santhica 27 (3,1 %), with no significant amounts of other cannabinoids. For varieties Fedora 17, USO 31, Tisza, Tiborszallasi, and Antal, CBD content in whole inflorescences was comparable to the declared values. Uniformity of Δ9-THC/CBD ratio was shown for the varieties Helena (1:31), Kompolti hibrid TC (1:33), Finola (1:29), Monoica (1:29), and USO 31 (1:33). Less uniform Δ9-THC/CBD ratio was observed in Carmagnola (1:1 to 1:32), KC Dora (1:1 to 1:33), Tiborszallasi (1:1 to 1:30), and Tisza (1:1 to 1:31).

No Conflict of Interest Research was supported by the Slovenian Research Agency and Slovenian Ministry of Agriculture, Forestry and Food (research project reference: V4-1611).
Statement on behalf of all authors. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this work.

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PC4-25 Untargeted metabolomics sheds light on the secondary metabolism of Malpighiaceae family

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DOI 10.1055/s-0041-1736863

The chemical characterization of plant species with medicinal interest is one of the main bottlenecks for the discovery and development of new therapeutic agents inspired in natural products. Malpighiaceae family, for instance, although described for important biological properties, is still considered chemically underexplored [1]. Therefore, this study aimed to perform an untargeted metabolomic investigation of a dataset comprising leaves of 39 genera and 139 species from all the major phylogenetic groups currently accepted for the family. The extracts were analyzed by UHPLC-ESI-MS/MS, followed by multivariate data analysis, library searches, molecular networking, and cutting edge *in silico* tools to disclose the corresponded chemical space [2, 3]. Several metabolites were annotated, and our results show that classes and subclasses of compounds were specific to particular phylogenetic clades or genera, raising evolutionary hypotheses for the production of metabolites by different groups. Our systematic approach allows a step forward for the Malpighiaceae chemical characterization and chemosystematics, while the associated findings will certainly contribute to future drug discovery studies using natural products.

Conflict of Interest; Funding (Source, ID)
The authors have no conflicts of interest to declare. This research was funded by CNPq, the Brazilian Fulbright Commission, and FAPESP

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PC4-26 Authentication of *Stephania tetrandra* commercial products using DNA mini-barcoding

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DOI 10.1055/s-0041-1736864

Plant-based medicine are used worldwide for their desirable pharmacological properties. Although herbal medicines are often perceived as a more natural and safer alternative to conventional and modern drug therapies, there are many herbal products that actually cause serious complications. A historic example was reported in Belgium in the 1990s. Multiple incidents of severe renal disease were reported and linked to the substitution of the relatively harmless *Stephania tetrandra* (Hang Fang Ji) with the nephrotoxic *Aristolochia fangchi* (Guang Fang Ji) [1]. Although, there are methods available, such as HPLC and Hyperspectral imaging, to detect *Aristolochia* presence in herbal remedies, these have limitations that lower the efficiency. Therefore, there is still a need for a quick and inexpensive method for distinguishing between Fang Ji herbal medicines [2]. In this research DNA mini-barcoding, a method of species identification using a short section of DNA from a specific DNA region, is used as an alternative method to test *S. tetrandra* commercial samples and several *Aristolochia* samples. All samples have been tested with specie-specific primers designed to detect specific Fang Ji species. Results showed that specie-specific primers are able to detect different Fangi Ji species. *Aristolochia* and *Stephania* specific primers were used with commercial samples demonstrating that DNA mini-barcoding is a valid alternative to traditional DNA barcoding when samples' DNA is degraded and difficult to amplify.

No conflict of Interest; this project was supported by De Montfort University HEIF funds

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PC4-27 DNA metabarcoding for quality control of basil, oregano and paprika

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DOI 10.1055/s-0041-1736865

Herbs and spices are among the most vulnerable products to fraud [1]. The standard analytical tests are accurate to detect specific marker compounds, but they cannot assess the entire species composition of multi-ingredient products. To overcome this limitation, untargeted diagnostic tools are recomanded to be used in order to ensure the quality and safety of such complex products [2, 3]. In this study, DNA metabarcoding was used for the authentication of 62 products, containing basil, oregano, and paprika, collected from different retailers and importers in Norway. Our results showed varying degrees of discrepancy between constituent species and those listed on the products labels, despite the high product authenticity. We recomand that integrating DNA metabarcoding into the toolbox of analytical methods for quality control and assesment of fresh and/or processed plant-based products can considerably improve their quality.

The data presented in this paper was generated in a project commissioned by the Norwegian Food Safety Authority (Mattilsynet), within the "Overvåkning- og kontroll-program for krydder 2018 (57237)" project. This work was partially supported by a grant of the Romanian Ministry of Research and Innovation, CNCS – UEFISCDI, project number 157/2020 within PNCDI III.

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PC4-28 Rapid discrimination of secondary metabolite production caused by endophytic bacteria on Alkanna tinctoria (L.) Tausch calli based on untargeted HPTLC metabolomics

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DOI 10.1055/s-0041-1736866

Rhizospehric microorganisms tightly interact with their host plant by influencing her growth and modulating her metabolome. As proof of a concept, the effect of the extracellular medium and bacteria cells homogenate of five strain suspensions belonging to *Chitinophaga sp*., *Xanthomonas sp*., *Pseudomonas sp*., *Allorhizobium sp*., *Micromonospora sp*. were tested on *in vitro* callus culture of

Alkanna tinctoria (L.) Tausch. For rapid discrimination of callus metabolome modification, a High-Performance Thin-Layer Chromatography (HPTLC) image-fingerprints analysis method was developed. As a result of the multivariate analysis, bacteria cells homogenate and extracellular medium induced the production of different kinds of secondary metabolites. *Chitinophaga sp*. and *Allorhizobium sp*. bacteria extracellular medium were revealed as promising "metabolome modulator" since the chemical fingerprint is close to the one elicited by phytohormonal elicitor: Methyl jasmonate. Crucial markers were then identified using a TLC-MS interface combined with UHPLC-MS analysis. In parallel, HTPLC-DPPH effect-directed analysis was performed and demonstrated the production of new antioxidant metabolites after 30 days of coculture. The developed HPTLC-based untargeted metabolomic method has proved to be a robust approach to monitor interactions among bacteria endophytes and their host plant. The results confirmed that endophytes play an essential role in the biotransformation/induction of compounds of interest.

This work has been financed by the EU H2020-ITN-MICROMETABOLITE project (Grant N °721635)

PC4-32 UPLC-Q-TOF MS/MS metabolomic analysis of *Crepis incana* polar extract, a Greek endemic representative of the tribe Cichorieae.

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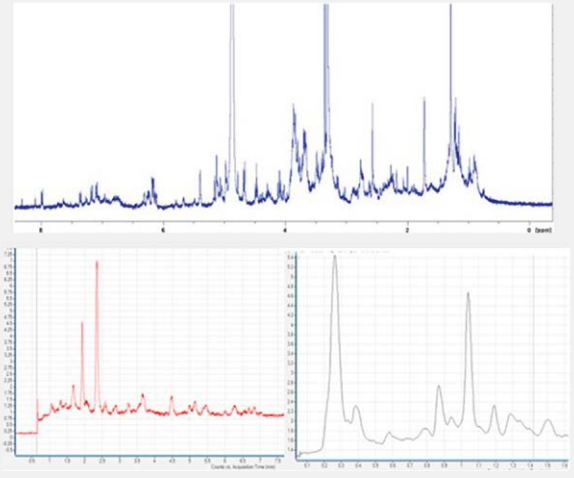
DOI 10.1055/s-0041-1736867

Crepis L. belongs to the Cichorieae tribe of the Asteraceae family [1]. In continuation to our previous studies on the pink dandelion *C. incana*[2], we report herein the investigation of the polar extract of the plant. Specialized natural products were identified via liquid chromatography-high resolution quadrupole mass spectrometry in both positive and negative ion modes and they were further confirmed through NMR spectroscopy. The metabolite profiling revealed the foremost presence of phenolic compounds, precisely, the extract was rich in luteolin and caffeic acid derivatives, both aglycons and glycosides. Moreover, a significant amount of a sesquitepene lactone, taraxinic acid β-D-glucopyranosyl ester, was traced down. Our study contributes to the characterization of *Crepis* polar extracts, which are limited in the literature. The metabolites accumulation varies significantly among species, thus further studies should be conducted.



▶ **Fig. 1** ^{1}H-NMR and UPLC-Q-TOF MS/MS.

Conflict of Interest; Funding (Source, ID)
The authors declare that they have no conflict of interest; The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 16274).

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PC4-33 Qualitative and quantitative analysis of pyrrolizidine alkaloids in crude extracts of industrial interest

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Pyrrolizidine alkaloids (PA) are reported as the most widely distributed naturally occurring secondary metabolites of high toxicological relevance. More than 660 compounds have been detected in floriferous plants. Their high occurrence and toxicity have triggered the development of different PA quantitative methodologies based on commercially available PAs. However, a limiting amount of standards are currently available and the effectiveness of the pre-purification steps used for their quantification remain uncertain.

The aim of our study is to shed light on the variability and the distribution of PAs in herbal extracts of industrial interest and to propose a validated quantification methodology for the evaluation of the total PA content excluding the pre-purification steps. Herein, we performed a comparative PA screening of eleven crude and SPE enriched extracts obtained from Boraginaceae, Fabaceae and Asteraceae families.

In total, 105 PAs were tentatively identifiedthrough a meticulous dereplication study of UHPLC-HRMS^{e} analyses by comparing our data with customized in-house libraries and literature data. In addition, a previously published quantification method was optimized to assess the total PA-content expressed as retronecine-equivalent, directly from their crude matrices assuring high sensitivity, recovery and reproducibility.

Our findings highlight a more accurate and exhaustive retronecine-based PA quantification methodology which may advantageously be exploited for the analysis of extracts of industrial interest. In addition, the effectiveness of the pre-purification steps, which are widely recommended in the literature for its benefits in both reducing matrix load and concentrating PAs from complex matrices, resulted ambiguous in relation to major PAs in the extracts.

PC4-34 Utilization of pomace oil production by-products to produce olive bioactive-specific enriched extracts and in-depth chemical characterization thereof.

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During pomace oil production several by-products are produced in mills and specifically wet olive pomace, dried olive pomace, exhausted olive cake, olive pomace skin and olive stones. According to the type of by-product, they are characterized by the presence of different classes of olive bioactive compounds, such as simple phenols and phenolic acids, secoiridoids, flavonoids, lignans and triterpenic acids [1]. However, despite their high commercial interest there is

only a limited number of studies regarding their detail chemical composition and the targeted recovery of bioactives. Thus, the initial aim of this work is the detailed chemical investigation of these by-products using HPLC-DAD, LC-HRMS & HRMS/MS and HPTLC analytical methods. Moreover, selective extraction and separation protocols were developed and applied to produce chemical class-specific enriched extracts, employing Ultrasound Assisted Extraction (UAE) and Fast Centrifugal Partition Chromatography (FCPC) techniques among others. Overall, in the context of the present study, it was possible to produce extracts enriched in secoiridoids or triterpenes or simple phenols from different types of olive pomace by-products which could have potential applications in the food, cosmetic and pharmaceutical industry.

Conflict of Interest; The authors declare no conflict of interest

Funding The present work was carried out and co-funded by the European Regional Development Fund (ERDF) and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH – CREATE - INNOVATE" (project TYREL code: 03053).

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PC4-35 Optimization of Oleuropein Extraction from Olive Leaves. Alternative approaches for the recovery of olive bioactive secoiridoids.

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Oleuropein (OLE) is the major secoiridoid of olive leaves with established beneficial effects on human health, proven by numerous scientific data, with cardioprotective properties to distinguish amongst others. Since olive leaves is one of the richest sources of OLE, there are several studies available in the literature regarding different methods and approaches for its isolation. In the present work, the impact of row material treatment (olive leaves drying conditions) and extraction method on OLE recovery from olive leaves, was investigated. Based on our results it was shown that the obtained OLE levels are strongly dependent on the drying protocol of leaves prior any treatment, as well as the method which is followed for the extraction, and more specifically the extraction solvent, the temperature and the pH. Additionally, it was evident that these factors also affect considerably the secondary metabolites content generally, qualitatively, and quantitatively. Indeed, based on the conditions used, new secoiridoids were detected, isolated, and finally identified by means of NMR and HRMS.

Overall, in the context of the present study, it was possible to develop an optimum protocol for the recovery of OLE from olives leaves, as well as to point out the high impact of different parameters related to starting material treatment and extraction protocols, towards the investigation of secondary metabolites in plants.

Conflict of Interest; Funding

The authors declare no conflict of interest. The present work was carried out and co-funded by the European Regional Development Fund (ERDF) and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH – CREATE - INNOVATE" (project HOLEA code: 03816).

8.5 Translational natural product pharmacology

PC5-1 Proanthocyanidines from EGb 761® improve impaired short term memory

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DOI 10.1055/s-0041-1736871

EGb 761®, a dry extract from Ginkgo biloba leaves, is used for the treatment of declining cognitive performance. The extract is adjusted to contain 22.0–27.0 % ginkgo flavonoids, 5.4–6.6 % terpene lactones and less than 5ppm ginkgolic acids. Further, EGb 761® contains about 7 % proanthocyanidines (PAC) [1] with a yet unknown contribution to the efficacy of EGb 761®. In the present work we aimed to assess the relevance of PAC constituents for the pharmacological activity of EGb 761®. One hour before testing spontaneous alternation in the T-maze, NMRI mice received perorally either vehicle, donepezil (0.3mg/kg), EGb 761® (10-300mg/kg), isolated PACs (3-300mg/kg), or two finished products with different Ginkgo extracts that showed eight-fold differences in PAC content but comparable levels of terpene lactones and ginkgo flavonoids (50mg/kg). 20 minutes prior to the session, mice were injected i.p. with 1mg/kg scopolamine to impair short term memory. Mice were observed for 20 trials and spontaneous alteration was documented. Both, EGb 761® (ED_{50} = 89mg/kg, E_{max} 90 %, n = 6) and its isolated PAC fraction (ED_{50} = 43mg/kg, E_{max} 71 %, n = 7-14) dose-dependently attenuated the scopolamine-mediated impairment. Interestingly, treatment with a Ginkgo product with a high PAC content extract (EGb 761®) showed better activity in reversing the scopolamine effect than a product with a low PAC content (63 % vs. 34 % inhibition).

In conclusion, we identified PACs as a novel, quantitatively relevant and pharmacologically active constituent of EGb 761®.

All authors are employees of Dr. Willmar Schwabe GmbH & Co. KG, Germany.

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PC5-3 Nutritionally related strategies for evaluation of vegetable oil and seedcake from seeds of a *Cynara cardunculus* Greek cultivar. From by-products to the functional food chain

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DOI 10.1055/s-0041-1736872

Cynara cardunculus is a native plant in the Mediterranean basin, known for its therapeutic properties of its leaves, and its applications on bioenergy sector [1]. The seeds of a Greek artichoke cultivar have been recently studied [1], expressing promising phytochemical and nutrient profile with interesting bioactivities.

In the framework of this study, the seeds' oil obtained from this cultivar by cold press was further examined. Sterol and fatty acid profiles, as well as total phenolic and lignan contents were evaluated and compared with highly consumed and respected edible vegetable oils (sesame, flaxseed and olive oil). Linoleic and oleic acids (ω-6 and ω-9, respectively) appeared as the most abundant fatty acids while is contained high amount of lignans (12.5 g/kg oil) expressed as arctigenin. Furthermore, the seedcake, obtained as seed oil's by-product, revealed as a rich source

of phenolics (111.4 mg GAE/g extract) (such as arctiin, tracheloside, cynarinine etc) showing promising antioxidant and AChE-enzyme inhibitory properties. Conclusively the eco-friendly delivery of *Cynara's* seed oil, is characterized from high % of mono- and poly-unsaturated fatty acids and lignans. Furthermore, Its nutritional characteristics (high energy and protein content), nominating it as a functional oil. The seedcake is also a promising pool of bioactive compounds with remarkable activities, which could be also used as a value-added crude material, rather than discarded as by-product.

The authors declare that there is no conflict of interest; This research received no external funding

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PC5-4 In silico, in vitro, and structural investigations on BAHD-enzymes from different species able to malonylate 21-hydroxypregnanes

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DOI 10.1055/s-0041-1736873

BAHD-acyltransferases represent a large enzyme family found ubiquitously in angiosperms. They are considered substrate-promiscuous, accepting various substrates ranging from small aliphatic alcohols to complex structures such as terpenoids or flavonoids. BAHDs catalyze the formation of esters using different acyl-CoA thioesters and are thus important enzymes of specialized plant metabolism [1]. It is assumed that one step in cardenolide formation, the 21-*O*-malonylation of 21-hydroxypregnanes, is catalyzed by a BAHD-malonyltransferase (21MaT).

To screen potential candidates able to malonate 21-hydroxypregnanes, we isolated cDNAs of BAHD-malonyltransferases from *Arabidopsis thaliana* (*At*PMaT1, *At*PMaT2), *Erysimum crepidifolium* (*Ec*MaT1) and *Digitalis lanata* (*Dl*MaT1). To predict substrate acceptance, homology models of these enzymes were generated. Docking simulations with those models implied that all tested enzymes, except *At*PMaT2, accept 21-hydroxypregnanes as substrates [2].

To verify the *in silico* results, the candidate genes were expressed in *E. coli* and the respective enzymes tested for 21MaT activity *in vitro*. All enzymes accepted 21-hydroxypregnanes as substrates. However, *At*PMaT1 turned out to have a remarkably higher specific 21MaT activity (20 – 700 times higher) as compared to the other enzymes tested.

To investigate this phenomenom, homology models of the various enzymes were compared.

*At*PMaT1 and *Ec*MaT1 show a high sequence identity but show considerable differences in enzyme activity *in vitro*. A comparison of the binding pockets revealed that their sequences only differ in two amino acids. To enlighten the catalytic role of these residues, site-directed mutagenesis experiments were performed and revealed that mutants of *At*PMaT1 show an up to 100-fold decrease in 21MaT activity.

Conflict of Interest; Funding (Source, ID)

This work was supported by grants of the DFG (German Science Foundation) for the project 427204405, the Cluster of Excellence "Engineering of Advanced Materials (EAM)", project EXC 315, and by the Central Institute for Scientific Computing (ZISC).

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PC5-5 Identification of parthenolide metabolites in human liver microsomes by LC-Q-TOF-MS/MS.

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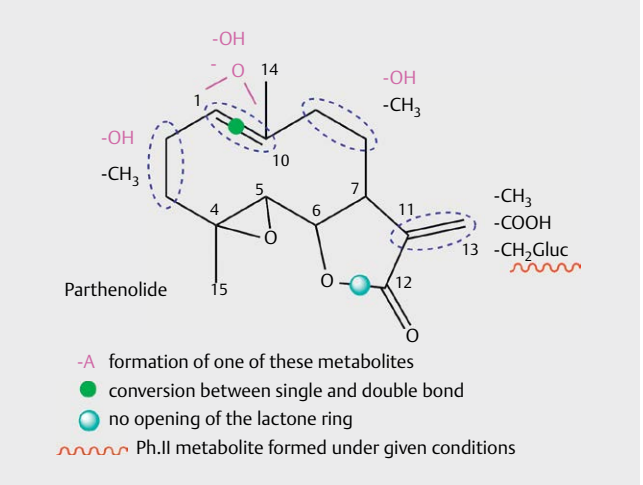
DOI 10.1055/s-0041-1736874

Sesquiterpene lactones (SLs) are highly active specialized metabolites, bearing an α-methylene-γ-lactone ring which is reacting through Michael type additions with sulfhydryl groups in biological systems. Parthenolide is a natural SL isolated from *Tanacetum parthenium* L. which has a long-term use in folk medicine, while due to its anti-cancer potential its semi-synthetic derivative was subject of clinical trials [1]. Although there are some data on the metabolic pathways and metabolites of a few SLs [2], parthenolide has not yet been investigated, thus this study characterizes its metabolites in human liver microsomes. Phase I, phase II (glucuronidation) as well as combined phase I + II metabolism were studied (Fig. 1). Metabolites were identified via liquid chromatography-high resolution quadrupole time-of-flight mass spectrometry. Eight metabolites were found, including four methylated (one lost the double bond at $\Delta_{10\text{-}1}$) and one hydroxylated. Moreover, no metabolites with an opened lactone-ring could be identified, however the exomethylenic double bond at $\Delta_{11\text{-}13}$ was subject of reduction, oxidation or glucuronidation.



▶ **Fig. 1**

Conflict of Interest; Funding

The authors declare no conflict of interest. Part of this work was supported by European Social Fund-ESF (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

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PC5-6 Effect of *in vitro* simulated digestion on the anti-*Helicobacter pylori* activity of different propolis extracts

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DOI 10.1055/s-0041-1736875

Helicobacter pylori (HP) is among the most common pathogens causing infection in human worldwide [1]. Oxidative stress and gastric inflammation are involved in the progression of HP-related gastric diseases, and can be targeted by integrating the conventional antibiotic treatment with polyphenol-enriched natural products. In this work, we have characterized three different propolis extracts and for the first time, we evaluated their stability under *in vitro* simulated gastric digestion, compared to their main constituents alone. The extract with higher stability to digestion (PPE) showed a MBC lower than 1 mg/mL on HP strains with different virulence factors. Moreover, the antioxidant and anti-inflammatory activity showed by PPE can contribute to its gastroprotective effect.

Finally, since urease is one of the virulence factor contributing to the establishment of a microenvironment which promotes HP infection, we evaluated the possible inhibition of this enzyme by using molecular docking simulations and *in vitro* colorimetric assay, showing that galangin and pinocembrin may be involved in this activity.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest.

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PC5-7 Neuroprotective and antidiabetic activities of *Thymus capitellatus* aqueous extracts

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DOI 10.1055/s-0041-1736876

Halt biodiversity loss is one of the main challenges present nowadays. Either due to climatic changes or anthropogenic activities, a warning number of species have been listed as threatened for a possible risk of extinction. For these reasons and due to particular edaphoclimatic conditions, *Thymus capitellatus*, a near-threatened species, can only be found on Portugal's southwest shore. Aqueous decoction (AD) extracts obtained from *T. capitellatus* aerial parts were analyzed to ascertain its phytochemical composition and evaluated for their neuroprotective and antidiabetic activities. HPLC-DAD analysis of the AD extracts revealed rosmarinic acid, salvianolic acids, and glycosidic derivatives of apigenin, luteolin, and quercetin, as the main components of this extract. Using acetylcholinesterase and tyrosinase inhibition assay it was observed that the extracts show potential neuroprotective effect. Regarding antidiabetic activity, selective inhibition of α-glucosidase was observed, in contrast with a low inhibition of α-amylase, showing a potential for reducing intestinal sugar absorption.

These results provide a new insight into *T. capitellatus* possible applications in pharmaceutical and nutraceutical industries and thus increasing the interest in its preservation.

Conflict of Interest and Funding

Authors declare no conflict of interest. Authors acknowledge funding from the Portuguese Science and Technology Foundation, and NORTE 2020, through European and National funds, under the projects UIDB/04033/2020 (CITAB), UIDB/00616/2020 (CQ-VR), as well as the PhD grant to C. M.-G. (SFRH/BD/145855/2019).

PC5-9 *Cirsium palustre* extracts as potential modifiers of colon motility in functional gut disorders – an *ex vivo* study

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DOI 10.1055/s-0041-1736877

Inflammatory bowel disease and many other functional gastrointestinal disorders represent a modern global threat to human health. Due to the complex etiology of the problem, the available treatment methods do not completely cure the disease and thus are not entirely satisfactory, and new treatment alternatives are intensively searched for. Thus, the study was aimed to verify the usefulness of *Cirsium palustre* (L.) Scop. (Asteraceae) extracts to modify colon dysmotility. The experiments were performed on an alternative experimental model of swine colon specimens, which can be used in translational medicine. Intestine preparations were incubated in modified Krebs-Henseleit solution under isometric conditions. The experiments included evaluating the effect of methanolic and 50 % methanolic extracts of *C. palustre* on the spontaneous and acetylcholine(ACh)-induced contractility of colon smooth muscle [1]. An analytical approach based on LC-ESI-MS was applied to obtain a metabolite profile of analyzed extracts. The results revealed significant and dose-dependent potency of both *C. palustre* extracts to modify colon motoric activity. Both extracts increased dose-dependently (0.00001-0.1 mg/mL), the spontaneous colon contractility reached over 1.5 fold enhancement of primary activity compared to the control treatment. The opposite effect of both extracts was observed in the case of ACh-evoked contractility. *C. palustre* decreased the magnitude of ACh-induced reaction dose-dependently. In the case of methanolic extract, the reduction was slightly more potent and resulted in a reaction amounting to approx. 60 % of control ACh contraction. The results of the performed study indicate that *C. palustre* can be considered a valuable, antispasmodic agent to address gastrointestinal hypermotility problems.

Conflict of Interest

The authors report no conflict of interest

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PC5-10 Cannabis terpenes demonstrate acute anti-seizure activity in rodent brain slices *in vitro*.

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Cannabis has received attention for its potential anti-seizure activity, however studies of cannabis have predominantly focused on the plant's cannabinoid constituents. This study aims to assess the anti-seizure potential of α-pinene and linalool, two monoterpenes commonly found in the *Cannabaceae* family of plants [1].

Extracellular local field potential (LFP) *in vitro* electrophysiology was employed to measure network neuronal activity in rat brain slices. Seizure-like events

were elicited using the bath application of the proconvulsant agent, 4-aminopyridine (100 µM). Following the establishment of baseline seizure activity, doses linalool (100 & 300 µM) or α-pinene (10 & 30 µM were added. Waveforms of seizure activity were recorded from the medial Entorhinal Cortex. The effects of each monoterpene on seizure duration (SD), first spike amplitude and power spectral density (PSD) were analysed. DMSO (0.03 % w/v) was used as a vehicle control. Addition of linalool resulted in a significant reduction in both SD and PSD when compared to baseline activity. Linalool also produced a significantly greater percentage decrease in PSD when compared with α-pinene or DMSO. α-Pinene failed to produce a significant change in any of the parameters measured at the concentrations studied. These results indicate that linalool possesses the ability to reduce both the duration and the area power of LFP seizure activity. This anticonvulsant activity may be attributable to modulation of glutamate neurotransmission and/or voltage-gated sodium channels [2, 3]. This evidence supports the potential use of linalool in the treatment of seizures in patients with epilepsy.

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PC5-11 A honokiol-enriched *Magnolia officinalis* Rehder & E.H. Wilson. bark extract alleviates neuropathic pain by inhibiting neuroinflammation

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DOI 10.1055/s-0041-1736879

Neuropathic pain is a chronic condition that influence patient's quality of life [1]. Current therapies are characterized by several side effects, which limits their prolonged used. *Magnolia officinalis* Rehder & E.H. Wilson bark extracts have been extensively used in traditional medicine. Its main constituent, honokiol, is an effective modulator of cannabinoid receptors [2]. In this work we evaluated the effect of a honokiol-enriched *M. officinalis* bark extract (MOE) in a mice model of peripheral neuropathy, investigating the mechanism of action in the spinal cord tissue. MOE 30 mg/kg reduced mechanical allodynia and thermal hyperalgesia 45 min after oral administration, without affecting locomotor activity and cognitive function. The anti-hyperalgesic effect was completely prevented by the co-administration with a CB1 antagonist (AM251). MOE reduced the inflammatory factors in spinal cord tissue of animal with neuropathy, compared to the control group and that these effects were completely reverted by AM251. These results highlight that *M. officinalis* could be considered a possible candidate for the management of neuropathic pain through the regulation of cannabinoid receptors, and encourage the search for honokiol structural analogs to be investigated as novel potential drugs against neuroinflammation.

Conflict of Interest; Funding (Source, ID)
The authors declare no conflict of interest.

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PC5-12 Ivy leaves dry extract EA 575® mediates biased β_2-adrenergic receptor signaling

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DOI 10.1055/s-0041-1736880

Background β_2-adrenergic receptor (β_2-AR) stimulation activates the G protein/cAMP pathway, which is opposed by the GRK2/β-arrestin 2 pathway. The latter is of disadvantage in the treatment of respiratory diseases. EA 575® is capable of mediating biased β_2-AR signaling.

Methods The impact of EA 575® on β_2-adrenergic signaling was tested in a dynamic mass redistribution assay in HEK wild-type and in HEK β-arrestin knock-out cells. GloSensor™ and PathHunter® assays were used to investigate cAMP formation and recruitment of β-arrestin 2. Additionally the influence of EA 575® on the NFκB transcriptional activity was determined in both HEK wild-type as well as HEK β-arrestin knock-out cells.

Results EA 575® inhibits the recruitment of β-arrestin 2 and thereby enhances G protein/cAMP signaling under β_2-stimulating conditions, as evidenced by a corresponding increase in cAMP formation. While β_2-AR-mediated inhibition of NFκB transcriptional activity is β-arrestin-dependent, EA 575® leads to significant inhibition of NFκB transcriptional activity in β-arrestin knock-out cells and thus via a β-arrestin-independent signaling pathway.

Conclusion EA 575® is the first active phytopharmaceutical ingredient for which biased β_2-adrenergic activation has been described. This shift towards G protein/cAMP signaling provides the molecular basis for the clinically proven efficacy of EA 575® in the treatment of lower respiratory tract diseases. In this light, EA 575® could potentially reduce β-arrestin-mediated adverse effects in new combinatorial therapeutic approaches.

Conflict of Interest; Funding
The authors declare no conflict of interest. This work was supported by a research grant of Engelhard Arzneimittel GmbH & Co.KG, Niederdorfelden, Germany.

PC5-13 The lichen compound evernic acid in combination with temozolomide regulates Wnt signaling in the glioblastoma cell line U-87

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Background Glioblastoma (GBM) is one of the most heterogeneous tumours and develops frequently resistance to chemotherapy, especially to temozolomide (TMZ). Evernic acid (EA) was shown to reduce TMZ dosages in a TMZ resistant glioblastoma cell line (U-87) [1]. Wnt is involved in resistance development.

Aim We investigated the effect of EA, TMZ and their combinations on Wnt (Wingless-related integration site) signaling in U-87 cells.

Methods Cells were treated with EA, TMZ and their combinations. After 24 hours of treatment RNA was isolated. Transcriptom analysis was performed by deep sequencing. Sequence alignment was performed by HISAT2-tool (data base: Homo sapiens hg38). Differential gene expression was calculated (DESeq2). Pathway enrichment and gene ontology analyses were performed in BioJupies.The Wnt inhibitory factor 1 (WIF1) release was measured by ELISA.

Results EA, TMZ and their combinations modulated the canonical Wnt signaling pathway (GO:0060070). Among these the combinations EA (35µM) -TMZ(320µM) ($EA_{35}TMZ_{320}$) and $EA_{20}TMZ_{580}$ downregulated the pathway significantly ($p<0.05$).

$EA_{35}TMZ_{320}$ downregulated Wnt5A (0.6f, $p<0.05$). Wnt3 was upregulated by EA_{45} (2.6f, $p<0.05$). All combinations downregulated FZD7, a receptor for the Wnt proteins (0.4–0.6f, $p<0.05$). TMZ_{600}, EA_{45}, $EA_{35}TMZ_{320}$ and $EA_{20}TMZ_{580}$ downregulated (TCF7L1), a downstream protein of the pathway.

Single compounds slightly upregulated the WIF1 protein, whereas combinations increased the WIF1 release. The combination EA (35µM)-TMZ(320µM) showed the highest upregulation of WIF1 (10.5 f, $p<0.001$).

Conclusion The combination of EA and TMZ is a potential candidate to reverse the TMZ-resistance of U-87 cells through the regulation of the Wnt pathway.

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PC5-14 Elucidation of multi-target mechanisms of STW5-II on functional dyspepsia

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Introduction STW 5-II, a combination of six plant extracts (*Iberis amara* L., *Mentha piperita* L., *Matricaria chamomilla* L., *Glycyrrhiza glabra* L., *Carum carvi* L., *Melissa officinalis* L.), is indi-cated for the treatment of Functional Dyspepsia (FD) and Irritable Bowel Syndrome (IBS) symptoms in Germany. Native compounds of these extracts have been previously identified by LC/MS. **Objective**: FD gene targets for the native compounds in STW 5-II were predicted followed by wet lab validations to characterize effects of STW5-II and its single herbs. **Methods**: Four databases were screened to identify gene targets involved in FD. Compound-target networks for FD were constructed to elucidate relationships between native compounds and FD targets. NCM460 colon cells were treated with STW 5-II, its individual herbs, or omeprazole. Gene expression (GE) analyses (deep sequencing) and RT-PCR were performed.

Results 96 genes were predicted as potential targets with AKT1, NOS3, HSP90A, TRPV and SMADs among the most common ones. *In silico* data were supported by GE-data (AKT1: 3.7fold(f); HSP90AA: 11.3f, TRPV3: 0.63 f, SMAD3: 5.1f ($p < 0.01$). RT-PCR confirmed regulations of AKT1, NOS3 and SMAD3 by STW 5-II. GO and reactome pathway analyses indicate, among others, significant upregulations of cell motility, epithelium development and lipid metabolism by STW 5-II. The individual herbs in STW 5-II contributed differentially to its overall activity, justifying the differential role of the six herbs in targeting different pathways in FD pathogenesis.

Conclusion *In silico* and *in vitro* studies confirmed that STW 5-II is a multi-target herbal formula acting on relevant targets of FD. Combinations of *in silico* and *in vitro* studies are useful to elucidate the complex molecular mechanisms of FD.

Conflict of Interest; Research was supported by the Steigerwald Arzneimittel GmbH, Bayer Consumer Health

PC5-15 Why are phytopharmaceuticals so successful in functional gastrointestinal disorders? Anti-inflammatory effects as a common mechanism of action

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Introduction In 1992 S.M Collins raised for the first time the question "Is the irritable gut an inflamed gut?" [1], suggesting that functional gastrointestinal diseases might be often caused by slightly increased concentrations of inflammatory mediators interfering with the enteric nervous system. Given that 53 herbal drugs have been rated for the therapy of gastrointestinal disorders by the HMPC of the EMA, the question comes up whether anti-inflammatory effects could be their common denominator. **Methods:** In order to pursue this assumption, database research [Medline] on anti-inflammatory effects of these and further herbal products was carried out.

Results For all these herbal products, anti-inflammatory properties could be identified. In contrast to the NSAIDs, herbal products have been shown to have protective effects on the mucosa of the gastrointestinal tract, associated with an increase of mucosa protective prostaglandins [2–4], as well in *in vitro* as *in vivo* models. **Conclusions:** Herbal drugs and preparations used in functional gastroenterological disorders have anti-inflammatory properties that can counteract functional disturbances.

Conflict of Interest; Funding (Source, ID)
OK is employee, KA was pharmacy intern at Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany, MTK and KN have been receiving honorary from of Steigerwald Arzneimittelwerk GmbH.

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PC5-16 Influence of St. John's wort extract STW3-VI, hyperforin and hyperoside on the signaling activity, density, and internalization of 5-HT_{2A} receptors

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DOI 10.1055/s-0041-1736884

Background Depressive disorders belong to the most frequent diseases. Antidepressant treatment try to normalize disturbed neurotransmitter systems. In this context activity, density, and trafficking of serotonin 2A (5-HT_{2A}) receptors are of high interest. Deeper insights into 5-HT_{2A} behavior are crucial for a better understanding of the effects of antidepressants like St. John's wort extracts.

Methods Influences of STW3-VI, hyperoside, and hyperforin on the signaling activity, receptor density and agonist-induced internalization were investigated in HEK293 reporter cells over-expressing different luciferase-based detection systems.

Results A six days pre-treatment with STW3-VI significantly and dose-dependently decreased 5-HT_{2A} receptor density under non-stimulating conditions by 12 % and receptor internalization after agonistic stimulation from 18.8 % to 8.1 %. The data suggest a decrease in 5-HT_{2A} receptor activity, which was confirmed by reduced intracellular calcium levels of up to 41 %. Pre-treatment with 1 µM hyperforin and 1 µM hyperoside for six days showed no statistically significant effects on those parameters.

Conclusion The data presented here demonstrate that 5-HT_{2A} receptors are an important target for understanding the complex mode of action of STW3-VI. The activity of 5-HT_{2A} receptors themselves and their crosstalk with other receptor systems, e. g., the 5-HT_{1A} receptor, is of importance in the treatment and development of depressive disorders.

Conflict of Interest; Funding (Source, ID)
SA and CK are employees, HH and SA have been receiving grants and travel support from Steigerwald Arzneimittelwerk GmbH.

8.6 Endophytes and microbes

PC6-3 Fungal community of *Dendrobium fimbriatum* (Orchidaceae): Selection of the most probable interacting fungi and their molecular compositions

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To increase our knowledge on the fungal community associated to Dendrobium fimbriatum (Orchidaceae), fungi were isolated from apparently healthy adult plants, and their ability to produce different metabolites (phytohormones, siderophores) and hydrolytic enzymes, as well as their anti-phytopathogenic activity, were evaluated ex situ.

A total of 25 cultivable fungal species were isolated and identified based on their ITS sequence by a sequence similarity search in GenBank. They belong predominantly to *Fusarium*, *Trichoderma*, *Colletotrichum*, *Curvularia* and *Didymella* genera. Among them, 3 fungi, initially isolated from the roots, *Fusarium sp.*, *Trichoderma yunnanense*, and *Curvularia sp.*, showed a high potential to act on plant growth and plant defense [1].

Their chemical profiles investigated by LC-MS/MS analysis and represented by molecular networking, highlighted the distinctiveness of *Trichoderma yunnanense* metabolites. Major constituents of the fungal extracts were putatively identified as harzianic acid, fusaric acid and their derivatives, and 2,4,6-octatrienoic acid

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PC6-4 Arbuscular mycorrhizal fungi (AMF) modulate metabolites production of Anchusa officinalis L. under semi-hydroponic cultivation system

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DOI 10.1055/s-0041-1736886

In this study, two independent plant growth experiments, associating *A. officinalis* to the AMF *R. irregularis* MUCL 41833, were conducted in a semi-hydroponic (S-H) cultivation system to assess the AMF impact on primary and secondary metabolism during a 9 (Exp. 1) and 30 days (Exp. 2) period of growth. The total fresh weight as well as the AMF root colonization was assessed in both experiments and the differences in the content of primary (PMs) and secondary metabolites (SMs) in shoots and roots as well as in exudates of M (mycorrhized) and NM (non-mycorrhized) plants were evaluated by an untargeted UHPLC-HRMS metabolomics approach combined with multivariate data analysis. The increased plant fitness, in terms of growth rate, observed for M plants in Exp. 1, was followed by an enhance production of PMs,

including organic acids and key amino acids. Similarly, SMs production has been significantly affected. 15 di-, tri- and tetra- meric C_6-C_3 derivatives of caffeic acid were mainly up-regulated in the roots of M plants while 4 oleanane-types saponins were overexpressed in the shoots. As a result of AMF symbiosis, herein, we describe for the first time, two salvianolic acid B derivatives and one new rosmarinic acid derivative, all presenting a common substitution pattern. An overexpression of methylated compounds was underlined in M plants suggesting that AMF has the potential to induce production of specific compounds.

This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement Nº 721635.

PC6-5 Gut Microbiota and Medicinal Plants for Mental Health: Is there a Link?

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DOI 10.1055/s-0041-1736887

For centuries, medicinal plants have been used in traditional medicine to support mental health and alleviate neurological disorders. There is a growing evidence that gut microbiota play an important role in the pathophysiology of mental disorders via modulating the microbiome-gut-brain axis. Therefore, the interaction between medicinal plants and gut microbiota could be relevant to explain their mental health-promoting effects [1]. This systematic review has been done on 2-steps: first, medicinal plants for which clinical studies on anxiety, depression, sleep disorders or cognitive dysfunction are available, were identified and second, studies on interaction of those plants with gut microbiota were retrieved. A literature search was performed using the online databases Pubmed and Embase, and a total of 887 publications were screened. 210 publications were analyzed as full-text documents, of which 104 studies met the inclusion criteria. In total, 34 mental health-related medicinal plants with in vitro and/or in vivo data on possible interactions with gut microbiota have been identified. Among the most intensively investigated herbs are *Schisandra chinensis* and *Panax quinquefolius* [2, 3]. In many cases, the herbal extracts were able to inverse gut dysbiosis and/ or to modulate the Firmicutes/Bacteroides ratio in various animal models [4]. Since many potentially relevant plant constituents cannot cross the blood brain barrier in their genuine form, pre-clinical or intervention studies need to consider the gut microbiota as an additional piece of the puzzle to explain the mode of action of medicinal plants on mental health.

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8.7 Regulation of herbal and traditional medicines

PC7-1 Functional gastrointestinal diseases in children: Pharmacoepidemiological data on STW 5 from the PhytoVIS data base

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DOI 10.1055/s-0041-1736888

Introduction Data from pharmacoepidemiological research can provide insight where clinical trials are difficult to conduct, as e. g., in children. [1].

This also applies to STW 5, a herbal medicinal product used for the treatment of functional GI diseases. It is approved for use in children, and there is a large body of data on overall 44 488 children from non-interventional studies available [2]. To broaden this evidence, data from the German PhytoVIS study, with 20,870 patients presumably the world's largest pharmacoepidemiological study on the use of herbal medicinal products [3], were evaluated.

Method A screening on paediatric patients using STW 5 was conducted.

Results Overall, 77 datasets from the paediatric population were evaluated (60 % female, 40 % male). Dyspepsia-associated symptoms (FD) were the largest group (90 %) while IBS could be assigned to 4 % and FD-/IBS associated to 6 % of the patients. In most cases, the onset of the therapeutic effect was within 60 minutes. In 60 % of the patients, the treatment was rated as very, in 30 % as moderately and in 10 % as minimally effective. 7 % of patients experienced slight adverse effects with no significant impairment and 93 % experienced no adverse effects at all.

Conclusion The results confirm the therapeutic usefulness and safety of STW 5 in children [2]. In general, the data from the PhytoVIS study turned out to be a valuable basis for studying the pharmacoepidemiology of children in Germany.

Conflict of Interest; Funding (Source, ID)

CS and JM have been interns, OK is employee of Steigerwald Arzneimittelwerk GmbH. KN received honoraries from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.

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PC7-2 Synergy research in gastrointestinal disorders: Herbal medicinal products at the forefront

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DOI 10.1055/s-0041-1736889

Herbal medicinal products are frequently used in gastrointestinal disorders, and are also a domain of combination products in this field. So, it is interesting, whether these are backed by studies that quantify synergy effects.

Methods A literature search on the combination effects of combination products in gastroenterology was carried out, focusing on studies that, following the work of Berenbaum resp. Chou and Talalay [1, 2], allow a quantitative determinantion of synergistic, additive and antagonistic effects.

Results Studies quantifying combination effects are available for two combination products, STW 5, a combination of nine, and STW 5- II, a combination of six plant extracts. A study of Ulrich-Merzenich et al. [3] shows that chamomile and peppermint extracts act synergistically with Iberis extract in an esophageal epithelial cell line with bile acid-induced inflammation. Studies of Hoser et al [4] showed that Iberis and lemon balm extract synergistically reduced the LPS-induced lactate dehydrogenase (LDH) release from CaCo-2 cells, while chamomile and Iberis showed additive inhibtory effects on LPS-induced TNFα release in THP-1 cells effects. Synergistic effects of Iberis and peppermint or caraway extracts have also been inhibiting acetylcholine (ACh) -induced contractions of ileum preparations of rats in vitro.

Conclusion A proof of synergistic effects is available for combination partners from two herbal preparations with clinically proven effectiveness in functional dyspepsia and irritable bowel syndrome, These are STW 5, which, in accordance with the pharmacological profile of its components [5], is preferred for acute and motility-related complaints, and STW 5-II, which is preferred for long-term complaints, based on the calming, anti-inflammatory and mucosal protective profile of its components. These effects support the concept of a multi-target therapy in these indications [10].

Conflict of Interest; Funding (Source, ID)

OK is employee of Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany, HS has been receiving honoraries from of Steigerwald Arzneimittelwerk GmbH.

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PC7-3 Cannabis for medical use – implementation of a new regulation in Germany

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DOI 10.1055/s-0041-1736890

Worldwide, regulatory frameworks for medical use of cannabis are under development. There is a basic international agreement, the"Single Convention on Narcotic Drugs", released by the United Nations in 1961 and amended in 1972 [1]. The Single Convention defines a broad framework, one of the key elements being the establishment of a national Cannabis Agency, if cultivation in the area of a state is intended.

The national regulation on cannabis for medical use in Germany was modified in 2017 in order to set an adequate framework to improve supply of severely ill patients. Key elements of the legislation were the legal base to prescribe cannabis-derived medicinal products to individual persons by medical doctors, establishment of adequate quality standards, options for reimbursement by the health-care system, a non-interventional data-monitoring and establishment of a national cannabis agency at BfArM. The establishment of cultivation and distribution of cannabis for medical use had to follow the laws of procurement. In a European tender three contract partners were identified for cultivation (including processing and packaging), in another European tender a contract partner for distribution to pharmacies. In total, the contracts will provide more than 10.000 kg of cannabis flowers of pharmaceutical quality to be supplied to patients. As a result of the implementation of this new legislation, the first cannabis for medical use grown in Germany was released to the market in July 2021 with BfArM acting as wholesaler and pharmaceutical company.

Conflict of Interest: The authors state, that there is no financial conflict of interest.

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PC7-4 Emergent quality issues in Chinese medicinal plants: Investigation of their contemporary occurrence and historical persistence through questionnaires and interviews of key informants.

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DOI 10.1055/s-0041-1736891

Introduction 88 % of the world's population use herbal medicine and Chinese medicinal plants **(CMP)** are the most prevalent. Though much advanced analytical testing and legislation was implemented to control CMP quality, problems such as adulteration, substitution and toxicity persist [1]. Most proposed future solutions are iterations of previous efforts, which raises the question if other alternative solutions should be considered?

Aim To investigate solutions to CMP quality issues by consulting with key informants **(KI)** using questionnaires and interviews

Methos 29 KI were purposefully identified and invited by email [2]. Their responses were thematically analysed [3].

Findings Respondents in the fields of; education (9), supply (5), manufacturing (2), research (2), and regulation (2), completed 5 questionnaires and 15 interviews. CMP quality issues Identified were, product integrity (authenticity, adulteration, contamination, potency, substitution) and toxicity. Reasons for CMP quality issues and suggested solutions were:

(1) Supply chain opacity. Reduced through educating stakeholders and regulators more specifically about CMP.
(2) Testing and legislation does not fully accommodate phytochemical variation. Improved through re-evaluating basic assumptions and accepting that good quality herbs can be variable.
(3) Value motivated opportunistic behaviour, improved by including human motivation in future solutions, such as incentives and profit sharing.

Conclusion Solutions to CMP quality issues were investigated and proposed.

No Conflicts of interest
PhD studentship sponsored by Brion Research Institute of Taiwan (Sun Ten Group) and Herbprime Ltd UK

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8.8 Medicinal plants and natural product research on Traditional Medicines

PC8-1 Hydrodistillation Assay and Essential Oil Optimising Yield Study – a Comparative Analysis of Essential Oil Content of Irish Grown and Commercially sourced Chamomile *Matricaria recutita*

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DOI 10.1055/s-0041-1736892

Essential oil (EO) was extracted from Irish grown chamomile and a commercial sample of chamomile by hydrodistillation using a Clevenger apparatus utilising a procedure calibrated during this study. Samples were found to contain a significantly higher concentration of EO than the commercial sample. The average volume extracted from the Irish grown samples and commercial sample was 0.765 mL/100 g and 0.3 mL/100 g, respectively. The highest EO content was extracted from the samples sourced from LI (Long Island), suggesting that *Matricaria recutita* may thrive in an environment exposed to harsh weather conditions. It was confirmed that the addition of a solvent during the hydrodistillation process did not affect the EO yield, as suggested by Clevenger[1]. To definitively determine the constituent profile of the samples and evaluate the quality, all samples should be further analysed using quantitative analytical techniques, e. g., HPTLC, HPLC, GC-MS.

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PC8-2 Quo Vadis? Plants of the Gods and Their Recently Discovered Therapeutic Applications

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DOI 10.1055/s-0041-1736893

Plant-based psychedelics, also known as serotonergic hallucinogens (e.g., Mescaline, psilocybin, and N, N-dimethyltryptamine), have an ancient history of medicinal use. The early reports on psychedelics in the 50s ignited the initial interest in the therapeutic use of psychedelic drugs in psychiatry [1]. Unfortunately, politically driven prohibitive legislatures in the mid-1960s and subsequent passage of the Substance Act of 1970 –which placed psychedelic drugs in the Schedule I category– effectively ended all major psychedelic research programs [2]. More recently, however, there has been a renewal in interest and promise of psychedelic research (Fig 1).
Accumulated research to date sheds light on the potential of psychedelics as a breakthrough therapy for different psychiatric disorders; particularly for conditions that conventional methods like selective serotonin reuptake inhibitors (SSRIs) proven to be semi- or non-effective (e.g., major depressive disorder, posttraumatic stress disorder, anorexia nervosa, etc.) [3].
Nevertheless, the therapeutic potential of psychedelics goes far beyond the mental health. During the last five years (2016-2021), 55 clinical studies have been conducted with psychedelics, not only in the field of psychiatry but also in chronic pain (e.g., cluster headaches) and general well-being (e.g., social connectedness). [4, 5]. These recent discoveries provide a scientific road map for investigating and applying natural psychedelics in medicine.
In summary, a new door has been opened for the diverse medical purposes of serotonergic psychedelics. These sacred compounds operate through unique mechanisms that show promising effects for various debilitating and lethal disorders and should be rigorously researched [1, 3].

Conflict of Interest; Funding (Source, ID)
N.A.

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PC8-3 Enhanced hypocrellin production in mycelium *Shiraia* culture by sodium nitroprusside

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Hypocrellins are main perylenequinone pigments isolated from fruit-bodies of bambusicolous *Shiraia* fungi, which have been used in traditional Chinese medicine. Hypocrellin A (HA) and B (HB) are developed as new non-porphyrin and reactive oxygen species (ROS)-generating photosensitizers in photodynamic therapy (PDT) for cancers, viruses and infectious microbes [1, 2]. *Shiraia* mycelium culture has become a promising production process of the new PDT agents [3, 4]. Nitric oxide (NO) is an important signal molecule to elicit the production of secondary metabolites in plant stress responses [5]. In this study, sodium nitroprusside (SNP) was used as a NO donor to induce hypocrellin production in *Shiraia* mycelium cultures. SNP application could not only enhance HA content by 178.96 % in mycelia, but also stimulate its efflux to the medium. SNP acted as a pro-oxidant by up-regulating the gene expression and activity of (ROS) generating NADPH oxidase (NOX) and antioxidant enzymes. Moreover, SNP treatment increased the proportion of total unsaturated fatty acids in the hypha membranes and enhanced membrane permeability. Gene ontology (GO) analysis revealed that SNP treatment could up-regulate biosynthetic genes for hypocrellins and activate the transporter protein major facilitator superfamily (MFS) for the exudation. The results can facitate further understanding signa-

ling molecules in the hypocrellin biosynthesis and provide a practical biotechnological strategy for enhanced hypocrellin production.

Funding

This work was supported by the National Natural Science Foundation of China (No. 82073955 and 81773696) and the Priority Academic Program Development of the Jiangsu Higher Education Institutes (PAPD).

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PC8-4 *Acmella uliginosa* (Sw) Cass. leaves extracts mitigate Complete-Freund Adjuvant (CFA) induced arthritis in rats via angiogenesis suppression

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Acmella uliginosa has been demonstrated to exhibit diuretic, antibacterial, antimalarial and anti-inflammatory activities. Since there has been no study reported on its anti-arthritic effect yet, this study was performed to investigate the anti-arthritic potential of its ethanol (AULE) and fractionated hexane (AULH) extracts on complete Freund's adjuvant (CFA)-induced arthritic rats for duration of 28 days. Both AULE and AULH were prepared at 1, 10 and 30 mg/kg respectively based on the results in our preliminary studies. A total of 54 SD rats were randomly induced via subcutaneous injection of CFA onto the right hind paw of rats except for rats in the normal group. Oral treatments were administered starting from day 9 until 28. Paw volume, severity of arthritis, and body weight gain were recorded. On day 28, the rats were sacrificed. The hind paw tissues were homogenized to estimate COX-2, 5-LOX and VEGF protein expressions. The results showed both extracts significantly ($P < 0.05$) reduced rats paw volume and severity of arthritis at all doses of extracts in a dose-independent manner, when compared to negative control. At lower dose; 1 mg/kg, both AULE and AULH showed highest percentages of inhibition (72.95 % and 72.60 %) respectively which demonstrated equal efficacy with indomethacin at 3 mg/kg (77.74 % inhibition). AULE and AULH also significantly decreased the protein levels of COX-2, 5-LOX and VEGF in arthritic rats, which validated that both AULE and AULH may exhibit anti-arthritic effects via angiogenesis suppression.

Conflict of Interest; None Funding (Ministry of Higher Education, Malaysia, GP-IPS/2016/9482000)

Statement on behalf of all authors. The abstract has been read and approved by all named authors

PC8-5 Apoptosis induction in C32 human melanoma cells by *Jasione montana* and its main phytoconstituents

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Jasione montana L. (Campanulaceae) is a common biennial plant, with ethnopharmacological report indicates its use in sleep disorders in children [1]. Because our previous study showed significant cytotoxic activity of the Et_2O fraction (JM4), we decided to evaluate on human melanoma cells (CRL-1585) the apoptosis induction ability of JM4 (25-100 µg/mL) and its main compound 22 (25 µg/mL) assessed as luteolin based on the LC-MS phytochemical profile. The characterization of apoptotic pathways through caspase activation proved that JM4 (100 µg/mL) influences the initiation of the external and intrinsic (mitochondrial) apoptotic pathway by activating caspase-8 (41.8 ± 0.5 %), caspase-9 (40.9 ± 2.4 %) and caspase-10 (43.7 ± 1.1 %), as well as activation of the effector caspase, caspase-3, directly responsible for apoptosis and DNA damage. The obtained results were almost twofold more significant than after treatment with vinblastine sulphate (positive control). Luteolin (22) (25 µg/mL) displayed a slightly weaker impact on the activation of mentioned caspases. The results of the intrinsic pathway of mitochondrial apoptosis agree with the previous reports documenting the ability of JM4 and 22 to induce mitochondrial membrane potential. Moreover, the alterations of cell cycle progression demonstrate JM4 (100 µg/mL) and 22 (25 µg/mL) to accumulate C32 cells in the S and G2/M phases and reduce the G1 phase. Our results indicated that the fraction from J. montana and its main compound, luteolin demonstrated significant proapoptotic potential and can effectively prepare with anticancer properties. Further studies of the phytochemical and biological assays are currently in progress.

Conflict of Interest The authors report no conflict of interest

Funding

(№ POWR.03.02.00-00-I051/16 from European Union funds, POWER 2014-2020, grant № 05/IMSD/G/2019.)

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PC8-7 Chemical analyzes of Matricaria pubescens and Matricaria recutita polar extracts and their protective effects on 3T3 fibroblasts

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Matricaria pubescens (Asteraceae), known as hairy chamomile, is endemic in North Africa and is used in rheumatic and muscular pains, coughs, allergies, ocular affections, dysmenorrhea, scorpion stings, dehydration and toothaches [1]. In Europe *M. recutita* is used for mild gastrointestinal problems, ulcers and inflammations of mouth and throat, for irritated skin and mucosae and for the

relief of common cold [2]. As a part of a project aiming at studying *Matricaria* sp. the analysis of *M. pubescens* from Algeria and *M. recutita* from Greece was undertaken. Methanolic and hydroalcoholic extracts were studied by HPLC-PDA-MS and chromatographic analyzes followed by 1D and 2D NMR. In *M. pubescens* more than 20 compounds have been identified up to now, among them quercetagenin-3-O-glucopyranoside, reported for the first time in *Matricaria* sp. and two polyamines previously reported in other Asteraceae sp. [3]. Fingerprint analysis and comparison of this species to the officially recognized in Europe *M. recutita* L. shows many similarities and justifies the ethnopharmacological uses in Algerian traditional medicine. *M. recutita* extracts from Greece were also found rich in phenolic constituents. The characterized extracts were screened for their protective effects on 3T3 fibroblasts. The cells were exposed to UVA light to 5-7J/cm^2 in presence of different doses of the extracts. Cell viability and oxidative stress were evaluated by neutral red absorption (540nm) and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester fluorescence (excitation at 485 and emission at 520nm). *Matricaria pubescens* methanolic and hydromethanolic extracts did not exhibit a significant anti-inflammatory activity. On the contrary, *Matricaria recutita* methanol extracts at low doses (0.1µg/ml, 1 µg/ml and 10 mg/ml) showed a considerable protective effect in the milder inflammation model (60min exposure to UV light).

Conflict of Interest

The authors declare no conflicts of interest.

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PC8-8 The seed oil from *Pistacia terebinthus* from Chios Island (Greece) as a promising source of functional crude material

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Pistacia terebinthus (terebinth or turpentine tree), (Anacardiaceae) is a small tree growing widely in the Mediterranean area. This wild *Pistacia* species has been used in folk medicine and human nutrition [1], while its seeds and oil have shown several bioactive properties.

This study aimed to evaluate the chemical profile of *P. terebinthus* seed oil gathered wild from Chios, due to its unique and long historical use in Greece for special culinary purposes. Regarding the evaluation of its sterol and fatty acid profiles (GC-MS), it is noteworthy that squalene was identified as the most abundant metabolite in the unsaponified fraction (approx. 80%), followed by β-sitosterol. In the esterified fraction, oleic acid (61.94%), palmitic, linoleic and stearic acids were also detected. The total phenolic content of the oil (3.6 g GAE/kg oil) has been further determined using Folin-Ciocalteu method.

The results presented herein appeared comparatively superior of previous reported data [2], due to the high oleic acid content. Furthermore, the main composition of fatty acids of the studied oil is close to the olive oil's, as well as the extremely high amount of squalene highlights its value. Squalene is a well-known skin health promoting agent.

In the framework of exploitation of forage from wild, as a sustainable and ecological friendly method for potential value-added materials, terebinth seeds and oil could be revealed as a rich source of unsaturated fatty acids, especially omega-9 and omega-6, beneficiary to human health, with interest applications towards food and/or cosmetic areas.

No conflict of interest; This research received no external funding.

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PC8-9 Number Significance of over-the-counter (OTC) herbal medicinal products (HMP): ATC code R

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Respiratory diseases, partially the flu, affect millions of people and are of global social significance. Common symptoms are cough, rhinorrhea and fever. The study focuses on the diversity of HMP classified in ATC code R in the current "List of medicinal products without prescription in the Republic of Bulgaria" [1].

The mentioned list has been used to estimate the HMP proportion, assess the ATC code and pharmaceutical form distribution and determine the significance of native plants used.

19% out of 218 OTC medicines classified in ATC code R are registered as HMP. Most of them are expectorants (R05CA). Syrups are the most common pharmaceutical from (39%). 16 of the 29 plants used to produce the active substances of the HMP are native to the Bulgarian flora. Only 34% of the 26 species used to treat respiratory disorders according to traditional medicine scources overlap with the ones found in the current study [2].

There is potential for creating new HMP shown by the biodiversity and the ethnopharmacological knowledge in the country.

Acknowledgements

The authors acknowledge the financial support of the EXTREME project, funded by the Bulgarian Ministry of Education and Science, D01-76/30.03.2021 through the programme "European Scientific Networks".

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PC8-10 Traditional Use of Common Selfheal as medicinal plant in the Balkans

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Common selfheal *Prunella vulgaris* L. (Lamiaceae) is in the focus of studies mostly as wide used herb in traditional Chinese medicine (TCM). There is fragmentary knowledge of it's folk use in Europe. The purpose of the study is to present the data on the common selfheal as a herbal remedy in Bulgaria and the Balkan region.

Data were collected using semi-structured interviews and from main sources that provide information for medicinal plants for the end of 19th and to the middle of the 20th century [1, 2].

In the study area, folk names mainly reflect the use of herb for wound healing. The aboveground part was only used (Prunellae herba) which contrasts with the dominant use of dried fruit spike (Prunellae spica) in TCM.

Totally 84 folk recipes were collected. The most frequently prepared formulation was an infusion (64%) which is used internally for cough and pain relief. Field data have shown its increased use as a haemorrhoid agent. Externally applied as infusion, decoction and maceration in olive oil as well as freshly crushed leaves, for treatment of different types of wounds and bites.

The study presents new data and shows a specific profile of traditional use in the study area.

Acknowledgements
The authors are grateful to the financial support of Bulgarian National Science Fund at the Ministry of Education and Science, Contract No 2901/KP-06-China/15/17.12.2020.
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PC8-11 *Prunella vulgaris* herbal products - diversity and opportunities

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The common selfheal, *Prunella vulgaris* L. (Lamiaceae) is Holarctic in distribution, where the morphological part "spica" is mainly used for treatment of many diseases, e.g., tuberculosis and mastitis. In recent years, interest in the fruit-spike from the medical plant *P. vulgaris* has been growing worldwide, leading to its inclusion in the European Pharmacopoeia in 2017.
The focus of this study is the products containing herbal drugs and/or herbal preparations of common selfheal, established from publicly available databases [1, 2]. They have been analyzed to determine their diversity, active substances and application.
Over 50 herbal products have been identified. The main application of the established dietary supplements (52 %) is as immunostimulants and in the treatment of skin diseases and superficial wounds, which corresponds to part of the experimentally established in the last decade antibacterial, antiviral, immunomodulatory etc. properties. Several types of cosmetic products (41 %) have been identified during the study where herbal preparations from *P. vulgaris* are used as a core ingredient in cleansing gels, hair balsams, in baby herbal cosmetic series with anti-inflammatory and antiallergic properties etc.
The analysis of the data and the absence of registered herbal medicinal products shows *Prunella vulgaris* as a herb with potential for the development of medicinal products.
Conflict of Interest; Funding (Source, ID)
The authors declare no conflict of interest; The authors acknowledge the financial support of the EXTREME project, funded by the Bulgarian Ministry of Education and Science, D01-76/30.03.2021.
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PC8-12 Restoring cardenolide accumulation in RNAi-mediated *DlP5βR1* and *DlP5βR2* knockdown shoot culture lines of *Digitalis lanata* and studying the effects of precursor feeding on cardenolide free dark-cultured *D. lanata* shoots

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Progesterone 5β-reductases (P5βR) catalyze the reduction of progesterone to 5β-pregnane-3,20-dione, which is a crucial step in the biosynthesis of the 5β-cardenolides. Two PRISE genes, termed *DlP5βR1* (AY585867.1) and *DlP5βR2* (HM210089.1) were isolated from *Digitalis lanata (Dl)*. RNAi-mediated *DlP5βR1* and *DlP5βR2* knockdown shoot culture lines provided experimental evidence for the participation of P5βRs in 5β-cardenolide formation [1]. These lines show reduced cardenolide (ca. 25 - 30 %) levels as compared with wildtype shoots of the same shoot culture clone (set to equal 100 %). Progesterone levels in RNAi-mediated knockdown lines were decreased compared to WT shoots and cardenolides levels could not be restored by exogenously added progesterone. We here tried to restore cardenolide content of the knockdown lines by feeding 5β-pregnane-3,20-dione, a cardenolide precursor downstream of progesterone. We observed a recovery of cardenolide content in *Dl P5βR1-RNAi* lines whereas the level in the controls was not further increased.
Cardenolide content in *Dl P5βR1-RNAi* lines reached around 2/3 (0.8 µmol × g − 1 DW) of the cardenolide level of WT shoots. Still high GSH levels in *Dl P5βR2-RNAi* lines seem to continuously disturb cardenolide formation. Cardenolide levels only increased from 0.3 µmol * g^{-1} DW to 0.5 µmol * g^{-1} DW.
Besides using a system of RNAi-mediated knockdown lines, we established cardenolid-free *D. lanata* shoot cultures by dark cultivation to study exogenously feed precursor effects on cardenolide formation [2]. Feeding 5β-pregnane-3,20-dione to *D. lanata* shoots kept in dark, cardenolide content remained unchanged low, but GSH and hydrogen peroxide levels decreased, indicating a reduced stress level.
Conflict of Interest; Funding (Source, ID)
No conflict of interest. On behalf of all authors.
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PC8-13 Liposomal formulations of *Alkanna tinctoria* root extracts for dermal applications

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Alkannins and Shikonins (A/S) are secondary metabolites with a well-established spectrum of biological and pharmacological activities, such as strong wound healing, regenerative, anti-inflammatory, antimicrobial and antioxidant. Their incorporation in liposomes can lead to higher therapeutic efficacy and also minimize side effects. The aim of this work was to encapsulate standardized oily extracts from the roots of *Alkanna tinctoria* in liposomes for dermal applications. Liposomes were prepared by using Pro-Lipo™ Neo (Lucas Mayer Cosmetics), a mixture of already organized phospholipids (pro-liposomes), which can be ordered to liposomes only with water addition. Experimental design (FCC) was employed to evaluate the effect of several parameters (drug/lipid ratio, water phase/lipid phase ratio and A/S concentration in the oily extract) on the mean particle size and encapsulation efficiency (EE) aiming to optimize the liposomal formulation. 32 samples were prepared, fully characterized and evaluated. The optimum formulation showed a mean particle size of 106 nm, z-potential value of -48.1 mV and EE % up to 72 %. The *in vitro* release profiles were satisfactory, and the formulations were stable for a 2 months period.
Funding
AEA, KNK and ANA acknowledge support of this work by the project "Upgrading the plant capital" (MIS5002803) implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

PC8-15 Phytochemical Analysis and Dermo-Cosmetic Evaluation of *Cymbidium* Sw. cultivation by-products. Circular economy in North Aegean island of Samos.

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The genus *Cymbidium* Sw. is one of the most popular orchid commercialized either as loose flower or as potted plant in floriculture worldwide. The plant was provided by "Garoufalis" greenhouses which produces annually more than 100,000 pots of 80 different varieties. The non- marketable parts are discarded (unsuitable flowers, leaves, pseudobulbs, roots), generating thus an enormous quantity of unutilized biomass.The above by-products (extracts and pure compounds) were investigated for their dermo-cosmetic potential. Initially, the unpolar, middle polarity and polar extracts of each part were evaluated for their antioxidant activity as well their inhibition to tyrosinase, elastase and collagenase enzymes. The middle polarity extract of pseudobulbs and roots presented important activity both in antioxidant and all three enzymatic assays. They were then submitted to chromatographic separation leading to the isolation of 14 secondary metabolites of which four phenanthrenes, two anthraquinones, two dibenzyls, two phenolic acid derivatives, two sterols, one dehydrodiconiferyl alcohol derivative and one simple phenolic compound. The isolated compounds were evaluated for their inhibitory activity in the enzymes mentioned. Dibenzyls (gigantol, tristin), showed anti-tyrosinase activity while phenanthrenes and anthraquinones (cymbinodin A, ephemeranthoquinone) presented anti-collagenase activity. None of the above exhibited significant activity in elastase. In conclusion, we observed that isolated metabolites act selectively on enzymes, but their combination (total extracts) shows activity on all 3 enzymes, assuming that total extract should be employed for dermo-cosmetic use rather than the isolated compounds. Hence, we re-evaluate the by-product as a high added value material from an island of North Aegean Sea.

Funding;The present work was co-funded by North Aegean Region and ERDF, (project code: 5021543).

PC8-16 Qualitative analysis and biological evaluation of propolis from lake Prespa region-Greece

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Propolis is a balsamic and resinous substance, produced by bees (*Apis mellifera*). It is characterized by a variable chemical composition, depending on the local flora, and it has shown significant bioactivities and commercial applications [1].

In the present study, five different propolis samples from the lake Prespa region (1-5) in NW Greece (the highest tectonic lakes in the Balkans) were analyzed for the first time. All samples harvested from fixed comb hives in five distinct areas, which characterized by rare local flora with protected species. Their analyses (GC-MS, NMR) showed comparable chemical profiles in Group A (samples 1-2, 4-5), rich in phenolic acids (ferulic and caffeic acids), flavonoids and chalcones (pinocembrin, pinostrobin), and only one sample (Group B, 3) with high amount in diterpenes (pimaric, communic acids, ferrunginol), which is in accordance with local flora. All studied samples have been evaluated for their total phenolic content, showing in Group A higher amount of phenolics (153-203 mg GAE/g extract) than B (39 mg GAE/g extract). Additionally, the extracts were evaluated for their antimicrobial activity against a panel of human pathogenic microorganisms. The assays revealed strong antimicrobial properties for B, probably due to its high diterpenic content.

Concluding, Group A could be classified close to European type of propolis, while B belongs clearly to the diterpenes' rich Mediterranean type. It is noteworthy that even in a restricted geographical area, as in Prespa Lake region, such a significant chemical diversity in propolis chemical profile has been demonstrated.

This research was funded by PoliPrespa (SARG-NKUA 17131).

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PC8-17 Chemical composition and biological evaluation of propolis from north Aegean Greek islands

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Propolis is a resinous material that bees collect from plants and it is characterized by a wide range of biological activities, depending on its chemical composition, which in turn depends on geographical diversity [1]. Greek propolis shares different characteristics than the typical European one [1] and in this study, thirteen samples from the NE Aegean islands: Lemnos, Lesvos, Chios, Psara, Oinousses, Samos, Ikaria and Fourni, were phytochemically analyzed for the first time. N Aegean islands characterized by a unique ecosystem with high plant diversification responses and endemism, as this area is standing as a bridge of Europe, Asia and Africa. All samples were analyzed (GC-MS, NMR) revealing a significant percentage of diterpenes (dehydroabietic acid, pimaric acid, abietic acid, totarol, sempervirol) in all samples, while flavonoids and chalcones were detected in low % in samples from Lesvos and Lemnos. The chemical analysis showed that N Aegean Islands' propolis belongs to the Mediterranean type, highly influenced by Conifer trees resin, due to the local flora. Furthermore, the total phenolic content was evaluated by the Folin-Ciocalteu method (23 to 173 mg GAE/g extract) and all propolis samples showed significant antibacterial activity against six Gram-negative and Gram-positive human pathogenic bacteria and three fungi, probably due to the large amounts of detected diterpenes, which are well known antimicrobial agents [1].

The authors declare that there is no conflict of interest; This research received no external funding.

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PC8-18 Determination of anti-tyrosinase activity of acetone extracts from selected *Potentilla* L. species.

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Potentilla L. species (Rosaceae) have a long history of use since antiquity to treat diarrhoea, skin infections and inflammations in the mouth and throat. However, the literature reports that the genus *Potentilla* represents also a rich source of the monomeric and oligomeric flavan-3-ols [1, 2]. Thus, the present investigation aimed to assess the composition of the secondary metabolites of acetone extracts from aerial parts of selected *Potentilla* species using LC-MS/TOF analysis. Furthermore, the assessment of the anti-tyrosinase activity was performed. The acetone extracts from aerial parts of *P. argentea* (PAr), *P. recta* (PRe), *P. grandiflora* (PGr), *P. norvergica* (PN) and *P. rupestris* (PRu) have been obtained. LC-MS/TOF analysis demonstrated the presence of catechin and epicatechin and their derivatives. The most potent anti-tyrosinase activity exerted PAr and PN with IC_{50} of 262.3 ± 0.285 and 309.1 ± 1.502 µg/mL. The results allow us to conclude that the application of *Potentilla* species may be effective in treating diseases with the hyperactivity of tyrosinase.

Conflict of Interest; Funding

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PC8-19 Chemical and pharmacological comparison of two chemotypes of *Psidium cattleyanum*: a rational approach for marker selection

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Psydium cattleyanum (PC; araçá) leaves are popularly used in Brazil for their anti-inflammatory properties. However, there are two types of PC: one which presents fruits with yellow epicarp and the one with red epicarp. This rises a question if the leaves of both types can be used interchangeable. To address this issue, 28 samples of the leaves of PC were obtained and extracted using the same method assisted by ultrasound. All extracts were analyzed by HPLC-DAD and the chromatograms were converted to the matrix **X**. The data were aligned and further transformed by column centering. This new matrix was analyzed by principal component analysis (PCA). Two chemotypes were detected; however, not related to fruit color. To identify which compounds could be responsible for this segregation, the loading plot of PC1 (54 % of the variation) was compared to the chromatograms.

Further UHPLC-Q-TOF-MS analysis, allowed to identify quercetin xylopyranoside and quercetin arabinoside as the main compounds responsible for the separation of a second chemotype. On the other hand, miquelianin (**1**), quercitrin (**2**), and hyperoside (**3**) were detected for both chemotypes. One representative sample of each chemotype, as well as **1**-**3** were evaluated *in vitro* for their anti-chemotactic activity, as an indirect measure of their anti-inflammatory potential. Both extracts and the isolated compounds exhibited similar IC_{50} (0.5 µg/mL). Taken together, these results indicate that both chemotypes can be used interchangeable and that **1**-**3** can be used as bioactive markers for the quality control of PC.

There are no conflicts of interest. CAPES – 001.
Statement on behalf of all authors.

PC8-20 Development and physicochemical characterization of nanoliposomes with incorporated oleocanthal, oleacein, oleuropein and hydroxytyrosol

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Oleocanthal (OLEO), Oleacein (OLEA), Hydroxytyrosol (HT) and Oleuropein (OLEU) are high-value secondary metabolites of *Olea europaea* (Oleaceae) with numerous biological activities. Nevertheless, their absorption during the oral administration or skin applications is limited due to low bioavailability. The incorporation of sensitive compounds in drug delivery nanosystems is a well-established method to overcome degradation and bioavailability problems.

The aim of the present work is the development and physicochemical characterization of nanoliposomes with incorporated OLEO, OLEA, HT and OLEU, after their chromatographic isolation in pure form, from extra virgin olive oil (OLEO, OLEA, HT) or olive leaves (OLEU), following previously developed processes [1–2].

Each pure compound was incorporated in liposomes, prepared from hydrogenated soya phosphatidylcholine (HSPC) lipid, by thin-film hydration preparation method. Their physicochemical stability of size, polydispersity index (PDI) and ζ-potential was evaluated during storage by light scattering methods.

The physicochemical characterization of liposomes was carried out by FTIR, XRD, TGA, DSC and rheology methods. Their morphology was illustrated by negative-staining TEM and Cryo-TEM, revealing well-dispersed round-shaped vesicles.

According to the results, liposomes of larger sizes exhibited higher encapsulation efficiencies of higher concentrations of compounds. According to release studies in simulated body fluid (SBF), OLEU and HT were released in higher percentage than OLEO and OLEA. Their release exponents indicated a Fickian diffusion, while the release mechanisms were well-fitted with Ritger-Peppas model. Taken into account all the obtained results, the proposed liposomes could be suitable carriers for targeted nutrient delivery in the human body.

Conflict of Interest: The authors declare no conflict of interest Funding:. This research was supported by the Fundamental Research Funds for The Central Non-profit Research Institution of CAF (CAFYBB2019GC001-12)

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PC8-22 Chemical composition and bioactivity of *Sonchus asper* (L.) Hill based traditional dish from Campania Region (Italy)

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Spontaneous edible plants, essential components of rural diet since ancient times, have now been almost replaced by limited cultivated varieties. The selection of more productive cultivars and the industrial cultivation has led to an impoverishment of the diet, both in terms of choice and nutrition. To promote the use of forgotten species used in Campanian tradition, a chemical-biological study

on *Sonchus asper* (L.) Hill (Asteraceae) was carried out. *S. asper* is a spontaneous plant consumed as vegetables in inland areas of South Italy. Previous investigations reported the presences of polyphenols, terpenes, carotenoids as main specialised metabolites [1]. The plant is used for several human disorder such as gastrointestinal infection, diabetes, and inflammation disease [2]. Raw and cooked plant was subjected to ultrasounds and microwave assisted extraction. The extracts were subjected to LC-HRESIMS/MS analyses to perform a quali-quantitative profile of bioactive molecules, antioxidant *in vitro* was also evaluated. Flavonoids, fatty acids, and phenolic acids were identified as major components, while lactone sesquiterpenes, glycosides and coumarins were found in traces. The presence of polyunsaturated fatty acids and polyphenols, both in the cooked and raw matrix confirms the nutritional value of the species [3], whose reintroduction in the diet would represent a healthy and sustainable choice.

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PC8-23 *In vitro* studies of antioxidant and estrogenic activities of *Trifolium pratense L*. extracts on human adenocarcinoma and non-tumorigenic breast cell lines

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DOI 10.1055/s-0041-1736911

Trifolium pratense L contains high concentrations of isoflavones, known for beneficial effects on human health attributed to their estrogenic, antioxidant and anti-cancer activities. The aim of this study was to determine the antioxidant and estrogenic effects of red clover aqueous and ethanolic extracts on adenocarcinoma and non-tumorigenic mammary gland cell lines. Both extracts were characterized by antioxidant spectrophotometric assays and the results were confirmed by Electron Spin Resonance spectroscopy. Their total phenolic content was determined and phytochemicals profile was assessed by HPLC analysis. No cytotoxicity and an increase of cellular proliferation rate was observed for normal cells treated with aqueous extract at 0.5mg/ml concentration. At the same concentration a significant anti-proliferative effect was induced by both aqueous and ethanolic extracts on adenocarcinoma cells. The cellular antioxidant activity (CAA) of red clover ethanolic extract was comparable with Quercetin at 500 µ M used as positive control. Strong estrogenic activity of aqueous extract was assessed by E-SCREEN test and by Western blot on adenocarcinoma cells. Our study has demonstrated that the Trifolium pratense L. extracts can induce anti-cancer effects, by reducing the cellular proliferation rate and the viability of tumour cells. Interestingly, at the same concentration on non-tumorigenic cells a significant pro-proliferative effect was observed. Moreover, red clover ethanolic extract, unlike the aqueous one, can have a cytoprotective activity against ROS generation in tumour cells.

Conflict of Interest: The authors confirm that there are no conflicts of interest;

Acknowledgements: This work was partially funded by Core Program PN 192901.03; and by the grant COP A 1.2.3., ID: P_40_197/2016

Statement on behalf of all authors. All the authors agreed with the text of this abstract

PC8-24 Phytochemical analysis- Biological properties of Heliotropium procubens from Panama

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Heliotropium (Boraginaceae) consists of approx. 300 species widespread worldwide, used widely in folk medicine (against inflammations, skin diseases etc) [1]. *H. procumbens Mill.* is an annual weedy herb, native in America and E to the West Indies. In the framework of our phytochemical studies on Boraginaceae plants, we report herein the chemical profile of the plant, revealing the presence of pyrrolizidine alkaloids, (LC-MS) among which several new natural products such as: helifoline and hydroxy-platynecine isomers (both saturated necine), 9-angleloyl-heliotridine (possessing a double bond in the 1,2-position -ring B), all in the forms of N-oxides and phenolic metabolites: flavonoids of luteolin, quercetin, kaempferol type and caffeic acid derivatives. All chemical structures were determined by spectral means (NMR).Antioxidant properties of methanol (ME) and water (WE) extracts were assessed *via* five different assays. Enzyme inhibition potential against key clinical enzymes involved in neurodegenerative diseases AChE and BChE, diabetes (α-amylase and α-glucosidase) and skin whitening (tyrosinase) were assayed. The extracts displayed considerable free radical scavenging activity against DPPH and ABTS radicals, with potential values for ME: 46.88, 68.31 and WE: 93.43 and 131.48 mg TE/g extr, respectively. *H. procubens* was found to harbor bioactive metabolites and hence could be served as a source of biological activities which could be further explored and exploited for potential applications.

The authors declare that there is no conflict of interest; This research received no external funding

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PC8-26 New terpenoid and phenolic metabolites from *Euphorbia gossypina* var. *coccinea* Pax

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DOI 10.1055/s-0041-1736913

Plants of the genus *Euphorbia* are known to possess considerable chemical, medicinal and economic importance [1]. Terpenes, including diterpenes and triterpenes, steroids, cerebrosides, glycerols, and phenolic compounds are the main constituents of *Euphorbia* species [2]. In the course of our work, *Euphorbia gossypina* var. *coccinea*, a much-branched, succulent, evergreen shrub native to Kenya, was investigated. It is used in folk medicine to treat swollen legs and general body pain. There is no literature data about the phytochemistry and pharmacology of the plant.The dried and ground plant material was extracted with methanol. After concentration, the extract was dissolved in 50 % aqueous methanol, and solvent–solvent partition was performed with hexane, chloroform and ethyl acetate. The chloroform phase was further separated by normal and reversed phase vacuum liquid chromatography and then it was purified by preparative thin layer chromatography and high-performance liquid chromatography. Structure elucidation of the compounds was carried out by NMR and MS spectroscopy as well as by comparison the data with literature values.
The compounds were tested for their antiproliferative activity against HeLa cell line using the MTT assay. Thirteen compounds, among them two new and one

known lignans, seven new and one known pregnane aglycones and glycosides and two flavonoids were identified from the chloroform extract of *E. gossypina* var. *coccinea*. Pregnane glycosides are substituted with thevetose, cymarose, digitoxose and glucose. All compounds were determined for the first time from the plant. None of the compounds showed antiproliferative effect against HeLa cell line *in vitro*.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. This work was supported by National Research, Development and Innovation Office, Hungary (K135845) and ÚNKP-20-4 (co-author N.K.) – New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Funds.

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PC8-27 Evaluation of Passive Permeability of *Withania somnifera* Plant Extracts

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Phytomedicine has seen a large push to not only corroborate ethnobotanical therapeutic use, but to optimize and enhance bioavailability of specific compounds within plants for use in modern day medicine. Many medicinal plants produce compounds that once purified, exhibit low permeability and thus poor bioavailability promoting doubt around the actual efficacy of these components as therapeutics. *Withania somnifera*, commonly known as Ashwagandha, is a powerful medicinal plant used to combat insomnia, inflammation, and neurodegenerative disorders. While permeability profiles have been determined for specific bioactive compounds in *W. somnifera*, including withanone [1] and withanolide A [2], little research has been published investigating potential additive and synergistic effects of whole extract preparations. This study will leverage an LC-MS based metabolomics analytical approach to investigate multiple extraction techniques, as well as the effect of stomach and intestinal fluid pre-incubations on the passive permeability of compounds and extracts from *W. somnifera* using the parallel artificial membrane permeability assay (PAMPA) [3].

▶ **Fig. 1** W. somnifera root certified from American Herbal Pharmacopoeia and Gentest Pre-coated PAMPA plate system.

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PC8-28 Investigations on the anti-inflammatory activity of *Cardiospermum halicacabum*

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Cardiospermum halicacabum L. (Sapindaceae), known also as balloon vine, is an important medicinal plant used in European phytotherapy and in Ayurveda for the treatment of a variety of inflammatory diseases like rheumatism, arthritis and atopic dermatitis [1]. The aim of this study was to investigate the anti-inflammatory effects of *C. halicacabum*. Enzymatic *in-vitro* assays using COX-1 from ram seminal vesicles and human recombinant COX-2 were performed in a 96-well plate format to test the inhibitory activities of the extract and its fractions (*n*-hexane, dichloromethane, ethyl acetate and *n*-butanol), as previously described [2]. Indomethacin and celecoxib were used as positive controls. NF-κB inhibitory activity of the extract and fractions of *C. halicacabum* was evaluated by SEAP (secreted embryonic alkaline phosphatase) reporter assay in the HEK-Blue hTLR4 cell line. BMS-3445541 was used as positive control. Cardiospermum extract showed potent COX-1 and COX-2 enzyme inhibitory activity with IC_{50} values of 24.9 and 4.1 µg/ml, respectively. All extract fractions exhibited a considerable COX-2 inhibitory effect at a concentration of 5.0 µg/ml, whereas only the *n*-hexane and dichloromethane fractions showed a relevant COX-1 inhibitory effect at 5.0 µg/ml. Cardiospermum extract and all of the fractions suppressed cell viability of HEK-Blue cells at all concentrations. Only the dichloromethane and *n*-butanol fractions at concentrations of 5.7 and 3.7 µg/ml, respectively, exhibited an inhibitory effect on SEAP without showing cytotoxicity. In both experiments the aqueous fraction did not exhibit any effect. Investigations are in progress to elucidate the anti-inflammatory active constituents.

Acknowledgement: We are grateful for the funding of the project by Dr. Willmar Schwabe GmbH & Co. KG.

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PC8-29 Glucolipid-enriched extract of *Osmanthus fragrans* flowers inhibits LPS-induced inflammation

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Osmanthus fragrans Lour., also known as sweet olive, is a plant native to East Asia. Its flowers have been traditionally used to treat various diseases accompanied by inflammation and pain [1]. The aim of this study was to investigate their anti-inflammatory properties and active constituents.

n-Hexane and CH_2Cl_2 extracts of *Osmanthus fragrans* flowers at concentrations of 20 µg/ml inhibited COX-2 mRNA expression by 48.8 ± 4.2 and 46.2 ± 5.3 %, respectively. Starting from these extracts, activity-guided fractionation led to a glucolipid-enriched fraction, mainly composed of digalactosyl-diacylglycerols (DGDGs) and monogalactosyl-monoacyl-glycerols (MGMGs). In total, four MGMGs and six DGDGs were identified by means of LC-MS. The corresponding fraction was not only able to inhibit COX-2 mRNA expression in LPS-stimulated PMA-derived macrophages, but also suppressed LPS-induced IL-8 secretion and E-selectin expression in endothelial cells. The active fraction inhibited proinflammatory effects of LPS, but did not suppress action of TNF-α and IL-1β, thus pointing to a selective inhibition of the TLR4 receptor.

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PC8-30 Chemical investigation of the medical wine "Oinos Tragoriganites" by LC-M

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In the Greek "Pharmacopoeia" of 1931, "Medicinal Wine" is defined as the wine in which a medicinal plant has been extracted. However, in the ancient texts of Plinius, Galen and Dioscorides, there are recipes including the co-fermentation of must with several medical plants, resulting in wines claimed to have a broad range of pharmacological properties. In continuation of our efforts to evaluate ancient Greek medical recipes, we investigated the *Materia Medica* recipe of "***Oinos Tragoriganites:*** *Let down four teaspoonfuls of tragoriganum (bound in a linen cloth) into four pints of must [grape pulp] for three months and then jar it. It is good for griping, convulsions, hernias, pains of the side, the movement of winds, and difficulty to digest*".

The plant material (*S. thymbra*) was collected in the area of Attiki and the model must and model wine were provided by the Agricultural University of Athens. The aerial parts of the plant were used and the fermentations/extractions were carried out under controlled conditions in duplicate. Experimental design included mixture analogies of: **a**) plant extraction in model wine and **b**) plant/must co-fermentation. The wine extracts and the co-fermented solutions were then eluted through macroporous resins of different polarity (XAD4 and XAD7), in order to remove the wine/must matrices (mainly sugars and organic/inorganic salts). The adsorbed materials obtained by ethanol elution, were analyzed by UPLC-HRMS/MS and differences were revealed concerning the chemical profile between the co-fermentations, the wine extracts and the hydroalcoholic extracts of *S. thymbra*.

Conflict of Interest

The authors declare no conflict of interest

PC8-31 Medicinal plants used traditionally for skin related problems in the South Balkan and East Mediterranean

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A review research was conducted to provide an overview of the ethnobotanical knowledge of medicinal plants and traditional medical practices for the treatment of skin disorders in Albania, Cyprus, Greece and Turkey. The investigation was focused on the detailed study of 126 ethnobotanical surveys conducted in these areas and the species used for skin ailments were singled out. The bibliographical analysis showed that 948 taxa belonging to 417 different genera and 111 different families are used in the treatment of skin related problems. The majority of the plants belong to the families of Asteraceae (11.3 %), Lamiaceae (7,6 %), Rosaceae (6,8 %), Plantaginaceae (5,3 %) and Malvaceae (3.7 %). Their usage is internal (decoction, infusion etc.), or external (compress, poultice, ointment etc.) to treat skin related ailments such as wounds and burns (21.9 %), hemorrhoids (15.0 %), boils, abscesses and furuncles (8.4 %) and eczema (6.0 %). Beside specific skin disorders, numerous species appeared to be used for their antifungal, antimicrobial and antiseptic activity (9.3 %). Literature evaluation highlighted that the most commonly used species in all four countries are *Plantago major* L., *Juglans regia* L., *Urtica dioica* L., *Hypericum perforatum* L. and *Plantago lanceolata* L. Finally, in order to relate this ethnopharmacological knowledge and trace its expansion and diversification through centuries, a comparison of findings was made with the use of the medicinal species mentioned in Dioscorides' "*De Materia Medica*" for skin disorders. Our findings confirm the primary hypothesis that people in Albania, Cyprus, Greece and Turkey are very close related in terms of traditionally using folk medicinal practices.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. This project is funded under the European H2020-MSCA-RISE-2018 (823973) project EthnoHERBS.

PC8-32 *Carissa spinarum* L. (Apocynaceae): a case study in ethnomedical research

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Introduction *Carissa spinarum* L. is distributed across Africa, Australia and parts of Asia. It is valued throughout for healing, diet and other practical uses. This study aims to analyse the ethnomedical data as a case study in bioprospecting research.

Materials and Methods The search includes electronic databases including PubMed and grey literature. 284 documents are included for analysis with 80 % of these being ethnomedical in focus.

Results This species grows in many habitats, climates and altitudes and is physically varied. It is listed as having 98 synonyms [1]. Nomenclature creates difficulties in data collation through errors in synonymy and spelling. All plant parts are used in healing and diet though the root is most commonly used medically and the fresh fruits eaten. Taken together, digestive ailments are the most commonly treated category. The respiratory, skin and musculoskeletal systems are also commonly treated. Within the ethnomedical data, key aspects of plant use are omitted including plant part (9 %), plant preparation (36 %), route (35 %) and ethnographic information on understandings of illness, healing and medical terms as used in the studies. Even minimal ethnographic information is lacking in 75 % of studies. The majority of analysed studies present decontextualised lists of species and represent the much criticised Phase I ethnobiological research [2].

Conclusions This analysis of *Carissa spinarum* research illustrates the lacunae commonly found in ethnomedical studies. The absence of such basic information may contribute to historically poor results from bioprospecting efforts.

Conflict of Interest; Funding (Source, ID)

The authors declare that they have no competing interests. Part funded by the TCD 1252 scholarship.

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PC8-33 Carmelite spirit for the treatment of stress-related gastrointestinal symptoms

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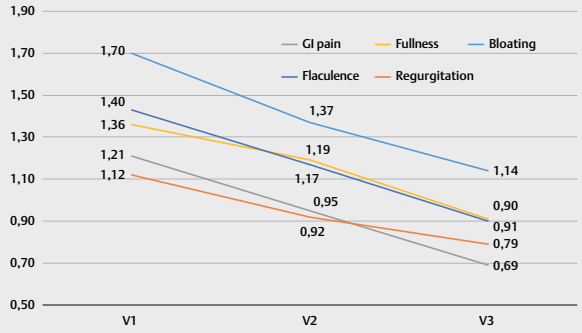
Background Carmelite spirits are traditionally used as medications for the improvement of well-being in cases of nervous complaints, including nervous gastrointestinal disturbances such as fullness and bloating.

Method The study was designed as an open, observational trial. 105 patients with inner restlessness and/or nervous gastrointestinal complaints were included and treated for four weeks with the Carmelite spirit "Klosterfrau Melissengeist". The preparation is registered as a traditional herbal medicinal product. It contains a distillate of a blend of *Melissa officinalis* L., leaves, *Inula helenium* L., roots, *Angelica archangelica* L., roots, *Zingiber officinale* Roscoe, rhizome, *Syzygium aromaticum* (L.) Merr. & L.M. Perry, flower buds, *Alpinia officinarum* Hance, root, *Piper nigrum* L., fruits, *Gentiana lutea* L., roots, *Myristica fragrans* Houtt, seeds, *Citrus* x *aurantium* L., fruit peel, *Cinnamomum cassia* (L.) D. Don., flowers and bark, and *Elettaria cardamomum* (L.) Maton, seeds. The effects on gastrointestinal symptoms were documented using the Gastrointestinal Quality of Life Index GLQI [1].

Results Significant improvements of gastrointestinal complaints were reported by 81.9 % of patients and for all of the 36 single symptoms. Of specific importance for the situation with nervous gastrointestinal complaints were the effects on gastrointestinal pain, fullness, bloating, flatulence, and regurgitation (all $p < 0{,}001$; Figure 1).



▶ **Fig. 1** Improvement of the frequency of GI complaints (0 = never, 4 = always).

Conclusions The results confirm the applicability of the GLQI as a tool in clinical studies. They support the indication of Carmelite spirit as a traditional herbal medicinal product for the treatment of nervous complaints of the gastrointestinal tract.

Conflict of Interest
The study was funded by MCM Klosterfrau Vertriebsgesellschaft mbH.

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PC8-34 Unlocking Nature's Pharmacy from Bogland Species

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DOI 10.1055/s-0041-1736921

The National Folklore Collection of Ireland (NFC) is an archive containing 740,000 pages of traditional knowledge (TK), collected by school children from their elders in 1930's post famine Ireland. The NFC provides a rich source of traditional medicinal knowledge (TMK), comprising of medicinal cures and practices that were orally passed down through generations. The archive can be accessed at Duchás.ie. The Boglands of Ireland which mainly constitute raised bogs and blanket bogs, offer unique biodiversity. The flora of the boglands have been used traditionally as medicines and much of the local healing knowledge of these plants is recorded in the NFC. In this study we have analysed the content of the archive to identify herbal medicines derived from bogland species. Searching the term 'bogs' using the website search engine, we retrieved 1,218 transcripts with the mention of boglands across 26 geographical regions. Examination of these transcripts resulted in 643 separate entries of TMK, which fell into several categories: 'religious', 'ritual', 'animal', 'plant', 'natural substance', and 'other' uses [1, 2]. Focusing on 'plant' entries, our analysis was comprised of three separate steps. (i) identification of all bogland species from our data along with their ethnopharmacological use, (ii) categorisation of the data counts into specific disease classification associated with the ailment, (iii) analysis of the top 6 cited bogland species through the geographical distribution of the entries and their traditional use. The top six species identified included Bog Bean, Bog onion, Coltsfoot, Broom, Bog onion and Yellow iris. The correlation of use of traditional medicinal use of these plants, with the current understanding of their chemical composition and bioactivity of these plants, offers support their use as healing plants in post famine Ireland.

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PC8-35 A scientific evaluation of Traditional Chinese Medicine (TCM) *Houttuynia cordata* Thunb (Yu xing cao 鱼腥草): a case study.

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Houttuynia cordata Thunb (Yu xing cao 鱼腥草) decoction (a traditional aqueous extract), is widely used in China to treat anti-inflammatory disease [1]. Previous studies have mainly focused on its organic extracts, therefore the study of chemical composition and biological activities of its aqueous extracts is requested. Sixteen samples purchased from Sichuan, Hubei and Anhui in China, prepared into traditional aqueous decoction, methanolic extracts and steam distillates, analysed by NMR, GC-MS and LC-MS. The anti-inflammatory activities of aqueous extracts were studied by *in vitro* model (TNF-α activated Caco-2 monolayers) and *in vivo* model (DSS-induced colitis murine model). Inflammation associated mediators (IL-1β, EGFR, MAPK, etc) were evaluated by qPCR, western-blot and ELISA. Results showed chemical difference among 16 *H. cordata* samples. Aqueous extracts showed anti-intestinal inflammation *in vitro via* an EGFR dependent MAPK (ERK) 1/2 signaling pathway, and a GMP product from Hubei presented the best effect. The beneficial effect was also seen *in vivo* at a molecular level within colonic tissues.

In conclusion, aqueous extracts of *H. cordata* from different geographical regions were shown to be chemically different and resulted in different levels of

therapeutic effect. The traditional use of *H. cordata* decoction to treat inflammatory disease such as IBD is validated.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. Thanks to Chinese Scholarship Council and Trinity NatPro Center for funding this project.

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PC8-36 Protective effects of Phytohustil® compared to its active ingredient *Althaea officinalis* L. root extract (STW42) on primary human epidermal keratinocytes

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DOI 10.1055/s-0041-1736923

Introduction Root extracts of *Althaea officinalis* L. (STW42) are used for the treatment of dry cough. Limiting the effect only to its polysaccharide components is not enough to explain its effects [1]. Coumarins and flavonoids, have anti-inflammatory/-oxidative properties and contribute to the beneficial effects of STW42 [1]. Keratinocytes act as the major barrier to agents that cause injury in the oral mucosa. The aim of this project is to investigate the anti-inflammatory/-oxidative properties of Phytohustil® on normal human epidermal keratinocytes (NHEK).

Material and methods NHEK were pre-incubated (5–6h) with Phytohustil®, its excipients, or its API STW42, followed by H_2O_2 (pro-oxidative) or LPS (pro-inflammatory) and further incubated (24h). Viability was measured by PrestoBlue®. Intracellular ROS were quantified with DCFDA. The release of IL-1β was determined by ELISA.

Results 50-1000µg/ml Phytohustil® or STW42 (24h-48h) revealed no cytotoxicity. Pro-inflammatory stimulation of NHEK with LPS (50µg/ml) induced an IL-1β release by 67 % compared to negative control. Pre-treatment with 100-400µg/ml Phytohustil® inhibited the IL-1β release by 18 %-35 % and STW42 by 35 % in comparison with LPS-stimulated NHEK. Pre-treatment (5h) with 1000µg/ml STW42 followed by co-incubation with 400µM H_2O_2 inhibited the H_2O_2-induced ROS production by 8- to 17-fold compared to treatment with 400µM H_2O_2 alone.

Conclusions Our results demonstrate the protective anti-inflammatory properties of Phytohustil® and STW42 and confirm the antioxidative properties of its active ingredient. The *in vitro* effects of Phytohustil® on NHEK may support its importance as a therapeutic drug for the treatment of irritated **oropharyngeal** mucosa and for the treatment of the symptomatic dry cough associated with this.

Conflict of Interest; Funding (Source, ID)

J.M. and OK are employees of Steigerwald Arzneimittelwerk GmbH, Darmstadt. The study was supported by Steigerwald Arzneimittelwerk GmbH, Darmstadt. Arzneimittelwerk GmbH, Darmstadt

Statement on behalf of all authors.

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PC8-37 Mountain tea (*Sideritis* species): an endless story

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Mountain tea, *Sideritis* species, is a well-known traditional tisane [1]. Previous phytochemical studies have showed a broad range of nutrients and specialized products. Furthermore, several research works have investigated various pharmacological activities of these plants.

In continuation of our research in genus *Sideritis*[2–8], we investigated *S. euboea* Heldr. which is found in Evia island (Greece). Given that *S. euboea* is considered as an endangered species, the present study explored the phytochemical profile and the biological activities of different extracts from cultivated populations of *S. euboea*. Overall, 47 chemical compounds were isolated and identified by using NMR methods [3, 4, 8]. The diterpenoid siderol exhibited great cytotoxic effects on three human cancer cell lines [4]. In addition, the anti-ageing activity of the ethyl-acetate residue and its specialized products were explored [8]. This study demonstrated that the cultivated *S. euboea* could be a potential agent for cosmetic formulations, confirming its anti-inflammatory, anti-tumour and anti-ageing effects.

Conflict of Interest: The authors declare no conflict of interest.

Funding: The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 16228)

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PC8-38 Healing plants of post famine Ireland

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DOI 10.1055/s-0041-1736925

As part of a study in the medical humanities we have we have investigated 10 % of the entries in the Irish National Folklore Collection of Ireland (NFC), accessed at Dúchas.ie, for entries which give detail on the use of healing plants in 1930's, post famine Ireland. The NFC is an early example of citizen science where school children collected information form elders in their communities related to Irish cultural practice, over a range of areas, including healing plants and ethnphrmacology [1]. Schools from 26 counties in the newly formed Republic of Ireland contributed to the collection. In this study 10 % of the schools in each county were selected, and the data that they submitted to the collection was systematically read and analysed, and entries related to herbs, wells, cures, and *piseóg* (*superstition*; a category which on examination housed descriptions of healing plants) were collected. The retreived sample set (6560) included cures derived from plants (2,967), animals (1710) and minerals (744). There were also details given across regions for the use of compound mixtures, mainly as poultices for topical application. The top ten healing plants were Dandelion (*warts, heart disease, TB etc*), Nettle (*measles, jaundice*), Dock (*nettle stings*), Gooseberry (*thorns for eye stys*), Plantain (*staunch bleeding),* tea (*headache, eye pain),* potato (*warts, burns, whitlows*), wild garlic (*coughs and colds*), common chickweed (swelling), and mallow (sprains/swellings) / comfrey (wounds, sprains). Some of the entries have detail relating to season for harvesting the plant material,

plant part used, preparation of the treatment and duration of administration. The main treatments were used for skin conditions.

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PC8-40 Evaluation of the renal toxicity potential of sub-acute exposure to the aqueous extract of *Alpinia officinarum* Hance. rhizome in rats

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In Persian traditional medicine, *Alpinia officinarum* rhizome is used for various diseases such as gastrointestinal and reproductive system disorders. However, there is not any study on the safety of this herb in continuous usage. This study was aimed to investigate the acute and sub-acute potential toxicity of *A. officinarum* aqueous extract in rats. The acute oral toxicity was evaluated in female Wistar rats by administering a single oral dose (2000 mg/kg) of the extract. Also, after a 28-days period administration of the extract (p.o; daily; 200, 400 and 800 mg/kg) in rats, biochemical and electrolyte parameters, oxidative stress biomarkers, histopathology and histomorphometry evaluations of renal tissues were performed. No mortality and behavioral signs of toxicity observed after administration of 2000 mg/kg. In sub-acute evaluation, the serum levels of creatinine, sodium (Na +) and total antioxidant capacity (TAC) increased dose-dependently. Also a significant decrease in level of calcium (Ca 2 +) was observed ($p < 0.05$). No significant changes were observed in the serum levels of Chloride, LDL, HDL, and cholesterol as well as oxidative stress biomarkers compared to control after 28 days. However, in histopathology and histomorphometry analysis, some alterations such as reduction of Bowman's space and segmental or global glomerular necrosis were observed especially at dose of 800 mg/kg. According to the results, it seems continuous oral consumption of *A. officinarum* rhizome extract in high doses might have renal toxicity. Further assessments should be performed to ascertain the safety/toxicity of *A. officinarum* in subchronic administrations.

There is no conflict of interests; Babol University of Medical Sciences (9808016).

PC8-41 COVID-19 Therapy with Traditional East Asian Phytotherapy: A Review

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Whilst Western research for the COVID-19 crisis only covers vaccination, in East Asia herbal prescriptions are applied for therapy. In Japan, Maoto (Ephedrae herba 4 g, Armeniacae semen 4 g, Cinnamomi cortex 3 g, and Glycyrrhizae radix 2 g, JPXVII) is used based on clinical evidence for its effect on early phase influenza (also RNA virus) comparable to oseltamivir [1]. The Health Ministry of Thailand has approved *Andrographis paniculata* (Jap. Senshinren) extracts for treatment of COVID-19 [2]. Its combination with Maoto, Maoto-ka-senshinren (+ 4 g), seems most promising for the treatment of viral pandemics. In China, the official guideline for COVID-19 treatment contains TCM prescriptions. The first mentioned Qing-Fei-Pai-Du-Tang (Jap. Seihai-haidokuto) is quite complex (21 drugs). Later, Shufeng Jiedu Jiaonang (Bupleuri radix 8 g, Forsythiae fructus 8 g, Glycyrrhizae radix 4 g, Isatidis radix 8 g, Patriniae herba 8 g, Phragmitis rhizoma 6 g, Polygoni cuspidati rhizoma 10 g, Verbenae herba 8 g) also entered the national COVID-19 treatment guidelines based on its immuno-modulatory and anti-inflammatory effects on SARS-CoV2 pneumonia in pre-clinical studies [3]. Fufang Yuxingcao Heiji (Forsythiae fructus 0.6 g, Houttuyniae herba 6 g, Isatidis radix 1.5 g, Lonicerae flos 0.6 g, Scutellariae radix 1.5 g) first gained prominence during the 2002 SARS epidemic [4]. With no Western medicine available, Eastern Herbal Medicine offers the most promising curative COVID-19 treatment.

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PC8-42 Ethnobotanical investigation of medicinal plants used to treat and manage childhood diseases in North West Province, South Africa

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In Southern Africa, childhood diseases such as diarrhoea, gastroenteritis and pneumonia remain common and contributes to the increasing high rate of child mortality. Even though a significant portion of population depend on traditional medicine for their healthcare needs, systematic documentation of valuable medicinal plants remains poorly explored. This study was aimed at documenting medicinal plants used for the treatment and management childhood diseases in the North West province, South Africa. An ethnobotanical survey was conducted with 101 participants through semi-structured interviews (face-to-face). Information such as local name of plants, medicinal use, method and part were collected and analyzed. A total of 44 medicinal plants from 25 families were identified. *Aptosinum elongatum* with 0.69 Cultural importance index (CI) and 70.2 Fidelity level (FL) values, *Bulbine frutescens* 0.20 CI and 20.7 FL and *Euphorbia prostrata* 0.316 CI and FL 30.6 were the most commonly used plants for treating and managing a variety of childhood diseases in the study area. *Aptosinum elongatum*also had the highest FL among the documented plants. Leaves and roots were the most commonly used plant parts. Decoction and maceration were the main methods of preparation and the plant remedies were mainly administered orally. Skin-related disease, sunken fontanelle, urinary related diseases were the most prevalent childhood diseases treated and managed by the participants.

The traditional health practitioners possess rich ethno-pharmacological knowledge and depend largely on naturally growing plants. The rich pool of plants and their diverse uses remain an essential drive for future research.

Conflict of Interest; Funding (Source, ID)
The authors declare that there is no conflict of interest. The opinions, conclusions/recommendations herein this study are based on the findings of the authors, therefore, the funder(s) accepts no liability whatsoever in this regard.
PTN is grateful for a doctoral bursary from the National Research Foundation in Pretoria, South Africa (Grant UID: 121525). The North-West University and the University of Mpumalanga (South Africa) provided additional financial support.

PC8-43 Anti-inflammatory properties of Arnica planta tota versus Arnica flos

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Arnica montana L. is traditionally indicated for treatment of blunt injuries like tissue damage, strains and bruises that are physiologically accompanied by local inflammatory processes. These include influx of inflammatory immune

cells and the release of pro-inflammatory prostaglandins and leukotrienes that are produced by cyclooxygenase 2 (COX-2) and 5-lipoxygenase (5-LOX), respectively, as part of the arachidonic acid (AA) pathway.

The Weleda Arnica 30 % ointment contains a unique ethanolic extract from the whole Arnica plant (Arnica planta tota). In contrast, most commercial products comprise extracts from Arnica flowers (Arnica flos) as active pharmaceutical ingredient. Arnica flos contains e. g., sesquiterpene lactones like helenalin or dihydrohelenalin that are known to be active inhibitors of both enzymes. Less is known about the anti-inflammatory efficacy of Arnica planta tota extracts. Thus we aimed to analyze the ethanolic extracts from Arnica planta tota and Arnica flos in terms of inhibition of recombinant human COX-2 and 5-LOX *in vitro*.

Arnica planta tota and Arnica flos concentration-dependently inhibited 5-LOX activity (IC_{50}: 8.33 µg/ml and 47.0 µg/ml, respectively) and COX-2 activity (IC_{50}: 5.5 µg/ml and 33.1 µg/ml) *in vitro*. Interestingly, the Weleda Arnica planta tota extract demonstrated superior inhibition of pro-inflammatory and pain-related enzymes in vitro. In this experimental approach the complex mixture of active compounds contained in the complete Arnica plant improved the anti-inflammatory and pain-related efficacy that is already known for Arnica flowers. However, further studies are required to further characterize potential therapeutic advantages of A. planta tota preparations.

Conflict of Interest

Röhrl J, Ammendola A and Künstle G are employees of Weleda AG, Switzerland

PC8-45 Unlocking Nature's Pharmacy – Assessing the Quality of Irish Echinacea plant materials

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Species of the genus Echinacea have been used extensively in traditional medicine in North America and Europe and many potential pharmacological effects have been attributed to a diverse range of constituents including caffeic acid derivatives, alkamides and polysaccharides. Echinacea has recently demonstrated some intereting activity against Covid [2].

Echinacea preparations range from tinctures, teas and juices, to tablets and oral solutions, as well as cosmetic preparations such as toothpastes and creams. Echinacea tinctures are licenced in Europe and are sold in pharmacies, health food stores, herbal dispensaries and online. Small producers in Ireland have recently been trialling *Echinacea* species, grown under organic conditions, to investigate their quality, with the intent of selling them in the future on international markets.

In this study we determine the NMR and HPLC metabolomic fingerprints of roots of *Echinacea pallida*, grown by Bord Na Mona at Mount Lucas in the midlands of Ireland, and *Echinacea augustofolia*, grown at the Shanbally Estate and Herbal Dispensary in Co. Tipperary. Most of the samples complied with the British Pharmacopoeia (BP), with most *E. pallida* samples having a mean echinacoside content > 0.2 %, and one of the *E. augustofolia* samples with a mean echinacoside content > 0.5 %. sample with > 0.2 %, echinoside. These results suggest that cultivation conditions in the midlands of ireland will support growth of commercially acceptable organic *Echinacea* samples.

PC8-46 Antibacterial potential of *Ipomoea littoralis* (Blume): An underutilized wild plant growing in Sri Lanka

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DOI 10.1055/s-0041-1736931

Sri Lanka is considered as a hot spot of biodiversity and there are many underutilized medicinal plants that have been not gained attention of scientists. Therefore the present study expected to scientifically validate, the anti-bacterial potential of the wild plant Ipomoea littoralis (Blume) growing in Sri Lanka. The different parts of the plant extracted with water, acetone, methanol and hexane. The antibacterial potential of each extract was assessed by agar well diffusion and broth dilution assays against four common pathogenic bacteria. Gentamicin was used as the positive control. The largest diameter of zone of inhibition against Shigella dysenteriae (ATCC 11835) and Escherichia coli (ATCC 25922) was exhibited by methanol extract of leaves and aqueous extract of stem respectively. The hexane extract of leaves and aqueous extract of stem indicated the highest inhibition against Staphylococcus aureus (ATCC 25923). The methanol and acetone leave extracts exerted maximum inhibition against Salmonella enterica (ATCC 14028). In broth dilution assay, the lowest minimum inhibitory concentration (MIC) observed for all bacterial species was 31.25 mg/ml. It was obtained for E. coil in aqueous and acetone extracts of stem, while the methanol and acetone extracts of leaves against S. enterica. The lowest MIC against S. aureus was exhibited by aqueous stem, hexane leaves and methanol stem extracts, while it was the methanol extract of leaves and stem against S. dysenteriae. The present study observed that the different parts of the wild plant I. littoralis possess promising inhibitory activity against multiple pathogenic bacteria, revealing its potential medicinal value [1, 2].

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8.9 Recent Advances in Medicinal Plant and Natural Product Research

PC9-2 A haematotoxicity evaluation of a natural polymer intended to be a nutraceutical and pharmaceutical drug delivery system.

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DOI 10.1055/s-0041-1736932

Nutraceuticals, are defined as foods or components of foods that have unique health benefits in the prevention and treatment of disease. Natural polymer research in the development of enabling excipients for long term administration of novel drugs,,has yielded remarkable results. Many chronic diseases are associated with haematological co-morbidities. The use of nutraceuticals, from polysaccharide food sources, versatile in their use as excipients, to complement

and augment the treatment outcome of drugs and for the promotion of health in such conditions is crucial. Even though nutraceuticals are primarily from food sources, in principle their safety should be evaluated. However a safety evaluation of some excipients has resulted in harmful haematological effects [1, 2]. A 90-day subchronic toxicity investigation, was undertaken to evaluate the effect of of cocoa pod husk (CPH) pectin in doses up to 71.4 mg/kg on indicators for haematotoxicity, direct, indirect and total bilirubin, and the spleen. A 90 day repeated dose administration showed no effects on critical indicators of anaemia, direct, indirect and total bilirubin levels,with no indication of leucopenia, lymphocytosis, lymphocytopaenia, thromboembolic disease and no abnormal histopathological changes in the spleen.There was however a transient elevation in mean corpuscular volume (MCV) at day 30. The data obtained from this study did not reveal any remarkable findings of toxicological relevance to the haematopoietic system [3, 4].

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest

The authors gratefully acknowledge the support of the University of Ghana Office of Research and Innovation (ORID) in providing a grant for this study

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PC9-4 *Plectranthus* spp. and their secondary metabolites for dermatological disorders treatment

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DOI 10.1055/s-0041-1736933

Many skin disorders such as hyperpigmentation, sagging and skin-aging have been linked to oxidative stress, resulting in an increased need of searching less cytotoxic, more powerful and safe alternative antioxidants with simultaneous cooperative activities.

This study represents a primary evaluation of *Plectranthus* spp. (Lamiaceae family) extracts and their isolated compounds as potential antioxidant agents capable of inhibiting the skin-related enzymes tyrosinase, collagenase and elastase, for the synergistic treatment of skin disorders.

Organic and aqueous extracts of *Plectranthus* spp. were obtained by microwave-assisted extraction method; their isolated compounds were screened and characterised by our team. Their in vitro antioxidant activity was tested using the DPPH activity assay. The anti-tyrosinase, anti-collagenase and anti-elastase was based on spectrophotometric methods.

The results showed that the antioxidant and enzyme inhibitor activities of the tested extracts can be explained by their main diterpenoid compounds such as 6,7-dihydroxyroyleanone and Parvifloron D.

Considering the obtained results, some studied *Plectranthus* extracts and their constituents represent promising bioactive agents with strong potential for cosmetic and/or pharmaceutical formulations in dermatology area.

Conflict of Interest; Funding (Source, ID)

This work was supported by FCT grants PEst-OE/SAU/UI4013/2014, UID/DTP/04567/ 2016, UIDB/04567/2020 and UIDP/04567/2020. E.M.D-M gratefully acknowledges being the recipient of a predoctoral FPU 2019 fellowship from the University of Alcalá.

PC9-5 Anti-SARS-CoV2 M^{Pro} activity of THC, CBD, and CBN and their structure-activity relationship (SAR)

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DOI 10.1055/s-0041-1736934

THC, CBD, and CBN are cannabinoids present in *Cannabis sativa* L. and responsible for cannabis biological activities, such as antiinflammation and antibiosis. Recently, an anti-SARS-CoV2 infection of THC, CBD, and CBN was reported from a cell-based assay by Raj et al. (2021), and anti-SARS-CoV2 main protease (M^{Pro}) was predicted as a primary mechanism[1]. Therefore, we conducted an in vitro anti-SARS-CoV2 M^{Pro} activity of THC, CBD, and CBN to confirm the predicted antiviral mechanism from this recent study[1]. As a result, THC and CBD were identified as bioactive agents with 100 % SARS-CoV2 M^{Pro} inhibition in an in vitro assay. CBN was inactive, showing 30 % inhibition only. IC_{50} value of THC and CBD was determined at 1.8 µM for CBD and 13.1 µM for THC. Our results are partially in line with the anti-SARS-CoV2 M^{Pro} prediction for THC and CBD by Raj et al. (2021)[1] and suggest a different anti-SARS-CoV2 mechanism for CBN.

This finding suggests a structure-activity relationship (SAR) among THC, CBD, and CBN. Main structural features are a flexible structure with bicyclic rings (C6-C6) and a cyclohexene substitution being present in CBD. This study is the first study reporting experimental data of an in vitro anti-SARS-CoV2 M^{Pro} activity of THC, CBD, and CBN and their structure-activity relationship.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. Funding (DAAD, grant number 57299294)

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PC9-6 Small molecules inspired from endogenous vitamin E metabolites induce a lipid mediator class switch from inflammation to resolution in innate immune cells

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DOI 10.1055/s-0041-1736935

Inflammation is a complex process driven by innate immune cells that produce pro-inflammatory lipid mediators during acute phase and specialized pro-resolving lipid mediators (SPM) in the resolution phase. Macrophages of the M2 subtype play a major role in inflammation resolution as they strongly express ALOX15 and ALOX15B and effectively release SPM, including resolvins, protectins and maresins. This lipid mediator class switch terminates inflammatory processes and restores tissue homeostasis. Current anti-inflammatory therapy largely targets pro-inflammatory cascades, without considering resolution. We have recently shown that endogenous metabolites of vitamin E cumulate in innate immune cells at inflammatory sites, limit inflammation by targeting 5-lipoxygenase (5-LOX) and raise systemic resolvin levels. Starting from an in-house library of 150 isolated and semi-synthesized vitamin derivatives, we searched for structurally optimized compounds that combine potent inhibition of 5-LOX with enhanced SPM biosynthesis. Lipid mediator profiles of activated

and non-activated macrophages (M1 and M2), peripheral blood mononuclear cells and neutrophils were assessed. We identified specific ω-oxidized tocotrienol derivatives that selectively trigger the biosynthesis of protectins among SPM while maintaining the 5-LOX inhibitory activity. These lead compounds are of high interest as tools for studying protectin function and open the door to a novel class of anti-inflammatory/pro-resolving drug candidates that induce a switch from leukotrienes to protectins.

PC9-7 Evaluation of the anti-tyrosinase activity of extracts and rare flavonoids from *Scleranthus perennis* L.

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Scleranthus perennis L. (Caryophyllaceae) is widely spread in Europe, Western Asia and North Africa. In folk medicine, the plant was used in veterinary to remedy animals that display fluctuating temperament. However, its phytochemical composition and pharmacological activity have not been established so far [1]. Our study aimed to analyzed anti-tyrosinase activity of extracts, fractions and the isolated compound obtained from *S. perennis*. The ethyl acetate fraction was phytochemically studied, leading to the bio guided isolation of rare C-flavonoid named as 5,7-dihydroxy-3'-methoxy-4'-acetoxy-flavone-8-C-*β*-D-xyloside-2''-O-glucoside [2]. The structure of compound was determined on the basis of spectroscopic data interpretation. All extracts, fractions and isolated compound were tested for their potential anti-tyrosinase activity. It was found that both diethyl ether and ethyl acetate fractions are the most effective in tyrosinase inhibition activity assays (110.09 µg/mL and 290.54 µg/mL, respectively). Moreover, isolated flavone showed comparable activity to the tested extracts or fractions. Correspondingly to our results, we concluded that all samples and isolated compound showed significant anti-tyrosinase activity. Obtained samples may protect the skin macromolecules against enzymatic degradation. Therefore, tyrosinase inhibitors block melanogenesis and prevent hyperpigmentation of the skin.

Conflict of Interest

The authors report no conflict of interest.

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PC9-8 *In vitro* and *in vivo* anti-influenza H1N1 activities of a hydroethanolic extract of *Cupressus sempervirens* cones

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Cupresssus sempervirens cones are rich in condensed tannins. A proanthocyanidin fraction exhibited *in vitro* antiviral activity against two retroviruses [1]. A standardized hydroethanolic extract of fresh cones extract [HCE] (Phytostandard® process, PiLeJe Laboratoire) showed virucidal activity using EN14476 + A1 standard against common respiratory viruses, including H1N1 [2].

To further assess the HCE properties on H1N1, we used two tests: 1) an *in vitro* cell model of the human airway epithelium cultured at the air liquid interface (MucilAir™) in which the virus and HCE were co-incubated during 3 hours on the apical surface, HCE being replaced every 24 hours during 4 days. Viral replication was assessed by quantitative RT-PCR; 2) An *in vivo* model in which mice were orally treated 14 days with HCE before nasal infection with H1N1. The HCE was administered for another 5 days after infection, then mice were followed for another 5 days.

In vitro, the HCE significantly delayed viral replication and reduced it according to dose and time. *In vivo*, the HCE significantly limited weight loss compared to infected mice without treatment at Days 7, 8 and 9. These results confirm and strengthen the antiviral properties of the HCE tested on H1N1. Further investigations will be needed to determine underlying mechanisms.

Conflict of Interest

GI, BV and DM are employees of PiLeJe.

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PC9-9 An *Echinacea purpurea* root extract modulates macrophage activation and polarization in response to infection

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Echinacea preparations are traditionally used for the prevention and treatment of upper respiratory tract infections. Although immunomodulatory properties have been described, notably on innate immunity, effects remain dependent on the phytochemical composition of the extract used, itself a function of the species and part of the plant used, as well as the method of extraction.

We investigated the immunomodulatory properties of a standardized hydroethanolic extract of fresh roots of *Echinacea purpurea* (patented process Phytostandard®; hydroethanolic root extract [HRE], PiLeJe Laboratoire) on peritoneal macrophage activation and polarization in response to LPS and *Streptococcus pneumoniae*. C57BL/6 mice were orally treated once daily with HRE or phosphate-buffered saline (n = 10/group) for 14 days and then **intraperitoneally injected** with LPS (0.5mg/kg). Twelve hours after LPS injection, activation of macrophages was assessed by flow cytometry and RT-qPCR. *Ex vivo* microbicidal functions of macrophages were
evaluated against *Streptococcus pneumonia* (phagocytosis and killing by fluorescence and CFU quantification, respectively).

Interestingly, the HRE decreased inflammatory response to LPS and induced changes in immune cell populations by increasing the percentage of small peritoneal macrophages and decreasing that of systemic classical monocytes. Regulation of macrophage gene expression was also observed; HRE decreases pro-inflammatory cytokines, increases C-type Lectin receptors and CD36, LTB4 metabolism pathway. Our findings show that bactericidal activity against *S. pneumoniae* was significantly increased in macrophages isolated from mice supplemented with HRE *versus* PBS.

Altogether, our results suggest that the HRE of *E. purpurea* tested strengthens innate immunity against invading pathogens while limiting the inflammatory process.

Conflict of Interest

BV, AM, GI and HS are employees of PiLeJe.

Planta Med 2021; 87: 1233–1328

PC9-10 Menthacarin® desensitizes TRPA1 channels

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Menthacarin® is a proprietary combination of 90 mg essential oil (EO) from *Mentha x piperita L.* and 50 mg EO of *Carum carvi* that has demonstrated efficacy in patients suffering from functional gastrointestinal complaints [1]. Transient receptor protein (TRP) channels are believed to play a relevant role in visceral hypersensitivity. Hence, modulation of TRPs represents an interesting potential therapeutic approach [2]. We aimed to characterize the TRP modulatory potential of Menthacarin and the relative contribution of its individual oil constituents in an *in vitro* study.

The activation of TRPA1, TRPM8 and TRPV4 by Menthacarin and its EOs constituents was assessed in human cells via determination of calcium influx by FLIPR technology. Menthacarin displayed activation of both TRPM8 and TRPA1, whereas no effect on TRPV4 was observed. TRPM8 activation seems to be mainly due to peppermint oil, whereas caraway oil was more potent on TRPA1.

Desensitization of TRPA1 by Menthacarin was assessed by repeated exposure of cells to the oil mixture, followed by assessment of the cross-desensitization to the TRPA1 agonist allyl isothiocyanate (AITC) using a FlexStation. Repeated administration of Menthacarin produced a concentration-dependent initial activation and subsequent desensitization of TRPA1 signaling. At high concentrations also cross-desensitization to AITC challenge was observed. In conclusion, Menthacarin activates TRPM8 and desensitizes cells to TRPA1 channel activation. These activities could underlie in part the clinical benefit in the treatment of functional gastrointestinal complaints.

All authors are employees of Dr. Willmar Schwabe GmbH & Co. KG, Germany.

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PC9-11 A novel triterpene glycoside from the sea cucumber *Holothuria atra*

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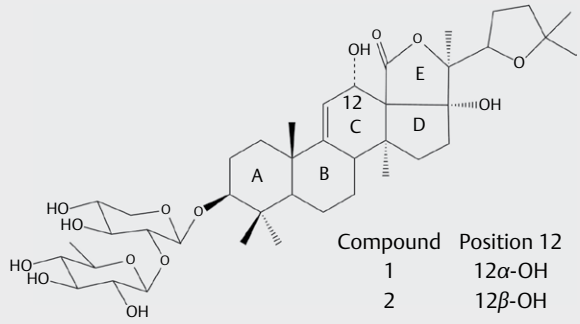
DOI 10.1055/s-0041-1736940

Sea cucumbers are marine invertebrates, commonly used as food, or applied in traditional medicine in Asia. They are known as a rich source of triterpene glycosides.

In the current work, the black sea cucumber *Holothuria atra* Jaeger, one of the commercially important species in Indonesia, was studied. An 80 % methanol crude extract was prepared and fractionated by means of column chromato-graphy (Diaion HP20). Semi-preparative HPLC-DAD-MS led to the isolation of two isomeric compounds.

Both compounds were submitted to extensive NMR, as well as HRMS analysis. The ^{13}C-NMR data of compound **1** were in agreement with those of desholothurin B (desulphated holothurin B) [1]. While the HRMS data of compounds **1** and **2** did not differ significantly, differences were observed for the ^{13}C-NMR data, especially for carbon signals of ring C. Based on 2D NMR, including NOESY, compound **2** was identified as a novel compound, the 12-epimer of **1**, and was therefore named 12-*epi*-desholothurin B.



▶ **Fig. 1**

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. The Indonesia Endowment Fund for Education (LPDP) and Indonesian Ministry of Research, Technology and Higher Education (RISTEKDIKTI) are thanked for the financial support of the research of Yunita Eka Puspitasari.

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PC9-12 Phytochemical investigation, characterization and antimicrobial evaluation of the extracts and isolated compounds of *Pistachia lentiscus* var *Chia* leaves

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P. lentiscus L. var *Chia* belongs to the Anacardiaceae family and it is cultivated only in the south part of Chios island, Greece. Although *P. lentiscus* is known for its resin, the leaves have been also used in traditional medicine. Thus, the aim of the present study was the detailed phytochemical investigation of *P. lentiscus* leaves and the evaluation of biological properties of total extracts as well as pure compounds. To that end, twelve compounds were isolated and purified from methanol leaf extract and were identified using spectroscopic and spectrometric methods (NMR, HRMS) belonging to phenolic acids, flavonoids and terpenes, with the most interesting being 2-hydroxy-1,8-cineole glucopyranoside which was isolated for the first time in Anacardiaceae family. In parallel, a dereplication attempt using UPLC-HRMS & HRMS/MS led to the detailed characterization of the metabolite profile of extracts under investigation. Finally, three total leaf extracts (MeOH, EtOAc, H_2O) and three pure compounds i.e. shikimic acid, 2-hydroxy-1,8-cineole glucopyranoside and myricitrin were evaluated for their antimicrobial properties. MeOH and H_2O extracts as well as, myricitrin and particularly 2-hydroxy-1,8-cineole glucopyranoside showed significant selective activity against pathogenic Mucorales, but not against Aspergilli, *Candida albicans* or bacteria [1, 2].

Conflict of Interest; Funding (Source, ID)

The authors declare that there is no conflict of interest. Plant UP, project code: 5002803; grant of Stavros Niarchos Foundation

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PC9-13 Primula veris from Epirus, Greece: a rich source of flavonoids. Development and validation of an HPLC method for the analysis of the flowers

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An HPLC-PDA method was developed for the determination of the flavonoids, in the flowers of *Primula veris* from Epirus, Greece. The aim was to investigate the chemical content of the over harvested *Primula veris* populations and to develop a suitable analysis protocol. Primula flowers from Epirus were particularly rich in flavonol triglycosides, derivatives of quercetin, isorhamnetin and kaempferol (**1**-**3**). Chromatographic isolations of the hydromethanolic (70 %) extract afforded a new naturally occurring flavonoid, isorhamnetin-3-*O*-*β*-glucopyranosyl-(1→2)-*β*-glucopyranosyl-(1→6)-β-glucopyranoside (**3**). Its structure elucidation was carried out by 1D and 2D NMR and mass spectrometry analyses. An HPLC-UV method was developed and validated according to ICH guidelines. Rutin was used as secondary standard and the correction factor for response was determined. The HPLC method was validated for linearity, LOD, LOQ precision and accuracy in three concentration levels. R.S.D. values ranged between 0.18 % and 2.67 %, for the intraday variation, within the acceptable limits. The inter-day variation ranged between 0.22 % and 3.37 % R.S.D. Accuracy was between 96.8 and 104.4 with RSD values ranging between 0.62 and 4.20. Finally, the recovery of the extraction was proved to be over 99.5 % after four extraction cycles, while the precision of the extraction expressed as RSD values varied between 1.58 and 4.66, within the acceptable limits. The developed assay was fast and simple and permitted the quality control of the herbal drug. The studied samples were particularly rich in flavonoids and the mean total content of flavonols ranged from 4.46-6.67 %mg, higher than the 3 % which is reported by the EMA [1].

Conflict of Interest; Funding (Source, ID);
The authors declare no conflicts of interest. This study is funded by the Region of Epirus, Greece. The authors are grateful to Assistant Professor Maria Halabalaki, National and Kapodestrian University of Athens for recording the High-Resolution mass spectra.

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PC9-14 Gram-scale isolation of Gentiopicroside from *Gentiana lutea* and evaluation of anti-proliferative activity against cancer and normal cell lines.

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DOI 10.1055/s-0041-1736943

Gentiopicroside (GPC) is a high added value glycosylated secoiridoid with numerous biological activities including hepatoprotective, and anti-inflammatory activities. The aim of this study was the development of an efficient method for the recovery of pure GPC from *Gentiana lutea* roots and the evaluation of its anti-proliferative activity against cancer and normal cell lines.

The separation process includes the combination of Centrifugal Partition Extraction (CPE) and Preparative High Performance Liquid Chromatography (prep-HPLC). CPE technique was firstly applied for the fractionation of methanol extract using Hept/EtOAc/EtOH/H_2O 1/8/3/5 (v/v/v/v) solvent system in elution-extrusion mode. 42gr were analyzed in 7h resulting in the recovery of 5gr of enriched GPC fractions which were then submitted to prep-HPLC to obtain 3,6gr of GPC in 97 % purity (HPLC, UPLC-HRMS, ^{1}H-NMR). This highly productive methodology with the advantage of industrial scalability provides in short time, large amounts of GPC, in pure form.

Its anti-proliferative activity was determined in the lung (A549, NCI-H1299), breast (MCF7, MDA-MB468), glioblastoma(T98G), rhabdomyosarco-ma (TE671),and colon (HT-29, DLD-1) cancer cell lines as well normal colon epithelial cells (CCD 841 CoTr) using the MTT assay. The dose-dependent growth inhibition effect was evident in all analyzed cancer cell lines. TE671 cells were the most sensitive and MDA-MB-468 cells were the least sensitive to GPC treatment with IC_{50} = 0,078mg/ml and 0,208mg/ml, respectively. A much weaker cytotoxic effect was observed against CCD 841 CoTr normal cells.

Conflict of Interest: Statement on behalf of all authors. There is no conflict of interest. **Funding**: (PlantUp, 5002803)

PC9-15 Integration of dereplication and regression analysis for rapid characterization of antiplasmodial compounds from the leaves of *Combretum paniculatum*

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DOI 10.1055/s-0041-1736944

The rapid development of modern analytical techniques and various chemometric approaches provide new perspectives for early metabolite identification in natural products research. These techniques represent a potential strategy to streamline the traditional and laborious process of isolating natural products through targeting of unknown active compounds before purification. In the present study, these innovative techniques have been applied on extracts of the leaves of *Combretum paniculatum*, which have demonstrated promising antiplasmodial activity during our preliminary studies, leading to a quick and effective identification of compounds correlated to this activity. The fractionation of crude extracts was carried out, followed by multivariate data analysis of liquid chromatography–high resolution mass spectrometry (LC–HRMS) profiles of the fractions obtained. In parallel, all fractions were screened against *Plasmodium falciparum* strain K1, and their cytotoxicity against MRC-5 cells was determined. Dereplication studies combining UPLC-MS/MS-based molecular networking, *in silico* analysis and NMR methods were employed to identify the important metabolites. Several compounds strongly correlated with antiplasmodial activity have been identified. Six compounds including rutin (IC_{50} 6.7 µM) and foliasalacioside F (IC_{50} 10.6 µM) have been isolated and their OPLS predicted score values were in agreement with antiplasmodial results found *in vitro*. These preliminary results provided clear evidence on the effectiveness of using these innovative methods (chemometrics and dereplication analysis) for the rapid identification of active metabolites in plant extracts. Further research aiming for the isolation of additional promising compounds which have shown a strong correlation with antiplasmodial activity is ongoing.

PC9-16 Sulfated galactan from *Acanthophora muscoides* reduces lipid accumulation during differentiation in 3T3-L1 cells

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Obesity is one of the most expensive current challenges for public health. Due to the large number of side effects of antiobesity drugs available today, there is an urgent need to develop new drugs that are both therapeutically potent and safe. This work aims to evaluate the effect of a sulfated galactan from *Acanthophora muscoides* (GSAM) on lipid accumulation in 3T3-L1 cells differentiated for 9 days [1], through staining with Oil Red O and analysis of protein expression by Western Blot. MTT assay showed that GSAM (6.25 – 400 µg/mL) reduced the viability of differentiated 3T3-L1 cells, by 22 %, only at the concentration of 400 µg/mL, compared to the control group. Through staining with Oil Red O, it was observed that GSAM (25, 50 and 100 µg/mL) reduced the lipid content of 3T3-L1 cells by 21.6 %, 28.5 % and 40.5 %, respectively, compared to the control group. Regarding the protein expression of important adipogenic transcription factors, GSAM (25, 50 and 100 µg/mL) reduced C/EBPα, by 17.6 %, 21.1 % e 23.6 %, and PPARγ, by 18.7 %, 24.2 % and 26 %, respectively, compared to the control group. Thus, our data suggests that sulfated galactan from *A. muscoides* inhibits lipid accumulation by controlling adipogenic factors in 3T3-L1 cells.

Conflict of Interest; Funding (Source, ID)
The Authors declare that there is no conflict of interest; CAPES, CNPq, FUNCAP.

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PC9-17 Analysis of photosensitizers from fungi via a photo-antimicrobial HTS

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Antimicrobial drug resistance is considered a serious threat to human health. Photodynamic antimicrobial chemotherapy (PACT), utilizing the synergistic effect of light and a chromophore, is an option to kill resistant microorganisms (MOs), causing non-specific multi target damage through reactive oxygen species [1]. Established photosensitizers (PS) are often based on dyes or porphyrin structures. Our goal was to discover novel structures aside from well-known scaffolds. We established a high-throughput screening (HTS) method, based on the European Committee on Antimicrobial Susceptibility testing (EUCAST), allowing to examine and compare potential PS under different wavelengths, light doses, and preincubation times. Fungi, a well-known source for traditional antibiotics, were only recently found to yield PS [2]. We examined the potential of six colorful representatives from the genus *Cortinarius* for photodynamic growth inhibition of three different MOs: *Candida albicans* (yeast), *Escherichia coli* (gram negative bacteria), and *Staphylococcus aureus* (gram positive bacteria). The most active extract was from *Cortinarius xanthophyllus*. Minimum inhibitory concentrations (MICs) were found at c = 7.5 µg/mL for *S. aureus* and at c = 75 µg/mL for *C. albicans* (λ = 478 nm, H = 30 J/cm^2, PI = 60 min). In summary, we present the application of an EUCAST based HTS for finding new PS in fungi.

FWF project "PhotoFungal" (P31950). The authors declare no conflict of interest.

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PC9-18 Benzylated dihydrochalcone MF-15 as a potent multitarget inhibitor of cancer cell growth

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DOI 10.1055/s-0041-1736947

Dihydrochalcones constitute a family of natural flavonoids that are well known for their anti-oxidative abilities. We investigated MF-15, a benzylated dihydrochalcone found in *Melodorum fruticosum* in an in silico profiling approach and discovered that this natural product possesses a wide array of bioactivities that also translate into anti-proliferation activity on different cancer cell lines. MF-15 was shown to inhibit 17β-hydroxysteroid dehydrogenase type 5 (87 % inhibition at a concentration of 10 µM) [1] and also to inhibit microsomal prostaglandin E synthase 1 with an IC_{50} of 1.8 µM. A Lipid mediator profiling in activated macrophages displayed a strong potential for upregulating of resolvins that resolve inflammation. In addition, we also confirmed inhibition of the androgen receptor by MF-15. These individual activities lead to a dose dependent inhibition of cell proliferation in castration resistant prostate cancer cell lines [1] as well as liver (Hep3B, IC_{50} of 6.38 µM) and thyroid cancer cell lines (TPC1,IC_{50} of 10.45 µM). In silico analysis of the binding modes of this compound interacting with different target proteins suggests that structural optimization towards higher bioactivity and selectivity is feasible. MF-15's impressive polypharmacological profile with the dual anti-androgenic effect, in combination with its anti-inflammatory properties render it an extraordinary lead structure from the class of dihydrochalcones.

The authors declare no conflict of interest; V.T. Is funded by the Austrian Science Fund (FWF) projectT942

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PC9-19 Quality differences of genus *Chrysanthemum* used as food and medicine from the global market

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DOI 10.1055/s-0041-1736948

Introduction Many studies have confirmed the pharmacological effects of Chrysanthemum flowers, but few have assessed the quality from a global market perspective [1], also ensuring product safety [2].

Material and Methods 16 *Chrysanthemum indicum* L. and 49 *Chrysanthemum x morifolium* (Ramat.) Hemsl. samples were obtained from the USA, EU and China. Some *C. morifolium* samples are labeled using their cultivar names. HPTLC was used to compare general chemical components among samples, and by ICP-OES to quantify heavy metal contamination.

Results The fingerprints of *C. indicum* samples are clearly distinguishable from those of *C. morifolium* based on the absence of a zone due to linarin. Different pattern found in their fingerprints do not always correlate with names of medicinal cultivars of *C. morifolium*. There are differences among the same cultivars collected on markets from different countries (USA and China).

As part of the heavy metal analysis, cadmium levels were most commonly above the accepted limits with no problems identified for lead and arsenic, as well as only one sample showing copper above the limit. In general, the USA samples showed the lowest rate for heavy metal contamination, but sample TAI-2 (*C. morifolium*) from USA showed by far the highest amount of cadmium. Generally, *C. indicum* have a higher excessive rates of heavy metals than *C. morifolium*.

Conclusions Some differences between USA and Chinese samples were observed, which might be ascribable to geographical reasons, e. g., soil conditions, degradation during transport or adulteration. Cadmium contamination is the most serious problem for heavy metal contamination.

Statement on behalf of all authors. I hereby confirm that the work submitted is my own. Any ideas submitted in this project are my own. Any ideas, quotations, and paraphrasing from other peoples work and publications have been appropriately referenced.

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PC9-20 Pholiols A-D and other triterpenes from *Pholiota populnea* and their activity against colon carcinoma

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DOI 10.1055/s-0041-1736949

Pholiota populnea (Pers.) Kuyper & Tjall.-Beuk. (Strophariaceae) is a saprophytic or sometimes parasitic mushroom species, which grows on poplars, willow and birch. The detailed phytochemical analysis of the hexane phase of P. populnea led to the isolation of four new lanostane diesters, named pholiols A-D (**1**–**4**), together with tetrahydroxy-squlene (**5**), ergosterol and 3β-hydroxyergosta-7,22-diene. The isolation was carried out by flash chromatography and HPLC, the structures were elucidated using extensive spectroscopic analyses. All compounds were isolated for the first time from this mushroom. The isolated fungal metabolites were investigated for antiproliferative activity against Colo205, Colo320 colon adenocarcinoma and non-tumoral MRC-5 cell lines. Combination with doxorubicin and efflux pump inhibitory activity of the compounds on Colo 320 cells were also assayed.

Among the tested compounds only ergosterol (**5**) showed antiproliferative activity against all cell lines with no tumour cell selectivity. P-glycoprotein efflux pump modulatory test on resistant Colo 320 cells indicated that pholiol A (**1**) and B (**2**), and **5** have the capacity to inhibit the efflux-pump over-expressed in the cells. Very strong synergism with doxorubicin was observed for linear triterpene **5**. Our results indicate that *P. populnea* is a promising source for finding new terpene metabolites with significant chemo-sensitizing activity on cancer cells.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest. This work was supported by NKFIH grant No. K135845, Hungary.

PC9-21 Protoflavone – spirooxindole hybrids exhibit promising, increased antitumor activity

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DOI 10.1055/s-0041-1736950

Protoflavones are rare natural flavonoids with a non-aromatic B ring, typically found in fern species. Several of these compounds show promising antitumor effects *in vitro* on various cell lines and *in vivo*. These protoflavones induce apoptosis and are capable to inhibit the ATR-mediated activation of checkpoint kinase 1, a crucial step in DNA damage response [1].

Spiropyrazoline oxindoles are found to induce apoptosis and upregulate p53 steady-state levels [2].

In this study we aimed to combine the aforementioned two compound groups with different antitumor mechanisms, gaining new hybrids with increased antitumor activity.

Utilizing modified sidechains and copper(I) catalysed azide-alkyne cycloaddition reactions we synthesized 8 new hybrid compounds so far.

The antitumor tests are in progress on multiple cell lines. Preliminary results look promising, as for example one of tested hybrid compounds showed 87.9 ± 1.1 % inhibition on MDA-MB-231 cell line at 5uM concentration, significantly higher than that of the fragments.

Acknowledgement: Supported by the ÚNKP-20-3 - New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund and FUNDAÇÃO PARA ciência e tecnologia (FCT) through COOPERAÇÃO CIENTÍFICA E TECNOLÓGICA FCT/NKFIH 2019/2020, UIDB/04138/2020 (IMED.ULISBOA), and PhD fellowships SFRH/BD/137544/2018 and SFRH/BD/117931/2016.

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PC9-22 Characterization of a *Malva sylvestris* L. flos preparation for beneficial properties suitable in the treatment of dry eye disease

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DOI 10.1055/s-0041-1736951

In dry eye disease (DED), inflammation is responsible for clinical symptoms such as itching, foreign body sensation, redness and pain. The release of pro-inflammatory mediators is triggered by an hyperosmolar environment due to loss of tear volume. Leukotrienes and prostaglandins produced by 5-lipoxygenase (5-LOX) and cyclooxygenase-2 (COX-2), respectively, are key mediators in inflammation. Visiodoron Malva® eye drops – authorized as a medical device for the treatment of DED – contains an ethanolic extract of *Malva sylvestris* L. flos (MS) that accounts for a reduction of surface tension thereby facilitating the wetting of the ocular surface. We report about novel investigations *in vitro* to explore the putative potential of MS in DED treatment to prevent inflammation and oxidative stress and the ability to promote wound healing. MS concentration dependently inhibited 5-LOX- and COX-2 activity *in vitro* (IC_{50}: 22.7 and 23.3 µg/ml).

In an ORAC (oxygen radical absorbance capacity) assay the scavenging efficiency was high against peroxyl- and peroxynitrite radicals (IC_{50}: 30.3 and 23.3 µg/ml), medium against hydroxyl radicals (308 µg/ml) and low against superoxide radicals (> 1.000 µg/ml). In a cell culture set-up MS significantly enhanced the regenerative response *in vitro* at 100 µg/ml. These data suggest that MS might exert an effective contribution in DED treatment by anti-inflammatory and

radical scavenging actions. MS might be further helpful promoting regeneration following ocular damage in DED. However, further investigations are needed to study bioavailability and efficacy *in vivo*.

Conflict of Interest

Künstle G, Röhrl J and Ammendola A are employees of Weleda AG, Switzerland

PC9-23 Identifying structural features for changing substrate preferences in two Plantago PRISEs (progesterone 5β-reductase and /or iridoid synthase)

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DOI 10.1055/s-0041-1736952

Cardenolides are drugs used to treat congestive heart failure. Recently their antiproliferative action was brought back into focus [1]. They are still extracted from plants, as their chemical structure impedes chemical synthesis. Though attempts have been made to produce cardenolides by plant tissue culture, farming foxglove still remains the sole source of cardenolides with a possible alternative approach based on microbial production systems. So far we engineered yeast and *E. coli* to produce intermediate products of cardenolide biosynthesis but still need to overcome enzyme bottlenecks [2]. Targets of enzyme engineering are the PRISEs (progesterone-5β-reductase/iridoid synthase-like enzymes), that catalyzes the reduction of progesterone to 5β-pregnane-3,20-dione, which is an important intermediate of cardenolide biosynthesis. PRISEs occur ubiquitously in the plant kingdom [3] and are enzymes with relaxed substrate specificity [4].

In biotechnology shaping enzymes with respect to their substrate specificity is a useful approach to optimize biosynthetic pathways. Thus, to overcome substrate shortages, we investigated substrate conversion *in silico* and *in vitro* of two *Plantago* PRISEs by creating mutants via site-directed mutagenesis. A PRISE of *P. media* showed a 70-fold higher specific activity with progesterone than its orthologue from *P. lanceolata*. Docking substrates into mutants prior to the site-directed mutagenesis, was used as additional tool to identify catalytically impactful residues, so that we were able to completely invert the substrate specificities of the two *Plantago* PRISEs for progesterone. Engineering substrate specificity into promiscuous enzymes may not only help to eliminate side reactions in biosynthetic pathway but also to understand metabolite-enzyme evolution [5].

Conflict of Interest; Funding (Source, ID)

No conflict of interest. On behalf of all authors.

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PC9-25 Design-of-experiment approach to optimize anti-inflammatory activity of volatile oil mix

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DOI 10.1055/s-0041-1736953

Design-of-experiment (DOE) approaches have been suggested before to study interactions between pharmaceutical drugs and may be useful to detect synergistic or antagonistic effects among phytochemicals. Moreover, the composition of phytochemical mixtures may be optimized for distinct biological activities, undesirable side-effects, cost, and/or other targets.

In the present study, a mix of five volatile phytochemicals known to occur in many essential oils derived from plants (carvacrol, thymol, methyl salicylate, D-carvone, L-menthol) was optimized for anti-inflammatory (AI) activity in an *in vitro* mouse macrophage assay using DOE. RAW264.7 cells were cultivated in 96-well microplates, stimulated by LPS, and nitric oxide (NO) production was measured as an indicator of inflammatory processes in the presence or absence of phytochemicals. A full factorial and subsequently a response-surface-design were created (Minitab 19), based on minimal and maximal concentrations of each phytochemical, derived from data from testing the single phytochemicals. An optimized ratio of phytochemicals was calculated based on the observed NO production.

Statistical evaluation of the DOE approach showed significant effects of the five phytochemicals alone, and of several interactions ($p < 0.05$). An optimized ratio of phytochemicals for AI activity reduced NO production by macrophages to 42 % of stimulated control cells, whereas the original, non-optimized mix reduced it to 60 %.

A DOE approach was applied for optimization of a volatile oil mixture towards AI activity *in vitro*. Furthermore, the design seems promising for identification of synergistic or antagonistic effects of mixtures to be tested *in vivo*.

Conflict of Interest:

The authors declare no conflict of interest.

PC9-26 The key-aroma constituents of oregano (*Origanum*) grown wild in Greece unraveled by headspace GC-MS and Raman spectroscopy

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DOI 10.1055/s-0041-1736954

In the present work we utilized two techniques, Gas Chromatography – Mass Spectrometry (GC-MS) and Raman spectroscopy, to decode the aroma constituents of different *Origanum* (oregano) plants grown wild in Greece. Aerial parts of flowering *Origanum* plants were collected from three localities of the Greek mainland (Mt. Belles, Mt. Pelion and Kassandra Peninsula) and one in the SE Greek Archipelago (island of Kos). Their taxonomic identification suggests that plants of the mainland, with a spike-like inflorescence and actinomorphic calyces, belong to two subspecies of *Origanum vulgare*, *viz.* subsp. *vulgare* (Belles) and subsp. *hirtum* (Pelion and Kassandra). Plants from the island of Kos, having a corymbose inflorescence and 1-lipped calyces, belong to the species *Origanum onites*. Essential oils were extracted from the plants using a Clevenger apparatus. The static headspace GC-MS method resulted in the identification of many terpenes. Raman spectroscopy was carried out using a laser at 785.0 nm to minimize fluorescence. Results indicated that *O. vulgare* subsp. *vulgare* contained the most terpenes among the samples; *O. onites* consists mainly of carvacrol, whereas *O. vulgare* subsp. *hirtum* of either carvacrol or thymol. A

comparison of the GC-MS and Raman spectroscopic methods was further performed.

Funding

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-04174)

PC9-27 Occurrence and smell of the "white" and "black" oregano (*Origanum vulgare*) in mount Belles (GR 1260001)

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DOI 10.1055/s-0041-1736955

Origanum vulgare L. plants are widely used since antiquity as food flavorings and infusions, while their essential oils (EO) are used nowadays in a wide range of applications. Mount Belles (Beles) is located in SE Europe, shared by Greece, N. Macedonia and Bulgaria. It lies in the Northern part of the NATURA 2000 site GR1260001 (LIMNI KERKINI - KROUSIA - KORYFES OROUS BELES, ANGISTRO – CHAROPO) [1]. In the frame of a wider project dealing with the morphological variation and EO composition of *O. vulgare*, aerial parts of flowering plants were collected from four geographical distinct localities of mount Belles. The dominant habitat type in each collection area was considered according to EUNIS [2, 3]. Based on a number of diagnostic - morphological characters (such as size and colour of bracts and corollas, number and size of leaf sessile glands), the plants were taxonomically identified as *O. vulgare* subsp. *hirtum* (Link) letswaart (white oregano) and *O. vulgare* subsp. *vulgare* (black oregano). The total EO yield (mL $100g^{-1}$ d.w.) and carvacrol content (% of identified peaks, by Headspace GC-MS) of subsp. *hirtum* plants collected from three localities of low to medium altitudes (< 450 m), where the habitat Pannonian-Balkanic turkey oak-sessile oak forests (code 91M0) dominates, was 1.8 and 92.5, 2.3 and 92.6, and 2.7 and 86.0, respectively. On the other hand, subsp. *vulgare* collected from a higher altitude (1335 m), where the habitat *Asperulo-Fagetum* beech forests (9130) occurs, had a much lower EO yield (0.1 %) and composition mainly characterized by sesquiterpenes.

Conflicts of Interest: The authors declare no conflict of interest

Funding:This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-04174

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PC9-28 Cytotoxic effect on human melanoma cell lines and tyrosinase inhibition of *Hottonia palustris*

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DOI 10.1055/s-0041-1736956

Our previous study on *Hottonia palustris* L. (Primulaceae) [1] lead to isolation and identification of rare methoxyflavone derivatives, including 5-hydroxyflavone, 5-hydroxy-2′-methoxyflavone, 5-hydroxy-2′,6′-dimethoxyflavone, as well as dibenzoylmethane [2], and sakurasosaponin. Thus, in the present study the activity of the isolated compounds, together with crude extracts and fractions was investigated, with a focus on an *in vitro* melanoma model. HTB140, A375, WM793 human melanoma cells, combined with normal skin keratinocytes HaCaT were used to examine the efficacy towards cancer cells differing in metastatic potential, as well as to verify the selectivity of action. Cell viability test was performed with the MTT assay, after 24h and 48h of incubation [3]. Butanolic fraction and the isolated sakurasosaponin revealed the highest cytotoxicity, with IC_{50} 7.15-16.63 and 2.04-4.5 µg/mL, respectively, and high selectivity. As melanoma growth is also associated with hyperexpression of tyrosinase, inhibitory activity towards this enzyme was investigated. Diethyl ether and ethyl acetate fractions revealed the highest activity, with IC_{50} 59.88 and 75.14 µg/mL, respectively. Such comprehensive anti-melanoma approach provides a more precise answer to the impact of the tested compounds on the viability of cancer cells, also their selectivity action on normal cells what may lead to preselection of substances with both high efficacy and safety profile.

Conflict of Interest

The authors report no conflict of interest.

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PC9-29 Overexpression of 3β-hydroxysteroid dehydrogenases in *Digitalis lanata* shoot cultures

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DOI 10.1055/s-0041-1736957

In recent years, the focus of cardenolide research shifted from their use in cardiac insufficiency towards their effects on different types of cancer and virus-induced diseases [1]. Plants are still the source for cardenolides. Therefore, there is still an interest in elucidating and manipulating cardenolide biosynthesis. We here focused on the investigation of 3β-hydroxysteroid dehydrogenases (3β-HSD) that are supposed to be involved in cardenolide biosynthesis, namely the oxidation of pregnenolone to isoprogesterone and the reduction of 5β-pregane-3,20-dione to 5β-pregnan-3β-ol,20-one.

Based on the Agrobacterium transformation protocol introduced by Klein et al. [2] we were able to generate D. lanata shoot cultures (OE lines) overexpressing 3β-HSD1 and 3β-HSD2 (DlHSD1 and DlHSD2). Overexpression was verified by qPCR and cardenolide content was quantified by UPLC. As cardenolide levels were not altered in OE lines as compared to non-transformed shoots (ca. 1.0 µmol/g dry weight), we checked for possible reasons for this observation. OE lines showed increased glutathione (GSH) levels as compared to controls (OE lines: ca. 300 µmol/g FW; WT: ca. 100 µmol/g FW). Reduction of GSH levels by feeding buthionine sulfoxime (BSO), an inhibitor of glutathione (GSH) synthesis, resulted in improved cardenolide formantion [3].

Therefore, we treated OE lines with BSO and also fed pregnenolone and 5β-pregnane-3,20-dione, assumed to be substrates of 3β-HSDs. While GSH levels could be normalized by this treatment cardenolide contents were barely effected. Similar results were reported for OE lines, expressing progesterone

5β-reductase, another gene involved in cardenolide biosynthesis [4]. We conclude that RNAi-mediated knockdown is a more effective approach to demonstrate participation of certain enzymes in biosynthetic pathways [2, 5].

No Conflict of Interest on behalf of all authors.

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PC9-30 Chemosensitizing properties of ß-caryophyllene oxide in hepatocellular carcinoma

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DOI 10.1055/s-0041-1736958

Hepatocellular carcinoma (HCC) is an aggressive malignant tumor, often associated with multidrug resistance, which limits the efficacy of conventional anticancer drugs [1]. In the last years, the possibility to affect tumour growth with multi-target therapies acting in synergy or on different targets has been highlighted. This approach enables to increase low-dose anticancer drug efficacy and limit systemic toxicity. In present study, the ability of the natural sesquiterpene ß-caryophyllene oxide (CRYO) to enhance the response of HCC to the first-line drug sorafenib (SOR) has been investigated.

The cytotoxicity of CRYO alone or in combination with SOR was evaluated by MTT assay in different HCC cells [2, 3]. Moreover, its ability to modulate both activity and expression of Pgp, MRP1/2 and STAT3 was measured[2, 3]. At last, the sorafenib chemosensitization by the natural sesquiterpene was assessed in a liver xenograft model [2]. Despite a low cytotoxicity in our cellular models, CRYO was able to synergistically increase HCC cell sensitivity to SOR. CRYO also inhibited Pgp and MRP1/2 activity and reduced the SOR-induced expression of Pgp, MRP1/2 and STAT3 (Ser 727). *In vivo* experiments highlighted the combination of CRYO and SOR was able to inhibit the tumor growth of about 58 %, despite a null effect of the anticancer drug alone.

In conclusion, our results highlight the possible role of CRYO as a chemosensitizing agent in combination with SOR and support its potential usefulness to reverse the sorafenib-induced multidrug resistance by targeting Pgp and MRP1/2 transporters, and/or the STAT3 pathway.

Conflict of Interest; Funding (Source, ID)

None

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PC9-31 Biological activity of aromatic plants cultivated in Northern Greece

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DOI 10.1055/s-0041-1736959

The biological activity of several aromatic plants (plant families: Apiaceae, Compositae, Lamiaceae) cultivated in Greece by Vessel company, is herein examined. All of them are of high economic importance, since they are used in a variety of products: from foods to cosmetics, perfumery and aromatherapy. Among them are the following six taxa (genera, species or hybrids): *Crithmum maritimum* L., *Helichrysum italicum* (Roth) G. Don, *Lavandula angustifolia* Mill., *Mentha* x *piperita* L. *(*hybrid between *M. aquatica* L. *and M. spicata* L.), *Thymus* L. *and Salvia sclarea* L.The plants and samples that had been cut at different cultivation timepoints from the regions of Chalkidiki (Tenedos) and Thessaloniki (Neo Risio), were evaluated for their antioxidant activity using two different assays:

(i) the interaction with the stable radical 1,1-diphenyl-picrylhydrazyl after 20 and 60 min and
(ii) the anti-lipid peroxidation activity. Their anti-inflammatory activity through the Lipoxygenase (LOX) inhibition was also determined. The biological results obtained from the examined species are very promising and usable.

Funding

This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T1EDK-04174)

PC9-32 Beetroot, beetleaves and rocket, as sources of nitrogen and sulfur containing compounds, attenuate lipid accumulation in adipocytes

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DOI 10.1055/s-0041-1736960

Nitrate and nitrite ions, found in green leafy vegetables, have been shown to have beneficial effects in the cardiovascular system, including cardioprotection, blood pressure reduction, antioxidant and anti-inflammatory activities, while also improving metabolic health [1, 2]. Likewise, H2S released from sulfur-containing compounds, like glucoerucin and glucoraphanin, has vasodilator and antihypertensive properties and stimulates angiogenesis [3]. Given their beneficial effects, various dietary supplements based on food plants rich in nitrogen and sulfur containing compounds have been developed. Herein, we studied extracts from beet (*Beta vulgaris*) and rocket (*Eruca sativa*), and evaluated their biological activity related to lipid accumulation in adipocytes. Beetroot, beetleaves and rocket were bought from local producers. Various, drying and extractions protocols were developed based on maceration with water, ultrasound

assisted extraction and supercritical fluid extraction, targeting to the production of environmentally friendly extracts, with high yields and especially high content in nitrate. The extracts were then evaluated for their ability to reduce lipid storage in cultured adipocytes. Beetroot juice and leaves extracts, as well as preparations from rocket attenuated fatty acid storage. We conclude that beet and rocket extracts and their combinations can be used for the preparation of dietary supplements with potentially beneficial effects in individuals with obesity and/or metabolic syndrome.

The authors declare no conflict of interest;

Funding: EPAnEK 2014-2020, VEGNO+, T2EΔK-00843

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PC9-33 Citral Containing Essential Oils as Potential Tyrosinase Inhibitors: a bio-guided fractionation approach to investigate the additive and/or synergistic contribution of minor compounds

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Excessive melanin production causes serious dermatological conditions as well as minor aesthetic problems (i.e. freckles and solar lentigo) The downregulation of tyrosinase, the key enzyme in melanin biosynthetic pathway, is a common strategy to treat such hyperpigmentation disorders and plant extracts have often proven to be valuable sources of tyrosinase inhibitors. Citral (a mixture of two structural isomers, *cis*- and *trans*-3,7-dimethyl-2,6-octadienal, also known as neral and geranial, in the typical 1/3 and 2/3 ratio) is an important fragrance ingredient which has shown anti-tyrosinase potential [1]. It is highly concentrated in the essential oils (EOs) of *Cymbopogon schoenanthus* (L.) Spreng., *Litsea cubeba* (Lour.) Pers., *Melissa officinalis* L. and *Verbena officinalis* L. however to the best of the authors' knowledge, only *L. cubeba* EO has been investigated as potential skin-whitening agent [2]. This work evaluates the *in-vitro* tyrosinase inhibitory activity of these EOs and studies through a bio-assay oriented fractionation whether the different chemical compositions influence the EOs overall inhibitory activities by possible synergistic, additive and/or competitive interactions among EOs components. The inhibitory activities of *C. schoenanthus* and *M. officinalis* EOs with negligible (+)-citronellal amounts were in-line with their citral content. Contrary, *L. cubeba* and *V. officinalis* EOs inhibited mushroom tyrosinase to considerably greater extent as they contained β-myrcene which additively contributed to the EOs overall activities. Similar considerations were done for *M. officinalis* EO with high (+)-citronellal content which increased citral activity potentially via synergistic interaction as it displayed no activity when tested individually.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest

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PC9-34 Essential oils bearing specialized metabolites with potential tyrosinase inhibitory activity

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Tyrosinase downregulation is a very widespread approach to reduce excessive melanin production, which is responsible for serious dermatological conditions as well as minor aesthetic problems (i.e., freckles and solar lentigo) [1]. Plants have been valuable sources of skin-whitening agents. To date, phenolic compounds have principally been studied while terpenoids have been relatively under-investigated as anti-tyrosinase agents [2]. This study explores the mushroom tyrosinase inhibitory activities of seventy-one essential oils which are complex mixtures of specialized volatile metabolites mainly including terpenoids. The investigated EOs have been subjected to *in vitro* enzymatic assay with Kojic acid used as positive control. Mushroom tyrosinase from *Agaricus bisporus* (J.E. Lange) was selected for this study. The chemical composition of the investigated EOs was determined by gas chromatography coupled to mass spectrometry. Among the investigated essential oils, the most promising ones were the EOs obtained by steam distillation from the leaves and flowers of *Citrus × aurantium L* (i.e. Neroli and Petitgrain EOs), citral containing EOs (i.e. *Cymbopogon shoenanthus Spreng* (L.), *Litsea cubeba* (Lour.) Pers, *Melissa officinalis* L. *and Verbena officinalis* L.), and β-myrcene containing EOs (i.e. *Juniperus communis* L. and *Pinus mugo* Turra EOs). Further studies are currently being carried out to comprehensively characterize all the EOs bioactive compounds and the potential antagonist/additive synergistic interactions responsible for the investigated biological activity of the EOs.

Conflict of Interest; Funding (Source, ID)

The authors declare no conflict of interest

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PC9-35 Phytochemical investigation of jointleaf rush (*Juncus articulatus*)

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Besides flavonoids, phenanthrenes are the characteristic secondary metabolites of Juncaceae species. These compounds are of great interest from structural, pharmacological and chemotaxonomical points of view [1, 2]. The aim of our work is the phytochemical and pharmacological investigation of Juncaceae species occurring in the Carpathian Basin. In the course of this work, the isolation of the secondary metabolites of *J. articulatus*, a perennial plant grows in moist areas, was performed. It is regarded as an environmental weed, it can form dense mats of vegetation and is a strong competitor with native species. The dried and ground plant material was extracted with methanol. After concentration, the extract was dissolved in 50 % aqueous methanol, and solvent-solvent partitions were performed with hexane, chloroform and ethyl acetate. Phenanthrenes are enriched in the chloroform phase; therefore, at first, this fraction was separated by column chromatography and then it was further purified by Sephadex LH-20 gel chromatography. As final purification, preparative thin-layer chromatography and high-performance liquid chromatography were used. Structure elucidation of the isolated compounds was carried out by NMR and MS spectroscopy as well as by comparison the data with literature values. To date, ten phenanthrenes, among them five new ones, were identified from jointleaf rush. The compounds are substituted with hydroxy, methyl, vinyl and acetylene groups. Moreover, two flavonoids, a glycerol derivative and vanillin were also isolated from the plant. All compounds were determined for the first time from *J. articulatus*.

Conflict of Interest; Funding (Source, ID) The authors declare no conflict of interest. This work was supported by National Research, Development and Innovation Office, Hungary (NKFIH; K128963).

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PC9-36 Antioxidant capacity in vitro of leaves and flowers extracts of Spathodea campanulata

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DOI 10.1055/s-0041-1736964

Background Reactive Oxygen Species (ROS) and reactive nitrogen species (RNS) are reactive molecules and free radicals produced in normal essential metabolic processes (endogenous sources) or due to external sources (e. g., ultraviolet, ionizing radiation, environmental toxins). Antioxidants are molecules that can donate an electron to a free radical without making themselves unstable; this causes the free radical to stabilize and become less reactive and prevent and repair damage caused by ROS/RNS. Plant phenolic compounds that share one or more phenol groups are the leading secondary plant metabolites with antioxidant properties. In this group, flavonoids are especially effective antioxidants and free radical scavengers. Aim: To determine the antioxidant capacity in vitro of Spathodea campanulata's leaf and flower extracts.

Material and Methods Aqueous (EAQSC), acetonic (EASC), and alcoholic extract (EOHSC) were obtained from Spathodea campanulata leaves and flowers. The total content of phenols, polyphenols, and flavonoids was quantified. The in vitro antioxidant capacity was determined using the compound DPPH (1,1-diphenyl-2-picrylhydrazyl) at different times. Results. Flower extracts contain a higher concentration of polyphenols, phenols, and flavonoids compared with leaf extracts. The highest antiradical efficiency in the leaf and flower extracts was observed in the aqueous extract, compared with the alcoholic and acetonic extract. In flower extract, the antiradical efficiency was twice that found in the leaf extract. Conclusion: Spathodea campanulata leaf and flower extracts are found to have antioxidant activity. Flower extracts have higher antioxidant activity compared to leaf extract.

PC9-37 The hydrophobic deep eutectic solvents for extraction of curcuminoids and ar-turmerone from the *Curcuma longa* L.

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The curcuminoids of *Curcuma longa* L. (CL) are curcumin (Cur) and derivatives such as bisdemethoxycurcumin (Bis) and demethoxycurcumin (Dem). Curcuminoids exhibit bioactivity against oxidative stress, inflammatory, infection, and cancer [1]. Curcumin and essential turmeric oils together give superior anti-inflammation than curcumin alone [2]. Although mentioned compounds play a significant function in biological activity, however, the extraction processes and utility of turmeric phytochemicals are challenged by their water insolubility, poor stability, and poor absorption. Therefore, tremendous strategies have been developed for the mentioned issues. The hydrophobic DESs were prepared using the mixture of menthol and a fatty acid [octanoic acid (OA), decanoic acid (DA), and dodecanoic acid (DDA)] at mole ratios (1:2, 1:1, and 2:1).

The DES compose of specific molar ratios of components, the hydrogen bond, and van der Waals forces are the interaction between menthol and fatty acid. The thermograms of DSC analysis indicated that the different mole ratios of menthol:OA and menthol:DA influence the shift of peak. These different ratios of components affect the extraction efficacy of curcuminoids and ar-Tur as well. The longer the hydrocarbon chain of fatty acid produced DES, with less effective the extraction of curcuminoids. The high yield of ar-Tur, on the other hand, was obtained with menthol:OA and menthol:DA. Among the investigated hydrophobic DES, the menthol:OA (2:1) demonstrated the highest extraction efficacy with Bis, Dem, Cur, and ar-turmerone concentration of 3.38 ± 0.24, 4.51 ± 0.42, 6.53 ± 0.58, and 6.48 ± 0.45 µg/mL, while the yields of mentioned compounds at 3.07 ± 0.01, 7.54 ± 0.37, 15.9 ± 0.27, and 6.21 ± 0.05 µg/mL, respectively, were obtained using methanol as the solvent. The results were obtained when 0.2 g of dry CL rhizome was extracted with 1 mL of solvent. Therefore, the hydrophobic DESs are suitable for the extraction of curcuminoids and ar-turmerone from the CL.

Conflict of Interest
There are no conflicts to declare.

Funding
Thailand Science Research and Innovation [contract NO. WU-FF64102]

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PC9-38 Phytochemical and pharmacological screening of Cyperaceae species and isolation of components of *Carex praecox*

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Cyperaceae is a cosmopolitan plant family with approx. 5000 species worldwide. The main constituents of sedges are phenolic compounds (flavonoids, phenolic acids, phenylpropanoids and stilbenoids) [1]. The aim of our work was the pharmacological screening of 26 Cyperaceae species native to Hungary. Based on the results of the antibacterial investigation, *Carex praecox* Schreb. was chosen to isolate its biologically active constituents.

The dried plant samples of Cyperaceae species were extracted with methanol. The extracts were evaporated, then solved in 50 % methanol and subjected to solvent–solvent partition with hexane, chloroform and ethyl acetate. All extracts were tested for their antibacterial effects by disc diffusion method against 8 bacterial strains. Dried aerial parts of *C. praecox* were extracted and separated with the same methods as in case of the antimicrobial screening study. Then the chloroformic extract was purified by various chromatographic methods, including VLC, MPLC, preparative TLC and HPLC. The structures of the isolated compounds were determined by a combination of NMR and MS measurements, and by comparing the data with literature values. In case of the antibacterial screening study, the ethyl acetate extracts proved to be the most active ones (inhibitory zones 15–20 mm). Among the tested sedges, *C. praecox* showed the highest antibacterial effect. As a result of the preparative work, to date 14 compounds, among them two novel and two known flavonoids, two novel and two known lignans, vanillin, benzaldehyde, *p*-cresol, dehydrovomifoliol and its derivative, and a chromene derivative were identified for the first time from the plant.

Conflict of Interest; Funding (Source, ID)
The authors declare no conflict of interest. The work was supported by the UNKP-21-3 (D.C.Z.) New National Excellence Program of the Ministry of Human capacities.

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PC9-40 Extraction of aphrodisiac chemicals from *Eurycoma longifolia* Jack using a natural deep eutectic solvent as a replacement for conventional solvents

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DOI 10.1055/s-0041-1736967

Eurycoma longifolia, also known as "Tongkat Ali", is an herbal medicinal plant found in Southeast Asia, including Thailand. The active compounds of *E. longifolia* root are classified into a group of quassinoids, canthin-6-one alkaloids, and beta-carboline alkaloids [1]. Eurycomanone is the major quassinoid found in *E. longifolia* root extracts, which has been reported to improve male sexual performance [2]. Natural deep eutectic solvents (NADES) are new solvents mainly composed of redundant primary metabolites found in nature. They're a potential alternative to toxic organic solvents in chemical processes, including extraction [3].

This study aimed to develop NADES for microwave-assisted extraction of aphrodisiac compounds from *E. longifolia* root. There were fifteen different NADES systems evaluated, each with three or four components. The analytes were identified and quantified after extraction using high-performance liquid chromatography with ultraviolet-visible detection (HPLC-UV) that has been developed. Eurycomanone, the aphrodisiac substance in *E. longifolia* root, was used as a biomarker for extraction. The HPLC-UV method was reliable and applicable for the determination of eurycomanone and other active compounds of *E. longifolia* root. The results showed that 50 % w/w of water: choline chloride: propionic acid (1:1:1) extracted the largest amount of eurycomanone, 1.19 and 19.60 times more than extraction with water and ethanol, respectively. The extraction efficiency of the other NADES was improved 1.03-1.18 times that of water and 17.02-19.47 times that of ethanol. This research shows the effectiveness of NADES in the extraction of bioactive chemicals from natural sources.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

This work was supported by the Personal Research Grant [grant number, WU-IRG-63-008], Walailak University, Thailand.

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PC9-42 Untargeted chemical and sensory characterization of *Swertia chirayita*: a bitter case study

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Bitterness, among all tastes, is the most difficult to decipher. Reasons are the chemical diversity of bitter-tasting compounds and the heterogeneous sensory response among the population. The very high bitterness sensitivity is further challenging regarding analytical methods. However, because of its pharmacological relevance, methodological developments are needed to decode bitterness. Relationship among chemical concentration and taste has been shown to be highly complex [1]. For this reason, we developed an untargeted methodology to deconvolute the tastes and composition of complex plant extracts. We selected *Swertia chirayita*, a well-known bitter plant with great chemical diversity as representative example. The extensive study of the fractions of the ethanolic extract was performed through 1D and 2D NMR experiments together with untargeted LCHRMS-MS analysis and CAD detection. After chemical clustering of the fractions, each cluster was evaluated with free sensory analysis, complemented by classical descriptive analysis [2]. This way, information gain was not limited to what has been named or annotated from both sensory or chemical point of view. Our data-driven, untargeted approach can be extended to any extract and allows an *a priori* characterization.

Attribution of the bitterness to well-known seco-iridoids was confirmed using free methods. Comprehensive qualitative and quantitative chemical characterization of the extract also allowed to highlight the contribution of minor compounds to the overall taste. Interesting insights into sub-threshold impact on taste were gained. These findings enable improved formulation of plant-based products.

Conflict of Interest; Funding (Source, ID)

The authors declare no competing interest

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PC9-43 Analysis and dermo-cosmetic evaluation of grape seed extracts.

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DOI 10.1055/s-0041-1736969

The current study focuses on the exploitation of grape seeds paste which is the major grape seed oil by-product, targeting on the discovery of extracts with advantageous dermo-cosmetic properties. Based on previous studies of our group [1], grape seed paste showed significant inhibition activity against tyrosinase, collagenase and elastase enzymes. Thus, it could be a potential exploitable product for cosmetic industry. The aim of this study was the production of extracts and enriched fractions with skin beneficial effects. For the extraction step two different techniques were employed. The first one, was ultrasound assisted extraction with ethanol and ethanol/water in ratio 1:1 v/v as diffusion solvents. The ethanolic extract due to its valuable chemical profile was scaled up on preparative level. Moreover, supercritical fluid extraction, with CO_2 and ethanol in percentages of 10 % and 20 % were employed on paste. All

the extracts were analyzed with LC-HRMS to record their chemical profiles. The ultrasound-ethanolic paste extract was fractionated with centrifugal partition chromatography following the system n-Hex/EtOAc/EtOH/H_2O 1:10:3:7 v/v/v/v. The final fractions of paste extract were analyzed with HPTLC and LC-HRMS, and were evaluated concerning their inhibition activity against tyrosinase, collagenase and elastase enzymes, in order to find the most effective fractions. Additionally, the anti-inflammatory and anti-oxidant effects of selected CPC fractions was evaluated in *in vitro* models. Specific fractions inhibited the tumor necrosis factor (TNF-a) induced IL-6 increase of the human endothelial (EA.hy.926) and fibroblast (DLF) cell lines. Also, the same fractions reduced TNF-a induced increase of oxidative species in human monocytes (U937).

Conflict of Interest; Statement on behalf of all authors. There is no conflict of interest; **Funding:** VitVin project (T1EΔK-04103)

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PC9-44 Inhibition of COX-2 mRNA expression by damask rose flowers

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DOI 10.1055/s-0041-1736970

Rosa x damascena Mill., commonly known as damask rose, is the most important scented rose species. It is industrially cultivated around the world and mainly used for fragrance production. However, preparations of damask rose flowers are traditionally also used to reduce inflammation and pain [1].

This study investigated the effect of damask rose flowers on gene expression of COX-2, one of the key enzymes involved in the production of inflammatory mediators. Damask rose flowers were successively extracted with *n*-hexane, CH_2Cl_2 and MeOH. All extracts were tested at a concentration of 20 µg/ml in LPS-stimulated, PMA-derived THP-1 macrophages.

n-Hexane extracts inhibited LPS-induced COX-2 mRNA expression by 57.37 ± 8.2 %. CH_2Cl_2 extracts exhibited a moderate inhibition rate of 31.84 ± 6.1 %, whereas MeOH extracts did not show a reproducible inhibition of COX- 2 mRNA expression.

The chemical composition of the *n*-hexane fraction has been investigated using LC-MS. Various flavonoids, fatty acids and (gluco-)lipids were detected. Further investigations regarding chemical composition of active fractions are in progress.

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PC9-45 Chemotaxonomic investigation of the natural hybrid *Origanum* x *lirium* and its parents focusing on the comparison of volatile and polar constituents

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DOI 10.1055/s-0041-1736971

The genus *Origanum* L. (Lamiaceae) is widespread mainly in the Mediterranean region, although 75 % of the species thereof are restricted to the eastern part. Nine species (eleven taxa) and three natural hybrids occur in Greece. However, there is an open discussion regarding their precise botanical classification. Interestingly, *Origanum* x *lirium* has been proposed both as a separate species as well as natural hybrid between *O. vulgare* subsp. *hirtum* and *O. scabrum*[1].

Thus, the aim of the current work is to contribute to the elucidation of this matter investigating the chemical composition of both essential oil and polar extracts of the above taxa collected from different geographical regions.

Based on the GC-MS and LC-HRMS data obtained and the comparative study which was performed it is evident that *Origanum* x *lirium* shares its chemotype with its parent species regarding both volatile and polar constituents. Moreover, it seems that the climatic conditions strongly influence its chemical composition. Overall, our study provides new information about the composition of the taxa under investigation and additionally supports the opinion of Ietswaart that *Origanum* x *lirium* should be considered as a hybrid and not a separate species but "on the way to species" [2].

Conflict of Interest; There is no conflict of interest

Funding: The authors are grateful to “PlantUP” (project code: 5002803).

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PC9-46 A tailor-made NaDESs development strategy for the enhanced extraction of hydroxynaphthoquinones from Alkanna tinctoria roots.

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DOI 10.1055/s-0041-1736972

The natural hydroxynaphthoquinone enantiomers (HNQs) are well-described pharmaceutical and cosmeceutical agents especially present in European *Alkanna tinctoria* (L.) Tausch roots [1]. In this work, eco-friendly natural deep eutectic solvents (NaDESs) were developed for the selective extraction of these compounds. An extensive screening was performed using more than sixty tailor-made NaDESs. The impact of the intrinsic physicochemical properties on the HNQs extraction efficiency as well as the specificity towards the different enantiomeric pairs was thoroughly investigated. As a result of a multivariate analysis, the most relevant mixture with the highest extraction efficiency was composed of levulinic acid and glucose (LeG) using a ratio of 5:1 (w/w) and 20 % of water (w/w). Further optimization of the extraction efficiency was attained by response surface methodology, using a temperature of 45 °C, a solid-to-liquid ratio of 30 mg/mL, and an extraction time of 50 min. A maximum extraction output of 41.72 ± 1.04 mg/g was reached for HNQs, comparable to that of the commonly used organic solvents. A solid-phase extraction step was also proposed for the recovery of HNQs and for NaDESs recycling. Our results revealed NaDESs as a highly customizable class of green solvents with remarkable capabilities for the extraction of HNQs.

This work has been financed by the EU H2020-ITN-MICROMETABOLITE project (Grant N °721635)

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PC9-48 Oleanane triterpenoid saponins from *Millettia pulchra* radix possessing protective effect on paraquat-induced neurotoxicity

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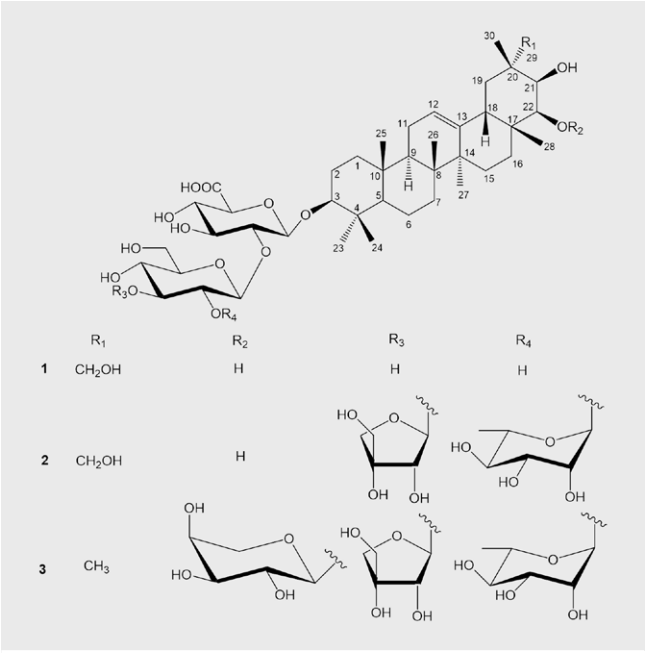
DOI 10.1055/s-0041-1736973

After biological activity screening, the *n*-butanol layer partitioned from the ethanol extract of *Millettia pulchra* Kurz radix proved a protective effect on paraquat (PQ)-induced neurotoxicity. Further purification of the potential *n*-butanol layer by Diaion HP20 column, ODS open column chromatography, and reversed-phase preparative HPLC chromatography, three undescribed oleanane-type saponin derivatives (**1**-**3**), along with five known flavonoids (**4**-**8**), were isolated. The structures of isolated compounds were elucidated by ESI-MS, 1D, and 2D MNR data. The sugar units of saponins were determined by combined use of aldoimidazole derivatization and mass spectrometric analysis. The biological evaluation revealed that **1** possessed significant preventing PQ-induced neurotoxicity (IC_{50} 3.0 ± 0.05 µM).



▶ **Fig. 1**

Keywords: *Millettia pulchra* Kurz, oleananes saponin, paraquat-induced neurotoxicity.

Conflict of Interest

The Authors declare no competing financial interests.

PC9-49 Antimicrobial activity of *Evernia prunastri* extracts and its isolates

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DOI 10.1055/s-0041-1736974

Aim Lichens are symbiotic organisms known for their antimicrobial activity. In this study we investigated the antimicrobial potential of different extracts from the lichen *Evernia prunastri* (L.) Ach.

Methods Lichen *E. prunastri* was sequentially extracted by hexane (Hex), dichloromethane (DCM) and acetonitrile (ACN). Antimicrobial activity was determined using the Minimal Inhibitory Concentration (MIC) method against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans*. The ACN extract of *E. prunastri* was separated by silica gel column chromatography. The active fraction was characterized by ^{1}H and ^{13}C-NMR spectroscopy. Analysis of structural similarity with main structures of antibiotics was performed by the Analyse Similarity/ Activity Cliffs tool.

Results The DCM and Hex extracts were both active against *S. aureus* (MICs: 4 and 21 µg/ml, respectively) but were less active against Gram-negative bacteria and yeast. The ACN extract exhibited activity on both *S. aureus* (MIC 14 µg/ml) and *C. albicans* (MIC 38 µg/ml) and was therefore further fractionated. Two fractions (V, VI) inhibited the growth of all tested microorganisms and, besides that, fraction VI showed a higher effect against *S. aureus*. Evernic acid was identified as a main component of this fraction.

Evernic acid as well as other compounds of *E. prunastri* did not have structural similarity to the main antibiotics representing a novel class of substances with antimicrobial activity.

Conclusion. Analysed compounds could possibly be candidates for development of novel antibiotics [1].

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PC9-50 NMR chemometrics reveal bioactive compounds from propolis extracts prior to isolation

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DOI 10.1055/s-0041-1736975

Propolis is a resinous natural product collected by honeybees from various buds and exudates, transformed and used for the protection of the colony. The chemical composition of propolis varies greatly, therefore, many different types of propolis have been identified around the world. In our previous study [1], 20 Greek methanolic propolis extracts were investigated and two separate group of samples were observed: **a**) propolis samples rich in terpenoids originating from Islands, and **b**) propolis rich in flavonoids from Mainland Greece, exhibiting strong tyrosinase and high collagenase inhibition, respectively. In this study, propolis sample (PR09) from Mainland and propolis sample (PR10) from Island group were selected for further investigation. Their methanolic extracts were fractionated by FCPC and the obtained fractions were evaluated *in-vitro* for their ability to inhibit tyrosinase and collagenase enzymes, subsequently. Their ^{1}H-NMR spectra were recorded. The biological activity was statistically correlated with spectral data through the HeteroCovariance Approach (*HetCA*). The analysis suggested that phenolic compounds (especially flavonoids possessing hydroxyl group at C-3' of ring B and caffeic acid derivatives) and terpenoids (abietane type diterpenes) contribute to the anti-tyrosinase activity. Polyphenols and caffeic acid derivatives seemed to be responsible for the anti-collagenase activity.

Conflict of Interest

The authors declare no conflict of interest.

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PC9-51 ICAM-1 bioassay for the evaluation of sesquiterpene lactones from *Calea* and *Centaurea* spp.

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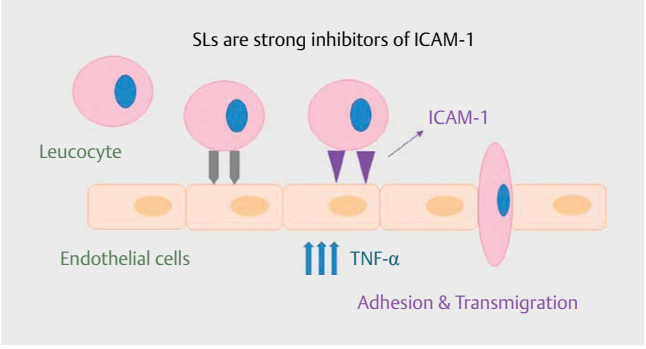
DOI 10.1055/s-0041-1736976

Sesquiterpene lactones (SLs) are highly active specialized metabolites, with well-known cytotoxic, anti-inflammatory, anti-microbial activities etc. This project unveiled that SLs isolated from endemic *Calea* and *Centaurea* spp. are strong inhibitors of the TNF-α induced ICAM-1 expression on endothelial HMEC-1 cells, in a dose-dependent manner, comparably to parthenolide used as positive control (Figure 1). The expression of the surface molecule ICAM-1 is triggered by cytokines and leads to endothelial adhesion and generation of intracellular signals on the endothelial cells during the inflammation process [1]. The lactone ring is essential for the mechanism of action of the strong inhibitors in comparison to the lower inhibitory potential of compounds lacking the α,β-unsaturated lactone moiety. The samples were firstly subjected to MTT viability test on HMEC-1 cells, in order to exclude any false positive results. No toxicity was observed, although Michael reactions usually also involve non-specific toxicity [2].

Further exploration of the SLs may offer a novel therapeutic approach to cope with inflammatory diseases.



▶ **Fig. 1**

Conflict of Interest; Funding
The authors declare no conflict of interest. Part of this work was supported by European Social Fund-ESF (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

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PC9-52 LC-MS based Alkaloid Profiling of Glaucium elegans and evaluation of its cytotoxic activities

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Glaucium (Papaveraceae) is a genus of about 28 species distributed worldwide, used traditionally for the treatment of various disorders. We aimed to investigate the major alkaloids of *G. elegans* by LC-MS profiling technique and determine its cytotoxic effects against some cancerous cell lines, in order to have a better insight for future studies and safety.

The plant was extracted with hexane, dichloromethane (DCM), and ethyl acetate. Since DCM extract contained alkaloids, it went for further investigations. A sensitive method coupling HPLC-PDA-ESIMS was optimized for metabolite profiling. Data preprocessing were performed using MZmine2. The alkaloids were identified by comparing their mass spectra with those in available databases.

Aporphines dehydrocorydine, dehydroglaucine, dehydrodicentrine, and oxoglaucine were the major components.

Moreover, benzophenantridines chelidonine and dihydrochelerythrine, and protopines dihydroprotopine and protopine were dominated alakloids. In Alamarblue assay, the fractions were not cytotoxic at concentrations 50-200 µg/ml in comparison with positive control. Accordingly, the plant can be used as a source of valuable alkaloids particularly aporphines with important therapeutic activities [1].

Conflict of Interest; None
Funding (Source, ID): Granted by Mashhad University of Medical Sciences (IR.mums.pharmacy.rec.1397.012).

Statement on behalf of all authors. All authors confirm that the results of the project is submitted for GA-69th Annual Meeting 2021, Bonn, Germany Virtal Conference

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PC9-53 Biological activities of *Sideritis cypria* Post extracts

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DOI 10.1055/s-0041-1736978

Sideritis cypria Post consists an endemic and endangered species in Northern Cyprus, used in folk medicine [1]. There are recent studies about its phytochemical profile, antioxidant activity, as well as its nutritional and mineral contents [1–4].

The present study focuses on the bio-guided investigation of the methanol extract of cultivated *S. cypria* driven by *in vitro* antioxidant, cytotoxic and antimicrobial assays. The crude extract revealed strong antioxidant properties which was ascribed to a high phenolic profile. On the other hand, the extract did not exhibit any cytotoxicity, though some fractions containing phytosterols, ent-kaurane diterpenoids and apigenin derivatives possessed strong cytotoxic activity on MDA-MB-231 breast cancer cells. In addition, apigenin derivatives were examined separately on the aforesaid cancer cell line, confirming their involvement in the cytotoxicity of the fractions.

Concerning the antimicrobial assay, neither the crude extract nor the fractions revealed any activity.

In continuation of this study, the dichloromethane extract of this cultivated species and its fractions were tested against AChE and BChE, showing moderate inhibition. Thus, the bioactive fractions were chosen for phytochemical analysis. Up to now, four ent-kaurane diterpenes were isolated. ^{1}H-NMR of the crude extract revealed the presence of triterpenes, diterpenes, flavonoids.

Conflict of Interest: The authors declare no conflict of interest.

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PC9-54 Biotranformation of cannabidiol by *Cannabis sativa* endophytic fungi

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DOI 10.1055/s-0041-1736979

Endophytic fungi can perform a variety of chemical reactions as biocatalysts and are effectively one of the most inventive synthetic chemists. Despite being widely regarded as such, the potential of *Cannabis sativa* mycobiota to biotransform cannabinoids is still an underexplored area.

To this end, twenty-five endophytic fungi were isolated from *C. sativa* leaves, stems and flowers. All strains were cultivated in three different solid media and the endophytes showing the largest growth rate were chosen for evaluation of their cannabidiol (CBD) biotransformation potential. The strain with the most promising chemical profile was then subjected to a scaled-up liquid fermentation process for the isolation of fungal biotransformation products.

After seven days of fermentation the liquid broth was filtered and both the medium and the lyophilized mycelium were extracted with EtOAc. The extracts were chromatographed using CC and the biotransformation products were purified using semi-preparative RP-HPLC. Isolated compounds were characterized by the extensive use of 1 & 2D NMR and HRMS spectra.

The fungal endophyte under investigation, belonging to the genus *Chaetomium* produced several metabolites of CBD. Among them, one previously undescribed glycosidic derivative of CBD and four new compounds belonging to the cannabielsoin type cannabinoids were isolated. In addition to them, one known mono-hydroxylated, two di-hydroxylated and one *O*-glycoside were purified. An antibiotic namely chochliodinol, characteristic of the specific genus, was also isolated. To conclude, it seems that with the aid of endophytes from *C. sativa*, cannabinoids can be transformed into a large inventory of novel compounds with potential pharmaceutical applications.

The authors declare that there is no conflict of interest

PC9-55 A holistic approach to sustainable use of Greek medicinal and aromatic plants for the production of innovative bioactive food and feed products

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DOI 10.1055/s-0041-1736980

Olive oil is one of the most important Mediterranean diet component, well known for its benefits in human health. It has been demonstrated that olive oil's aromatization process is a promising alternative to increase shelf life, introduce new products to the food industry, and offer consumers new prospects [1]. At the same time, the process of aromatization of olive oil with Medicinal & Aromatic Plants (MAPs), results to the production of a significant amount of plant by-products. The aim of this study was the evaluation of the aromatized olive oils and the production of innovative products for animal nutrition.

Initially, *Origanum vulgare, Thymus vulgare*, *Salvia officinalis* and *Rosmarinus officinalis* were extracted with olive oil by microwave technique and analyzed by Head Space Microextraction and GC-MS. Afterwards, in order to evaluate the by-products, the plant material remaining after the extraction with olive oil of the superior and inferior quality of *O. vulgare* and *T. vulgare*, was studied. Specifically, the hydroalcoholic extracts were evaluated for their antioxidant activity and total phenolic content and were chemically screened by means of HPTLC.

According to the results, MAPs' by-products revealed similar chemical profile to the crude herbal material as well as strong antioxidant capacity, which is related to the detected high phenolic content, indicating their usefulness for animal nutrition. Moreover, GC-MS analyses of the aromatized olive oils by MAPs, revealed the presence of their essential oil's major compounds (carvacrol, thymol, *γ*-terpinene and *p*-cymene), underlying the high added-value of the enriched aromatic olive oils.

Authors declare no conflict of interest. The study was funded by the national project "FeedMAP" (EPAnEK-2014-2020).

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PC9-56 Handling of complexity: Describing the mechanisms of action of a multi-target therapy in functional GI diseases by multi-step clustering derived heatmaps

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DOI 10.1055/s-0041-1736981

Introduction In functional gastrointestinal diseases (FGDs), a multitude of concomitant causes and likewise also targets for therapeutic interventions have been identified [1]. Therefore, from a translational perspective, a multi-target approach is a promising therapeutic strategy. For assessing the underlying complex mechanisms of action, a novel approach has been developed [2] and is now applied to another natural product, STW 5-II (NP) consisting from extracts of lemon balm leaves, caraway fruits, peppermint leaves, bitter candytuft total plant, chamomile flowers and liquorice roots, with proven efficacy in a multitude of clinical studies.

Methods All data from preclinical studies on mechanism of action of combination NP and its components were retrieved. Study results were sorted in relation to the different etiologic mechanisms, and multi-step clustering was conducted to reduce data complexity, followed by visualization in the form of 2D histograms/heatmaps.

Results The evaluation of the data showed that NP is active in etiologic factors involved in FGDs, especially in functional dyspepsia and irritable bowel syndrome, like hyper- and hypomotility, acidity, inflammation and hypersensitivity, but also in inflammatory gastrointestinal diseases.

Conclusions Multi step clustering allows the allocation of the specific actions of NP to the different components and the visualization of its anti-inflammatory, mucosa protective and calming actions, which are in accordance to its use in long lasting functional gastrointestinal diseases.

Conflict of Interest; Funding (Source, ID)

OK and HA-K are employees of Steigerwald Arzneimittelwerk GmbH. GL and KN received fees from Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany.

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PC9-57 *Urtica urens* dry extract as contained in Combudoron® prevented inflammatory edema *in vivo*

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DOI 10.1055/s-0041-1736982

Combudoron®, an herbal remedy containing ethanolic extracts from *Urtica urens* (Urtica) and *Arnica montana* (Arnica), is authorized (according to the anthroposophical under-standing of humankind and nature) for the treatment

of insect bites, sunburns, general first and second-degree burns, and radiation injuries, respectively. All of these pathologies share initial inflammatory reactions eventually leading to typical symptoms such as redness, pain and edema formation. Leukotrienes produced by 5-lipoxygenase (5-LOX) as part of the arachidonic acid (AA) pathway are key mediators for the onset and promotion of inflammation and preparations from Arnica and Urtica are well known for their anti-inflammatory properties. Both extracts were analyzed for inhibition of recombinant human 5-LOX and the anti-edema efficacy of both extracts were determined in vivo using the AA-induced ear edema model in mice. The right ears of ICR mice were topically treated with 0.6-12 mg Urtica or 0.6 and 6 mg Arnica dry extract before and after challenge with 2 mg AA. Ear swelling was measured 60 min after AA challenge. Urtica and Arnica concentration-dependently inhibited 5-LOX activity in vitro (IC_{50}: 59 and 3.5 µg/ml, respectively). However, in vivo Urtica but not Arnica revealed a dose-dependent and significant anti-edema activity starting at a dose of 1 mg/ear. These experimental data suggest that Combudoron® may be effective in the prevention of edema formation and thus may ease inflammatory symptoms in the treatment of e. g., mild burns and insect bites. However, inhibition of 5-LOX might not be solely accountable for this effect and further studies are required to define the mechanisms of action of both extracts.

Conflict of Interest

Röhrl J, Ammendola A and Künstle G are employees of Weleda AG, Switzerland

PC9-58 Olive branches as a source of valuable bioactive compounds. Comparative studies on phenolic composition and antioxidant activity of olive leaves and branches

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DOI 10.1055/s-0041-1736983

Olive by-products is at the center of commercial interest due to their high content in bioactive compounds. Till today, most of the studies have focused on olive leaves, olive mills by-products as well as edible olives debittering process by-products while there is only few information available about olive branches which is also a substantial agricultural by-product [1, 2].

In the present work, a systematic study towards the investigation of the phytochemical content of olive branches was performed. A high number of samples of different olive trees varieties and geographical regions of Greece were investigated using HPLC-DAD and LC-HRMS & HRMS/MS methods. Additionally, a comparative study was performed between leaves and branches of the same olive trees, in regard to their phenolic composition and antioxidant activity.

Based on our results, it is evident that olive branches, which are highly underestimated amongst olive by-products, comprise also a rich source of bioactives, with significant antioxidant activity.

Conflict of Interest; Funding

The authors declare no conflict of interest. The authors are grateful to Operational Programme “Central Greece 2014-2020 “PYTHOLEO” (project code: STER1-0018066

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PC9-59 Phytochemical analysis of *Gnetum montanum* stems' ethanol extract and *in vitro* evaluation of tyrosinase and elastase inhibitory activity.

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DOI 10.1055/s-0041-1736984

Gnetum montanum Markgr.(Gnetaceae) is used in Vietnamese traditional medicine for the treatment of rheumatoid, arthritis and bronchitis [1] while recently has been reported as an effective plant in the treatment of gout in Vietnam [2]. Our study focused on isolation and identification of natural compounds from stems of G. montanum and evaluation of their inhibitory activity on tyrosinase and elastase enzymes.

The dry stems were initially extracted with ethanol 96 % and subsequently the ethanol extract was partitioned with $CHCl_3$, EtOAc and H_2O and the fractions were evaluated in vitro for their capacity to inhibit tyrosinase and elastase enzymes. The EtOAc fraction presented high anti-tyrosinase and anti-elastase activity with IC_{50} = 0.011mg/ml and 0,072mg/ml, respectively and was chosen for further phytochemical analysis.

UPLC-HRMS/MS analysis of the above fraction resulted in the identification of over than 60 secondary metabolites, which mainly belong to stilbenoids, lignans, flavonoids, simple phenolics, and alkaloids. The combination of Centrifugal Partition Chromatography (with a series of 4 biphasic systems composed of n-hex-EtOAc-isoPrOH-H_2O x/y/4/6) and prep- HPLC or prep-TLC led to the isolation of 13 natural compounds, of which three aroma acids (*p*-hydroxybenzoic acid, 3-(4-hydroxyphenyl)-propionic acid, vanillic acid), three flavonoids (hesperidine, vitexin, nobiletine) and seven stilbenoids (2b-hydroxyampelopsin F, isorhapotogenin, resveratrol, ε-viniferine, gnetol, gnemontanin G and gnetanin A). To the best of our knowledge, this is the first report of the trimeric stilbene gnetanin A. All isolated compounds were elucidated by using 1D and 2D NMR spectroscopy as well HRMS/MS spectrometry and then their inhibition activity against tyrosinase and ellastase was investigated

Conflict of Interest; Statement on behalf of all authors. There is no conflict of interest; **Funding**: MediHealth project (Horizon2020-MSCA-RISE-2015-691158)

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PC9-60 Cell and Tissue Culture Models for Pharmacological Investigation of an Herbal Combination of Myrrh, Coffee Charcoal and Chamomile Flower Extract in the Context of IBD

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DOI 10.1055/s-0041-1736985

Many patients suffering from inflammatory bowel diseases (IBD) show interest in phytotherapy. However, herbal medicines often lack evidence on their efficacy and mode of action. Clinical data suggests the application of a traditional herbal medicinal product consisting of myrrh, coffee charcoal and chamomile flower extract in IBD maintenance therapy [1].

This investigation aimed to gather more information on underlying pharmacological mechanisms and active components.

Effects of coffee charcoal extract and secondary plant metabolites on pro-inflammatory mediator release (TNF, IL-6, MCP-1) were assessed in a cell model of human LPS-challenged THP-1 macrophages. Chlorogenic acids, especially cryptochlorogenic acid, showed the most distinct anti-inflammatory effects.

A co-culture model of the inflamed intestinal mucosa was used to evaluate effects of myrrh, coffee charcoal and chamomile flower extract on the inflammatory crosstalk between intestinal epithelial cells (IEC, 9:1 Caco-2 and HT29-MTX) and immune cells (THP-1 macrophages), and inflammation-induced barrier dysfunction. The individual extracts and their combination reduced the secretion of inflammatory mediators (IL-6, TNF, MCP-1, IL-8, PGE_2) to a varying extent. Myrrh, coffee charcoal and the ternary combination also enhanced the IEC barrier function.

To confirm and expand these findings in a translational tissue model, the feasibility of organotypic slice cultures of human biopsy specimens from IBD patients was evaluated. Preservation of characteristic tissue structure, viability and inflammatory cell presence was observed histologically, while a spectrum of detectable mediators was found in the culture medium after 24h and 48h. Thus, the tissue model appears as promising approach for further pharmacological investigations of the herbal combination.

This research was funded by Repha GmbH Biologische Arzneimittel. L Schiller, B Lipowicz, C Vissiennon are employed by Repha GmbH Biologische Arzneimittel.

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PC9-61 Studies on mechanism of genotoxicity of selected pyrrolizidine alkaloids in HepG2 cells

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DOI 10.1055/s-0041-1736986

Introduction Pyrrolizidine alkaloids (PAs) have been discovered in food, food supplements and herbs/spices. Certain PAs are hepatotoxic and genotoxic and are potentially harmful to humans. Here we investigated the chemical structure effect relationship of several genotoxic PAs.

Methodology The genotoxicity of PAs of different esterification types, such as europine, retrorsine and lasiocarpine, was determined in HepG2 cells using the cytokinesis-block micronucleus (CBMN) assay. DNA-crosslinking activity of different PA-types was investigated using a modified comet assay. The role of metabolic enzymes such as cytochrome P450s was investigated by using the inhibitor ketoconazole.

Results An increase of micronucleus formation was found with all tested PAs of different chemical structure. The lowest concentrations at which significant induction of micronuclei were detected were 3.2µM for lasiocarpine (open diester type), 32µM for retrorsine (cyclic diester type) and 100µM for europine (monoester type). In the modified crosslink comet assay, the diester type PAs reduced tail formation after hydrogen peroxide treatment, while an equimolar concentration of the monoester europine did not significantly reduce DNA migration. In addition, micronucleus induction by lasiocarpine was abolished after pre-treatment with the cytochrome P450 inhibitor ketoconazole [1].

Conclusion The results strengthen the hypothesis that the genotoxicic potential depends on the ester type of the PAs with the open diester PA-type being the most potent while the monoester PA-type being the least potent. In the modified comet assay, the crosslinking activity was also related to the ester type of PAs.

Conflict of Interest; Funding (Source, ID)

OK is employee of Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany, HS of Phytolab GmbH & Co. KG, Vesterbergsgreuth, Germany. The study has been financially supported by Phytolab GmbH & Co. KG and Steigerwald Arzneimittelwerk GmbH.

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PC9-62 *In vitro* anti-inflammatory potential and chemical profiling of *Entada africana* from the Republic of Benin

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DOI 10.1055/s-0041-1736987

Entada africana Guill. et Perr, a plant of the Fabaceae family, has been reported to exhibit potential analgesic efficacy in the management of inflammatory-related conditions in traditional medicine in the Republic of Benin [1, 2]. However, there is still a lack of knowledge about the constituents of the plant's leaves. The objective of the present study was to assess the anti-inflammatory effect of a hydroalcoholic leaf extract of *E. africana* and its fractions and to identify its active compounds. Fractions were obtained by chromatography on Sephadex® LH-20 using a step gradient from ethanol, methanol/water, and methanol to acetone. Subsequently, fractions were screened for their phenolic profile using thin-layer chromatography and UHPLC-DAD. Then, *in vitro* anti-inflammatory assays were carried out with fractions FC3, FC4, FC7, FC8, and FC22 using a TNFα-stimulated human keratinocyte cell line (HaCaT) as a skin inflammation model. FC7 and FC22 significantly decreased the release of IL8 with IC_{50} 91.74 µg/ml and IC_{50} 63.47 µg/ml, respectively. In addition, fractions FC3 (IC_{50} 54.13 µg/mL), FC4 (IC_{50} 87.55 µg/ml), and FC22 (IC_{50} 72.91 µg/ml) had a significant influence on IL6 release. Out of these fractions, ten phenolic compounds were isolated by MPLC on RP-18 and preparative HPLC. Structures were elucidated by HRESI-MS and NMR. The results obtained, support the anti-inflammatory effect of *E. africana* and its application in traditional Beninese medicine. These compounds will then be analyzed for their *in vitro* activity to identify compounds responsible for the pharmacological effect.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding: DAAD (German Academic Exchange Service, Funding programme /- ID: 57299293).

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PC9-63 Mitochondrial Analysis as a Potential Tool for Functional Assessment of Medicinal Plants and Natural Products

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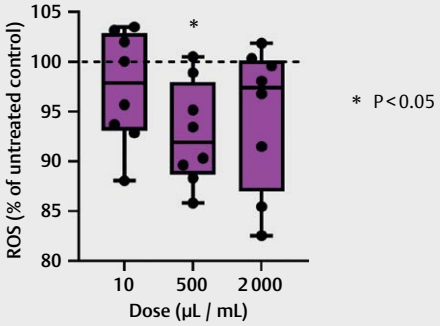
DOI 10.1055/s-0041-1736962

Introduction Analysis of mitochondrial function presents a novel opportunity to assess medicinal plants and natural products. Changes in these organelles in response to medicinal herbs can provide a better representation of their biological activity and potential clinical effectiveness than the current methods of chromatography, spectrometry and genetic analysis [1]. Mitochondria provide the energy for most cellular activities through oxidative phosphorylation [2] which generates Reactive Oxygen Species (ROS) that are important in both health and disease [3, 4]. Mitohormesis is the cellular protective response triggered by mitochondrial stress [5]. Phytochemicals are thought to participate in mitohormetic responses by up-regulating endogenous stress response pathways and antioxidant defenses [6]. Here we test the potential use of mitochondrial functions to assess medicinal herbal tea.

Methods MCF7 and MCF10A cells were seeded and treated for 24 hours with a medicinal herbal tea (Pukka Three Licorice Tea extract). Various assays assessed mitochondrial function including:

- Cell proliferation (MTT)
- ROS (DCFDA)

Results MCF10A cells exposed to 500 µL/mL Pukka Three Licorice Tea extract for 24 hours experience a significant ($p < 0.05$) drop in ROS compared to control (Figure 1).



▶ **Fig. 1**

No significant differences in ROS were observed in MCF7 cells. Cell viability was unaffected in either cell line.

Discussion Future tests include:

- Membrane potential (TMRE)
- Oxygen consumption rate (SeaHorse Flux Analyzer)
- Quantity, morphology and dynamics (fluorescent microscopy)
- Adenosine triphosphate (luciferase)
- Calcium stress signalling (Rhod-2)
- Citric acid cycle (citrate synthase)

Conclusion Mitochondrial assays can be used to generate a functional profile of how natural products interact with complex biological systems.

Conflict of Interest; Funding (Source, ID)
The author (SW) declares that he received funding from Pukka Herbs Limited Research. The funder was however not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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PC9-64 Modulation of the Neurothrophic Activity by Ballota nigra L. Crataegus oxycantha L., Passiflora incarnata L. and Valeriana officinalis L. and their Combination in vitro

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DOI 10.1055/s-0041-1736988

Introduction Neurotrophins such as the brain derived neurotrophic factor (BDNF) as well as cytokines play an important role in neuroprotec-tion, the control of the central nervous system, and change with different sleep patterns. We questioned whether the plant extracts of *Ballotra nigra L, Crataegus oxycantha L, Passiflora incarnata L.* and *Valeriana officinalis L.* alone or in combination can modulate the pro-inflam-matory cytokines, neurothrophines or neuro-transmitters in vitro.

Methods: Synchro-nized human neuroblastoma SH-SY5Y cells were treated with the different plant extracts (5-400µg /ml) alone and in combination. As reference drug Lorazepam (LZ) was used. Resazurin was used to determine cell viability. TNF-alpha and IL-1ß were estimated in the supernatant by ELISA. An unbiased transcript-omic approach was used to determine the overall gene expres-sions induced by the different plant extracts/ combination. Deepsequencing data were eva-luated with different tools including Principal ArComponent Analysis (PCA) and differential gene expressions.

Results: Neither the single plant extracts nor the combination or the refe-rence drug LZ reduced the cell viability in the tested concentrations. The treatments did not stimulate TNF-α or IL-1β releases. The gene expression profiles of the four plant extracts, their combination and LZ could be clearly distin-guished in the PCA –Analyses. The subsequent detailed gene expression analyses revealed that cytokines, neurotrophins (e. g., BDNF) and neu-rotransmitters were among the 2.500 genes with the most variable expression. The four plant extracts and the combination exhibited different expression profiles, also in comparison to LZ.

Conclusion: The four plant extracts and their combination target in SH-SY5Y cells relevant genes for the modulation of the neurotrophic activity.

Conflict of Interest; Funding:
This work was supported by the Steigerwald Arzneimittelwerk GmbH, Bayer Consumer Health

PC9-65 Cyclotides from Brazilian medicinal plant species *Anchietea pyrifolia*

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DOI 10.1055/s-0041-1736989

Cyclotides are cysteine-rich peptides from plants with molecular mass in the range of 2.8-3.7 kDa, with six conserved cysteine residues involved in the formation of an unique arrangement called cyclic cystine knot (CCK) motif. This arrangement is responsible for their thermal, chemical, and enzymatic stability [1]. *A. pyrifolia* (Violaceae), popularly known as "cipó-suma" is a medicinal plant used by local population of Brazil as depurative and for the treatment of skin disorders [2]. Thus, in this study, the roots were extracted with a hydromethanolic mixture (6:4; v/v) for 24 hours (4 times). The hydromethanolic extract was resuspended and submitted to solid phase extraction (SPE-C18-cartridges) to obtain the rich-peptide fraction. This fraction was submitted to LC-ESI-IT-MS analysis that confirmed the presence of peptides in mass range of 2800-3700 Da. HPLC chromatography allowed to isolate five cyclotides from the roots. The peptide sequencing was performed by enzymatic digestion with endoproteinase Glu-C, trypsin, chymotrypsin and followed by MALDI-TOF-MS/MS analysis. The spectra were examined based on the presence of both *b*- and *y*-series of ions, three new cyclotides (anpy A-C) and two previously known ones (cyO4 and cyO17) were identified and their hemolytic activity tested. Anpy A was the most hemolytic with HD_{50} of 22μM.

Conflict of Interest; Funding (Source, ID)
The authors declare no conflict of interest; and are thankful to FAPESP for the scholarship to Fernández-Bobey (grant #2019/26550-3) and Thematic Project (grant# 2016/16212-5). We also thank to INCT-BioNat (grants# 465637/2014-0 and 2014/50926-0).

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PC9-66 Panasenoside as a marker for the discrimination of Ginseng root and leaf extracts

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DOI 10.1055/s-0041-1736990

Both, Ginseng roots and leaves contain ginsenosides. Therefore, there is a chance that these extracts are mixed up. We have investigated roots and leaves of *Panax ginseng* C.A. Meyer and *P. quinquefolius* L. and found mainly quantitative differences in the content of ginsenosides. However, there was one characteristic blue fluorescent compound detected in Ginseng leaves, which could not be found in the roots. The compound has been isolated by preparative TLC and was finally identified by ms and nmr as kaempferol-3-O-glucosyl(1-2)galactoside, called panasenoside. Panasenoside was known as a constituent of leaves of *P. ginseng*[1]. However, this is the first report *P. quinquefolius*. Panasenoside can be used as a marker for Ginseng leaf extracts and can be easily identified by HPTLC or HPLC.

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PC9-68 From inflammation to depression. How can St. John's wort help?

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DOI 10.1055/s-0041-1736991

Introduction That inflammatory processes can be involved in the etiology of psychic depression is now widely accepted [1, 2]. An IFN-α-induced raise of TNF-α and IL-6 can cause depressive symptoms, and even the microinflammation in long COVID syndrome is often connected to depressive symptoms [3]. Therefore it seems relevant to re-assess, whether data confirm the relevance of anti-inflammatory mechanisms of action in herbal treatment options in depressive episodes, with focus on St. John's wort [4].
Methods A broad and systematic literature review was conducted [Medline, EMBASE] and evidence was assessed.
Results As well for St. John's wort extracts as for a number of its constituents, especially flavonols and hyperforin, in vitro and in vivo data show numerous anti-inflammatory mechanisms of action. Recent examples are data on the phagocytic and migratory activity of microglia cells [5] or on pituitary-derived AtT-20 cells in vitro [4].
Conclusions Overall, also most recent data show potential mechanisms of action of St. John's wort extracts, which could be one of the key mechanisms of actions [4] of St. John's wort in psychic depression, especially with inflammatory etiology.

Conflict of Interest; Funding (Source, ID)
OK and CK are employees, NM is pharmacy intern at Steigerwald Arzneimittelwerk GmbH, Darmstadt, Germany, KN has been receiving grants and travel support from of Steigerwald Arzneimittelwerk GmbH.

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PC9-70 New indole diketopiperazine alkaloids from a soil-derived fungus *Aspergillus ochraceopetaliformis*

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DOI 10.1055/s-0041-1736992

Prenylated indole derivatives are hybrid natural products containing both aromatic and isoprenoid moieties and are widely spread in terrestrial and marine

organisms, these compounds are mainly found in the genera of *Claviceps*, *Penicillium* and *Aspergillus* of Ascomycota [1, 2]. Tryptophan from the primary metabolism is a key precursor for the biosynthesis of the prenylated indole alkaloids and acts as the biogenetic origin of indole rings.[3] Besides L-tryptophan, most of these metabolites contain a second amino acid and form cyclic dipeptides with a diketopiperazine structure or a derivative thereof. However, their final chemical structure may be quite complex. Inspection of the natural indole diketopiperazine IDKP alkaloid chemical structures shows the pervasive presence of a prenyl group on the indole ring. The prenyl group consists of an allylic subunit with at least five carbons [4].

Several prenyl groups can be found at the periphery of these molecules and it is proposed to increase lipophilicity to favour interactions with biological membranes and bioactivity.[5] Prenylated IDKP alkaloids thus possess specific properties that make them good candidates for lead discovery and drug development. During our continuous efforts towards the discovery of new and bioactive secondary metabolites from terrestrial *Aspergillus ochraceopetaliformis*[6] which was collected at Giza province, Egypt, four new diketopiperazines named as 8-hydroxyechinulin (**1**), 27,28-epoxyechinulin (**2**), (12*R*)dehydroechinulin (**3**) and neoechinulin F (**4**), along with ten known compounds (**5**-**14**) were isolated. The compounds identified in this work were isolated using multiple chromatographic techniques, and the structures were established based on a detailed analysis of 1D and 2D NMR data combined with HR-ESIMS. The study of the stereochemistry of the isolated compounds was based on 1D and 2D NOESY experiments, Marfey's analysis, specific optical rotation measurements and thorough comparison with literature data. All isolated compounds were evaluated for their antimicrobial activities against six Gram-positive and Gram-negative bacteria as well as against three human pathogenic fungi.

Conflict of Interest: no conflict of interest.

Funding (Source, ID): No external fund

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PC9-71 Effect of thymol on lipid oxidation and fatty acid composition of rabbit meat

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DOI 10.1055/s-0041-1736993

As the bioactivity of thymol, a major constituent of *Thymus vulgaris* L. in animal organism is still not clear, present study provides new insights for understanding the processes of absorption and deposition of thymol in the rabbit organism in connection with ist protective role against oxidative deterioration in muscle tissue. After weaning at 35 days of age, 48 rabbits were randomly divided into two experimental groups (control and with thymol addition 250 mg/kg feed). Thymol was administered for 21 days (TA) and withdrawn for seven days (TW). Significant correlation (r_s = -1.000, $p < 0.01$) between thymol content in intestinal wall and plasma pointed on intensive absorption of thymol from intestine. During period of TA we detected decreased level of lactate dehydrogenase in muscle, which points to the sparing effect of thymol on cellular metabolism. Rabbits need adequate amounts of essential fatty acids (FA), which are primarily represented by linoleic and α-linolenic acids in their diet. We observed increase in FA and, mainly α-linolenic acid during TA, which was probably caused by block the oxidation of the lipids because of strong antioxidative properties of thymol. During TW, linoleic acid, as well as n-6 polyunsaturated FA decreased, which could be the reason for the insufficient effect of thymol in connection with its low deposition in the muscle tissue. We confirmed sufficient absorption of thymol from the gastrointestinal tract but further studies are needed to clarify the biotransformation and bioavailability of thymol in the rabbit with respect to the specific digestive characteristic, caecotrophy.

Funding

Scientific Grant Agency of the Ministry for Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences (VEGA 2/0009/20), the Austrian Federal Ministry for Science, Research and Economics, OeAD, Ernst Mach Grant Action Austria-Slovakia.

PC9-72 Adaptation and validation of a UV spectrophotometry method for quantification of tannins in *Cupressus sempervirens* L. cone extracts

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DOI 10.1055/s-0041-1736994

Analytical methods used for quality control of plants and plant extracts are based on the identification and quantification of chemical markers that are well-known compounds of the plant of interest [1]. *Cupressus sempervirens* L. is a tree native of the Mediterranean Basin (Europe, West Asia). In traditional medicines, aerial parts are used especially cones still green. European Pharmacopoeia described a UV spectrophotometry method for quantification of tannins [2]. This method is not specific to a particular plant. Our objective was to apply it to cypress and to validate the quantification. Fresh cones, a specific dried extract and a liquid extract, were matrices used for the validation of the process. The assay was composed of three main steps: the first was the dosage of total polyphenols, the second was the adsorption of polyphenols by hide powder and the last was the dosage of non-adsorbed polyphenols by hide powder. The quantity of tannins resulted from the difference of these two dosages. The results were expressed as pyrogallol. The method was found to be linear for pyrogallol in a wide range (0.012–0.038 mg/ml). In the absence of a reference sample, verification of the performance of this method was based on the pyrogallol linearity study and a reproductibility study (intermediate precision conditions).

This validated method offers a new reliable tool for quantification of tannins for *C. sempervirens* sourcing and extract standardization. The method is now part of our routine analytical techniques that are applied for quality control.

Conflict of Interest

BV, BL, CC, SN, GI and DM are employees of PiLeJe.

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PC9-73 Investigation of the Ecological Relevant Bioactivity of the Cyclic Depsipeptide FR900359

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DOI 10.1055/s-0041-1736995

Natural products are specialized metabolites, that show unique and specific biological effects under laboratory conditions. The significance of these effects in the ecosystem is in many cases not further investigated.

FR900359 (FR) is a complex non-ribosomal peptide and a potent and specific inhibitor of $G\alpha_q$ proteins, important signal transducers in eukaryotes [1]. FR was first isolated from the leaves of the plant *Ardisia crenata*, and later found to be produced by the unculturable endosymbiont *Candidatus* Burkholderia crenata living in the nodules of the leave margin. We verified that FR, produced only in the margin of the leaves, has an insecticidal effect, and may thus protect the plant from insect feeding [2]. We recently discovered a cultivable soil bacterium, *Chromobacterium vaccinii*, harbouring the FR gene cluster which is produced under laboratory conditions.

In this work the ecological relevance of FR produced by *C. vaccinii* is further investigated. Therefore, we tested FR on a plant pathogenic cyst nematode and provided evidence for its influence on the hatching of the nematode. As soil bacteria play an important role for plant health, deciphering the mechanisms leading to the protection of the plant may present new options for agriculture.

The authors declare no conflict of interest. We thank the DFG (FOR 2372 grants KO 902/17-1 and 17-2 to G.M.K.) and the DBU (Ph.D. scholarship 20018/568 to W.H.) for funding. P.G. has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 727715

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PC9-74 Antiviral activity of green tea and fruit juices against SARS-CoV-2, influenza, and other viruses

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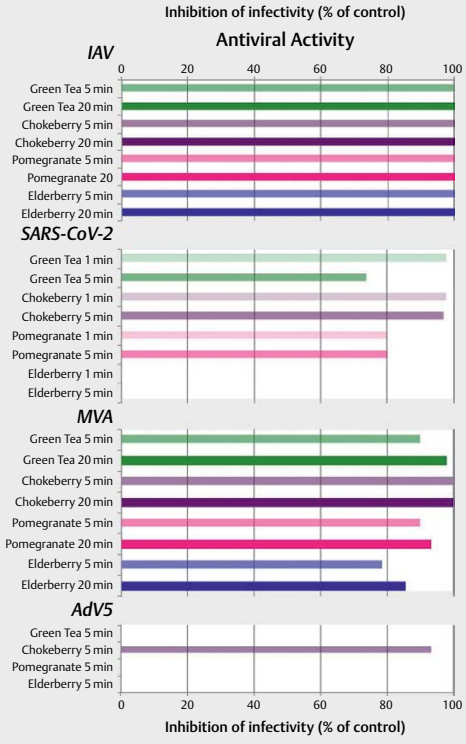
DOI 10.1055/s-0041-1736996

We here analyzed the virucidal activity of green tea (Camellia sinensis), black chokeberry (Aronia melanocarpa), pomegranate (Punica granatum) as well as elderberry (Sambucus nigra) juice, against different respiratory viruses. The results [1] show that they have remarkable virucidal properties. The severe acute respiratory syndrome-causing coronavirus-2 (SARS-CoV-2), influenza A virus (IAV) were inhibited in their infectivity up to 99.99% by the above plant extracts. Since viral replication, symptoms and transmission occur in the nasal and oropharyngeal regions, lowering viral titres as early as possible could be a proactive strategy to prevent infection, dissemination, severe disease progression and spread. Long-term use of the tested products in the form of mouthwashes and gargles are largely unproblematic and could be a suitable pre- and post-exposure prophylaxis during the current COVID-19 pandemic. In addition, the ability to swallow food, unlike conventional mouthwashes, is practical in many situations. Following in vivo studies [2,3] in people infected with SARS-CoV-2 and their rapid virus clearance by gargling, inhaling and sucking, the transferability of the in vitro results shown to the pandemic situation is promising and in-vivo studies should follow.



Antiviral activity of natural products against MVA, IAV, SARS-CoV-2 and AdV5 after indicated contact times. MVA: Modified vaccinia virus Ankara; IAV: influenza A virus (A/H1N1/Brisbane/59/2007); SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 (BetaCoV/France/IDF0372/2020); AdV5 adenovirus type 5.

▶ **Fig. 1**

Conflict of Interest; Funding (Source, ID)

Bruno Frank & Uwe Kessler are partner of CogniVerde GmbH Groß-Umstadt, Germany; Financial support by CogniVerde GmbH

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Index

Planta Med 2021; 87: 1233–1328

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J

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Planta Med 2021; 87: 1233–1328

Planta Med 2021; 87: 1233–1328

Notice
This was corrected in the online version on 27.01.2022
In this article the authors have been corrected.

Correct
Lystvan K, Belokurova V, Salivon A, Kuchuk M

Planta Med 2021; 87: 1233–1328